

JCEM

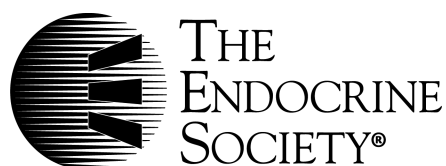
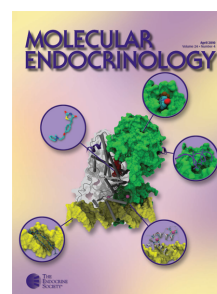
THE JOURNAL
OF CLINICAL
ENDOCRINOLOGY
& METABOLISM

Endogenous Sex Hormones and Cardiovascular Disease in Men

Majon Muller, Yvonne T. van der Schouw, Jos H. H. Thijssen and Diederick E. Grobbee

J. Clin. Endocrinol. Metab. 2003 88: 5076-5086, doi: 10.1210/jc.2003-030611

To subscribe to *Journal of Clinical Endocrinology & Metabolism* or any of the other journals published by The Endocrine Society please go to: <http://jcem.endojournals.org/subscriptions/>



CARDIOVASCULAR ENDOCRINOLOGY

Endogenous Sex Hormones and Cardiovascular Disease in Men

MAJON MULLER, YVONNE T. VAN DER SCHOUW, JOS H. H. THIJSSSEN, AND
DIEDERICK E. GROBBEE

*Julius Center for Health Sciences and Primary Care (M.M., Y.T.v.d.S., D.E.G.) and Endocrinology Laboratory (J.H.H.T.),
University Medical Center Utrecht, 3508 GA Utrecht, The Netherlands*

Unlike women, men do not experience an abrupt reduction in endogenous sex hormone production. It has, however, become clear that an age-associated decrease in the levels of (bioactive) sex hormones does occur. Whether endogenous sex hormones have an impact on cardiovascular disease has for many years remained largely unknown, but during the last decade more attention has been drawn to the importance of testosterone, estrogens, and adrenal androgens in etiology, prevention, and treatment of male cardiovascular disease. The pur-

pose of this article is to summarize the evidence currently available on the association between endogenous sex hormones and cardiovascular disease in males. Published studies dealing with the relationship between circulating levels of sex hormones and cardiovascular disease in males were reviewed. The studies reviewed in this article suggest that circulating endogenous sex hormones and estrogens have a neutral or beneficial effect on cardiovascular disease in men. (*J Clin Endocrinol Metab* 88: 5076–5086, 2003)

ABUSE OF EXTREMELY high doses of anabolic steroids has been linked to sudden cardiac death, leading many to believe that the physiologically high levels of androgens in men may have a deleterious effect on the male cardiovascular system (1). However, recent reports contradict this theory, claiming that androgens may have cardiovascular benefits in men (2, 3).

The age-related decline in sex hormones in men (4) is hypothesized to promote age-related health problems. For many years, little was known about sex hormones and cardiovascular disease (CVD), but in the last decade the importance of testosterone (T) and dehydroepiandrosterone (DHEA), including its sulfate (DHEA-S), in CVD in men had been recognized.

This review summarizes current evidence on the association between sex hormones, endogenous as well as exogenous, and intermediate or clinically manifest indications of CVD and conditions in men.

Search strategy and selection criteria

Data for this review were identified by searches of MEDLINE and PubMed with the search terms “androgens” or “sex hormones” in combination with the terms “cardiovascular” and “males” or “men.” We then searched these publications using the terms “diabetes,” “cholesterol,” “obesity,” “atherosclerosis,” “hypertension,” and “vascular function.”

Abbreviations: BT, Bioactive (bioavailable) T; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lpa, lipoprotein-a; MI, myocardial infarction; NO, nitric oxide; OR, odds ratio; PWV, pulse wave velocity; T, testosterone; tHcy, homocysteine.

We focused on publications in the past decade but did not exclude commonly referenced and highly regarded older publications. Relevant articles not identified with the search strategy described above but referenced in the bibliographies of these papers could also be included. Several recent review articles and book chapters were also included because they provide comprehensive overviews.

Sex hormone biosynthesis and metabolism

Circulating steroids of importance in men are T, dihydrotestosterone, and estradiol. T is mainly produced by the testicles, whereas DHEA and DHEA-S are mainly produced by the adrenal glands. Both organs produce androstenedione (5). Figure 1 presents the several pathways for androgen and estrogen biosynthesis.

Androgens and estrogens circulate either free (1–2% of T) or bound to the serum proteins, albumin (binds 40–60% of T) and SHBG (binds 40–80% of T, which is biologically inactive) (6). Bioactive (bioavailable) T (BT) includes both free and albumin-bound T. Androstenedione and DHEA circulate weakly bound to albumin. DHEA-S binds strongly to albumin.

Measuring hormone levels

Reported concentrations of sex hormones vary widely, which may in part be due to the choice of laboratory assays used (7). Although indirect methods have been the gold standard, direct assays can provide accurate relative results except for low concentrations (6) (Table 1).

There is no real consensus as to which forms of hormones should be measured to best evaluate androgen status, and there is a need to standardize the procedures used to measure

FIG. 1. Pathways for androgen and estrogen biosynthesis. Circled numbers represent the following enzymes: 1, 20,22-desmolase (P-450scc); 2, 3 β -hydroxysteroid dehydrogenase and Δ^5, Δ^4 -isomerase; 3, 17-hydroxylase (P-450c17); 4, 17,20-desmolase (P-450c17); 5, 17-ketoreductase; 6, 5 α -reductase; and 7, aromatase.

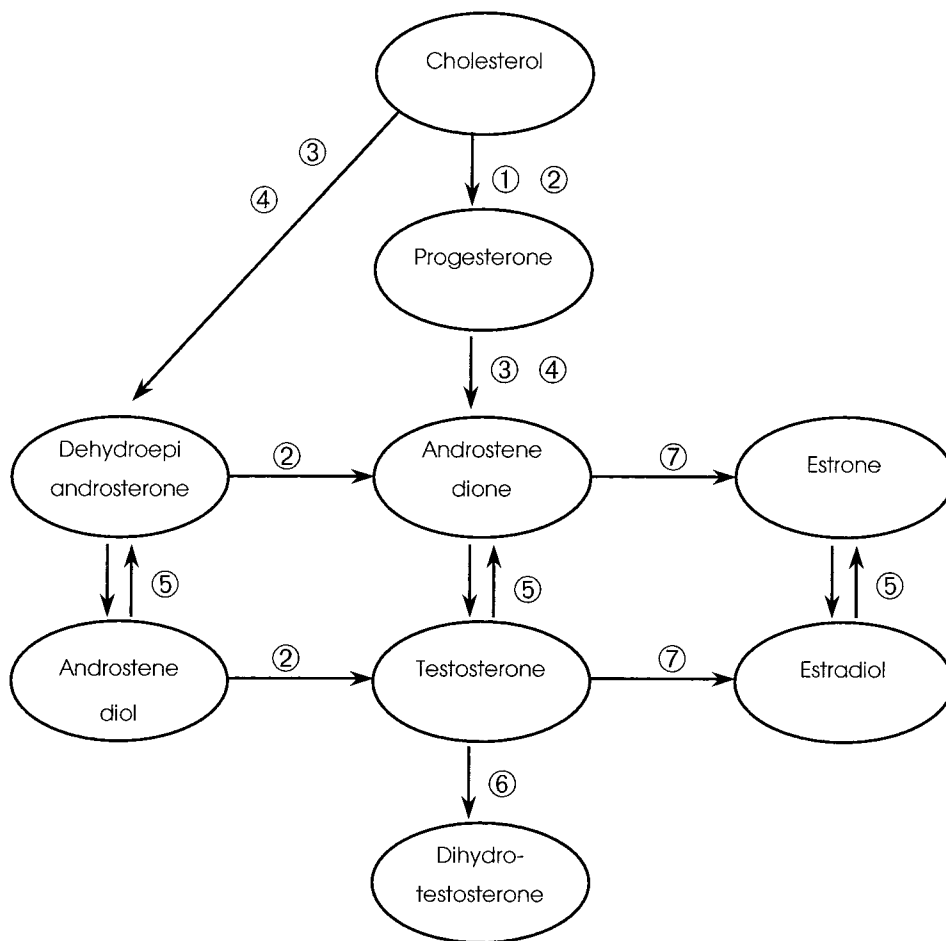


TABLE 1. Available assays of total T measurements

Assay	Pros	Cons
Direct immunoassay	<ul style="list-style-type: none"> • Use of small serum sample volumes • Reproducible at higher levels • Rapid and easy to use • Useful in research studies 	<ul style="list-style-type: none"> • Cross-reactivity • Problems at low levels concerning reproducibility • Not standardized for EDTA plasma
Indirect immunoassay	<ul style="list-style-type: none"> • Reproducible at high and low levels • Useful in both clinical care and research studies 	<ul style="list-style-type: none"> • Not easy to automate and laborious • Cross-reactivity (except if a chromatography separation step is included)
Chromatography and mass spectroscopy	<ul style="list-style-type: none"> • “Gold standard” • No cross-reactivity • Useful in both clinical care and research studies 	<ul style="list-style-type: none"> • Laborious

these hormones (6). Recently several studies compared results of T assays. They have suggested that free T and BT are the most practical methods to determine hypogonadism. BT has been suggested to be the assay of choice in older persons in whom SHBG increases and substantial variation of albumin levels may occur (8, 9). Due to episodic secretion, diurnal variation, and week-to-week variability of T and BT, the utility of a single value in making the diagnosis of hypogonadism is problematic (9). For epidemiological purposes a single value was found to be adequate (10).

Risk factors for CVD

During the past decades, several studies on the association between endogenous sex hormones and known cardiovascular risk factors have been conducted. It has been suggested that low levels of T and DHEA-S are associated with an unfavorable risk profile; however, the results have been conflicting. Table 2 summarizes the associations of observational studies between endogenous sex hormones and cardiovascular risk factors.

TABLE 2. Associations between endogenous sex hormones and cardiovascular risk factors: results from observational studies

Sex hormones	Cardiovascular risk factors	Direction of effect	Refs.	
T ↓	Blood pressure, systolic	↑	38–40	
	Blood pressure, diastolic	↑/=	38–40	
	Cholesterol, total, LDL	↑	12, 13, 16, 19–22, 24, 35	
	Cholesterol, HDL	↓	11–14, 17–23, 35	
	Triglycerides	↑	16, 23, 35	
	Body mass index, waist circumference	↑	12, 13, 27, 31, 35	
	Insulin	↑/=	13, 29–36	
	Glucose	↑/=	29–32, 34–36	
	Fibrinolytic activity	↑	47, 48	
	Estradiol ↓	Blood pressure, systolic	↓/=	12, 15, 42
		Blood pressure, diastolic	↓/=	12, 15, 42
Cholesterol, total, LDL		↑/↓/=	12, 19–22	
Cholesterol, HDL		↑/↓/=	12, 19–22	
Triglycerides		↑	12	
Body mass index, waist circumference		↓/=	12, 31	
Insulin		=	36	
Glucose		=	36	
DHEA-S ↓		Blood pressure, systolic	↓	44, 45
		Blood pressure, diastolic	=	44, 45
	Cholesterol, total, LDL	↑/=	13, 16	
	Cholesterol, HDL	↓/=	13, 16	
	Triglycerides	↓/=	16	
	Body mass index, waist circumference	↑/=	13, 31	
	Insulin	=	13	
	Glucose	=	13	

↑, Significant increase; ↓, significant decrease; =, no significant association; ↑/=, both significant increase and no significant association has been reported; ↓/=, both significant decrease and no significant association has been reported; ↑/↓/=, all associations have been reported.

Lipids. High-density lipoprotein (HDL) cholesterol is inversely associated with the risk of CVD. Conversely, low-density lipoprotein (LDL) and lipoprotein-a (Lpa) are associated with a high risk of CVD. Cross-sectional studies have found high T levels to be associated with high HDL-cholesterol levels, low LDL-cholesterol, and low triglyceride levels (11–22). A longitudinal analysis of the Multiple Risk Factor Intervention Trial confirmed this relationship (23). Furthermore, this study showed that a decrease in endogenous T is associated with an increase in triglycerides. Smaller dense LDL-cholesterol particles were found to be associated with a low total T level and SHBG (24). Estradiol was found to be associated with apolipoprotein E (15). Correlations between physiological levels of total and free T, estradiol, DHEAS, and SHBG and Lpa have not been found (25), although supplementation with anabolic steroids may reduce Lpa (26).

Insulin, glucose metabolism, and body composition. It has been postulated that in hypogonadal states there is a preferential deposition of abdominal adipose tissue. Increased accumulation of adipose tissue leads to an increase in aromatase activity and hence a higher conversion of T to estradiol, which results in a further depression of T concentrations and an increased deposition of abdominal fat (27). Intervention studies demonstrated that correction of relative hypogonadism in men with visceral obesity seem to decrease the abdominal fat mass and reverse the glucose intolerance as well as lipoprotein abnormalities (28, 29). There is extensive experimental evidence showing that sex steroids and insulin interact in tissues. In cross-sectional studies of sex hormones and diabetes, the total T concentration was found to be lower in men with impaired glucose tolerance (29–32). The traditional view of sex hormones increasing insulin resistance has been challenged in women by studies showing that insulin

stimulates androgen production in the ovary (29). Furthermore, it has been suggested that insulin stimulates T production and suppresses SHBG production in men (33). On the other hand, results of prospective studies show that low levels of SHBG and T play a role in the development of insulin resistance and subsequently the development of type 2 diabetes (34–36). At physiological levels T and estradiol are thought to be involved in maintaining normal insulin sensitivity. However, outside these physiological levels, these steroids may promote insulin resistance (29, 37).

Blood pressure. Concerning the association between sex hormones and blood pressure, research findings suggest a relationship between essential hypertension and impaired T levels in men (38–40). This may be due to the use of anti-hypertensive medication, which can lower sex hormone levels (41). Another important caveat is that the lower T levels observed in the aforementioned studies may merely reflect increased stress. Other findings suggest that in men with hypertension, renin profile may be related to estradiol levels (42). It is hypothesized that sex hormones increase arterial pressure by causing a hypertensive shift in the pressure-natriuresis relationship, either by having a direct effect to increase proximal tubular reabsorption or by activation of the renin-angiotensin system (43). Furthermore, total and free estradiol levels were found to be associated with systolic and diastolic blood pressure (15); however, these results were not confirmed by other studies (12, 42). Inconsistent results have been found concerning the association between endogenous DHEA-S levels and hypertension status. Epidemiological observations of positive associations between DHEA-S and blood pressure levels have raised some concern (44–46). However, studies in experimental animals do not

suggest that DHEA-S administration causes hypertension (45).

Coagulation and fibrinolysis. Fibrinolytic activity is inversely associated with cardiovascular risk. Research demonstrated that because of the increase in several prothrombotic factors, men with lower androgenicity seem to be at greater risk for CVD (47, 48). Furthermore, T supplementation may increase blood fibrinolytic activity and produce clinical improvement in patients with occlusive vascular disease (49, 50).

Other risk factors. An elevated plasma level of homocysteine (tHcy) is an independent risk factor for CVD. There are indications that plasma tHcy is influenced by sex steroids. Androgen administration in transsexual (female) subjects increases tHcy levels (51). In contrast, short-term, high-dose T administration did not affect tHcy levels in normal men (52). There is increasing evidence for the role of inflammation in promoting atherogenic risk; elevated C-reactive protein levels have been shown to predict cardiovascular outcomes. Postmenopausal conjugated equine estrogen replacement leads to increased C-reactive protein levels in women (53); however, androgen supplementation does not alter serum inflammatory markers in men (54, 55).

Coronary heart disease [angina pectoris, myocardial infarction (MI)]

Published studies of the relationship between circulating levels of T and DHEA/ DHEA-S and coronary heart disease (CHD) in men were reviewed by Alexandersen *et al.* (56). Up to 1996, they retrieved one randomized intervention trial (57) and eight prospective (58–65) and 30 cross-sectional studies (11, 14, 44, 66–92). More recently, additional studies have been published and are summarized here (93–98). Of 33 cross-sectional studies, 21 reported lower concentrations of T, BT, and/or DHEA(-S) in patients with CHD than in healthy men (14, 66, 69–76, 79–86, 93, 96, 98). In 12 other studies (11, 44, 67, 68, 77, 78, 87–92), similar levels of these sex hormones were found in controls and patients with CHD, and one study (68) showed elevated levels of DHEA(-S) in patients. One study (97) reported hyperestrogenemia to be related to thrombotic occlusion of the coronary arteries in MI; the mean serum estradiol level in the men who had had an MI (38.5 ± 8.8 pg/ml) was higher ($P = 0.002$) than the level in men who had not had an MI (31.9 ± 7.1 pg/ml). However, a causal interpretation of these findings is inherently restricted by the cross-sectional nature of the design.

No significant association between serum T and CHD was observed in the prospective studies (58, 60, 62, 64), whereas either no (61, 63) or an inverse (59, 65, 94, 95) association was found between DHEA-S and CHD. One study (94) showed that men with serum DHEA-S in the lowest quartile at baseline (<1.6 μ g/liter) were significantly more likely to incur ischemic heart disease by follow-up [odds ratio (OR), 1.60; 95% confidence interval (CI), 1.07–2.39].

Most studies, however, have been carried out in selected populations generally involving small numbers (44, 61, 67–69, 71–74, 76, 79–81, 83, 84, 87, 88, 90, 92, 97, 98). Moreover, to assess the independent association between endogenous sex hormones and CHD, analyses should have been adjusted

for age and other cardiovascular risk factors; however, only a few studies adjusted their analyses properly (14, 44, 59, 60, 64, 65, 81, 93, 94). Results of the studies that presented adjusted ORs and relative risks of the association between endogenous sex hormones and CVD are joined together in Fig. 2. It would appear that there is a small beneficial effect of T and DHEA-S on CVD and a neutral effect of estradiol on CVD; however, presented studies used different end points and different study designs. Firm conclusions about the relation between endogenous sex hormones and male CVD cannot be drawn.

Generalized atherosclerosis (carotid intima-media thickness, aortic calcification)

Data on atherosclerosis and sex hormones are scarce, and those available have yielded contradictory results. In an Asian study (99), DHEA-S and DHEA concentrations were significantly lower in subjects with aortic calcification than in those without it (60 and 35%, respectively). However, in a large-scale cohort study, the development and progression of carotid atherosclerosis over 5 yr, monitored by high-resolution duplex ultrasound, was not related to age- and sex-adjusted endogenous DHEA(-S) concentrations (100); the OR of incident/progressive atherosclerosis comparing a 50% increase in DHEA-S levels was 0.99 (95% CI, 0.89–1.11). In one population-based study (13), an inverse association between levels of T and aortic atherosclerosis was observed, with a 60% reduced risk of severe atherosclerosis for men in the highest total T tertile, compared with the lowest tertile. Recently a study among independently living elderly men showed that lower serum T and estrone levels were found to be associated with increased carotid wall thickness (101); a decrease of total T with 1 nmol/liter was associated with an increase in intima-media thickness with 0.04 mm (95% CI, 0.01–0.08).

Vascular function (flow-mediated dilatation, pulse-wave velocity)

Impaired vascular reactivity is an important early event in atherogenesis and may determine dynamic plaque behavior in patients with coronary artery disease (102). Flow-mediated dilatation, measured in the brachial artery, has been used to investigate endothelium-dependent arterial dilatation. Arterial compliance or elasticity can be measured by pulse wave velocity (PWV). Decreased central arterial compliance decreases coronary artery perfusion and increases cardiac workload (103). There are several indications that low serum levels of androgens lead to deterioration of endothelial function, although reported associations have not always been in the expected direction. However, the Asian study reported low levels of DHEA and DHEA-S to be associated with a high PWV (with 2 m/sec increase in PWV for 50% decrement in DHEA or DHEA-S level), suggesting that high levels of DHEA(-S) have beneficial effects on vascular elasticity (99), which agrees with the data on clinically manifest cardiovascular end points. These adverse hemodynamic effects may increase cardiovascular risk in this patient group.

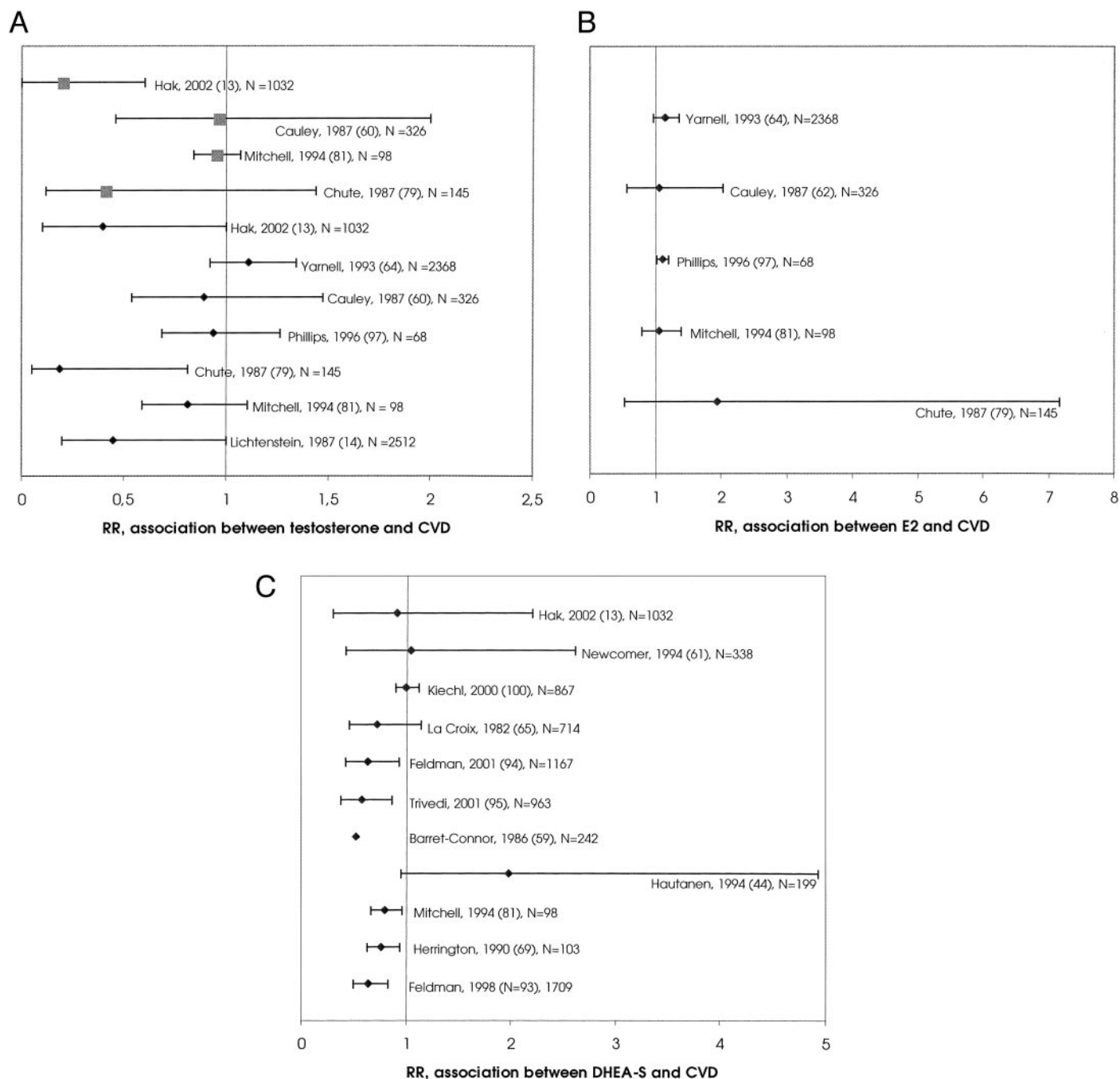


FIG. 2. Association between endogenous sex hormones and all cardiovascular end points in men of observational studies giving adjusted relative risk estimates (RR). In addition, sample sizes are presented. A, Studies of total (*small squares*) and free (*large squares*) T and CVD. B, Studies of estradiol (E2) and CVD. C, Studies of DHEA-S and CVD.

Peripheral arterial disease

To our knowledge, only one study (104) has been published on the association between endogenous sex hormones and peripheral arterial disease. The Edinburgh Artery Study, a large-scale prospective survey, found that after 5 yr of follow-up, 40 men had developed peripheral arterial disease. In a nested case-control study, total T, SHBG, and estradiol appeared to have a protective effect, whereas estrone appeared to have an adverse effect. None of the associations

reached statistical significance, but the power of the study may well have been too low to allow firm conclusions to be drawn (104).

Sex hormone supplementation

A possible role for sex hormone supplementation in men has been investigated in several studies. A few studies have shown that, in both young and middle-aged men, androgen administration is associated with a reduction in visceral fat

accumulation in the abdomen (28, 105). Several studies in the 1940s (106–110) showed beneficial effects of T therapy (± 25 mg/wk im during 1–11 months) on both ischemia and exercise tolerance. In one intervention study involving patients with CHD, orally administered T undecanoate significantly improved angina pectoris, as judged by patients' symptom records and electrocardiogram ST-T segment changes and Holter monitoring, compared with placebo (57). Moreover, short-term administration of T (2.5 mg iv in 5 min) induced a beneficial effect on exercise-induced myocardial ischemia in men with CHD (111). Compared with placebo, T increased time to 1-mm ST-segment depression with 12% and total exercise time with 19%. Similar results were observed in an intervention study of 22 patients treated with 2.5-mg T patches for 12 wk (2). In an intervention study involving 11 patients with CHD, acute iv administration of T (2.3 mg) enhanced endothelium-dependent flow-mediated vascular reactivity, compared with placebo ($6.86 \pm 3.72\%$ vs. $3.16 \pm 1.90\%$, respectively; $P = 0.005$) (3). However, in another study (112), acute T or placebo infusion in 32 men with stable CHD had neither a beneficial nor deleterious effect on the onset and magnitude of stress-induced myocardial ischemia.

Compared with age-matched controls, men with complete androgen deprivation had a significantly higher central PWV (14.2 ± 2.7 m/sec vs. 11.8 ± 1.6 m/sec, respectively; $P = 0.02$) (113), suggesting stiffening of the large arteries. Furthermore, androgen deprivation increased the augmentation index from 24% to 29% after 3 months (114), which indicated that induced hypogonadism increases cardiovascular risk. In contrast, men with androgen deprivation have markedly greater endothelium-dependent dilatation of the brachial artery, compared with a healthy control group ($6.2 \pm 3.0\%$ and $2.0 \pm 1.9\%$, respectively; $P < 0.001$), supporting a deleterious effect of T on vascular function (115). In accordance with this, transsexual genetic females, treated long term with high doses of androgens, had impaired endothelium-independent vasodilatation, compared with untreated healthy female subjects (116). The abuse of androgenic steroids in young male athletes had been associated with premature MIs and strokes (117).

Recently Price and Leng (118) published a Cochrane review on whether exogenous steroid sex hormones are an effective treatment for male patients with lower limb atherosclerosis. Only two, small-scale trials were available, in which T was administered over relatively short periods, and different methods for assessing peripheral arterial disease were used. The authors concluded that there is currently no evidence that male patients with peripheral arterial disease benefit from T treatment (118). Winther (49) showed that high doses of T increased blood fibrinolytic activity and produced clinical improvement in 30 patients aged 46–76 yr suffering from ischemic disease of the lower limb.

In an intervention study of 18 healthy elderly men, oral estrogen reduced tHcy, fibrinogen, and plasminogen activator inhibitor-1 concentrations and favorably influenced very low-density lipoprotein, LDL, and HDL subclass levels without increasing markers of thrombotic risk. Breast tenderness occurred in four men and heartburn in five but did not require discontinuation of treatment (119). Men with prostatic carcinoma had drastically decreased Lpa levels after

estrogen treatment but higher Lpa levels after orchidectomy (120), thus showing the role of androgens after aromatization but possibly only at supraphysiologic levels. In male-to-female transsexuals, long-term estrogen supplementation improves vascular function, compared with men [(mean \pm SE) $11.5 \pm 1.3\%$ vs. $5.2 \pm 1.0\%$, respectively, $P < 0.005$] (121). The effect of high-dose conjugated estrogen (5 mg/d) was studied in the Coronary Drug Project, but estrogen treatment was discontinued because it nearly doubled the incidence of nonfatal MI in the treatment group (122).

It may be stated that estrogen as well as T administration had both beneficial and deleterious effects on cardiovascular functioning, depending on dose, population, and end point.

Mechanism of action

Most of the reviewed studies suggest that the naturally occurring adrenal and testicular androgens may have a beneficial or a neutral effect on cardiovascular diseases. The antiatherogenic mechanism of sex hormones is largely unknown, but several hypotheses have been proposed (Table 3).

Testosterone. Some data suggest that T may affect the development of CVD by modulating risk factors such as diabetes (36), insulin resistance (36), obesity (27), hypercholesterolemia (23, 24), and hypertriglyceridemia (23). It is hypothesized that an increase in triglycerides is mediated by changes in hepatic triglyceride lipase (123). Alternatively, T may directly affect HDL-cholesterol by increasing the hepatic production of apolipoprotein A-I, the major protein constituent of nascent high-density lipoprotein particles (23). Several lines of evidence support an association between hypogonadism and insulin resistance in men. Insulin resistance can be produced by castration of male rats and is reversed with subsequent T replacement (124). It is not known whether the observed relationship between low plasma T is direct or indirect because the relationship between T and insulin is not fully understood.

Androgen receptors are distributed throughout the cardiovascular system, having been found within human and animal systemic arteries, including aortic, coronary, pulmo-

TABLE 3. Mechanism of actions of endogenous sex hormones on the cardiovascular system

Sex hormone	Mechanism of action on cardiovascular system
T	<ul style="list-style-type: none"> • Favorably modulating cardiovascular risk factors • Androgen receptors in arteries, vascular cells • Vasodilatation through potassium channels, calcium antagonism • Vasoconstriction through thromboxane release • Conversion to estradiol
Estradiol	<ul style="list-style-type: none"> • Favorably modulating cardiovascular risk factors • Estrogen receptors in arteries and vascular cells • Local aromatase action • Increasing NO synthase activity in vascular endothelium • Direct effects on vascular smooth muscle

nary, and carotid arteries (125, 126). It is possible, in view of their location in the media, that steroids, acting through their receptors, modulate smooth muscle tone in some vascular beds (127). They may also in some way influence development of atherosclerosis because smooth muscle migration and proliferation appear to play a major role in the pathogenesis of this disease. Furthermore, androgen receptors have been found in peripheral vascular, ventricular, and atrial mammalian cells and normal human megakaryocytes and platelets (126, 128). The identification of androgen receptors on the megakaryocyte lineage may enable new strategies for investigating the prothrombotic effects of androgens (128). Vascular androgen receptors may mediate the effects of T on the arterial wall. T up-regulates the expression of arterial androgen receptor mRNA and is associated with a significant reduction in neointimal plaque development (129).

Furthermore, T has been shown to dilate the coronary, aortic, and brachial vasculature by both endothelial-dependent and independent mechanisms (130). Because genomic pathways typically mediate the hormonal effects of steroids, steroid hormone-induced responses generally take at least 1–2 h to occur. However, recent evidence has suggested that there are nongenomic pathways of steroid hormone action (130–132). It has been demonstrated that T induces endothelium-independent relaxation in isolated rabbit coronary artery and aorta and porcine coronary myocytes, a relaxation that is not mediated by prostaglandin I₂ or cyclic GMP (130). Potassium conductance and potassium channels may be involved in the mechanism of T-induced relaxation (131, 133). Furthermore, research findings (134) suggest that T acts as a coronary vasodilator by a calcium antagonistic action. T was recently found to impair coronary vasodilatation in response to adenosine in an isolated perfused rat heart model (135). Such effects were mediated acutely and most likely through thromboxane release. Thromboxane acts through membrane surface receptors to aggregate platelets and constrict vascular smooth muscle (136). Any vascular effect of T, therefore, is likely to be a balance of vasodilatation by endothelial and nonendothelial effects and vasoconstriction due to thromboxane and possibly other mediators. Another direct effect of androgens that should be considered is their anabolic effect on cardiac myocytes, which may lead to modulation of left ventricular mass (126, 137). T may attenuate early atherogenesis, at least in part, by being converted to estradiol by the enzyme aromatase, which is also expressed in endothelial cells. This resulted in increased local concentrations of estradiol without significant increases in circulating estradiol levels (138). The relevance of these mechanisms in relation to physiological levels of androgens remains to be elucidated (Table 3).

Estrogens. In men estrogen is not solely an endocrine factor but instead is produced in a number of extragonadal sites and acts locally at these sites. These sites include the vascular endothelium, aortic smooth muscle cells, and numerous sites elsewhere in the human body. Within these sites, aromatase action can generate high levels of estradiol locally without significantly affecting circulating levels (139). The following hypothesis has been postulated concerning the role of es-

trogens in men: Estrogen interacts with the vascular endothelium, causing an increase in nitric oxide (NO) synthase activity and the release of NO, which is now considered to be beneficial to the vascular system (140). The effects of estrogen on hemostasis and thrombosis, however, appear to be dose dependent. Estrogen at high concentrations has direct effects on smooth muscle in the blood vessel wall via a number of ion channels such as calcium channels (140). It has been shown that low-dose oral estrogen in men favorably influences very low-density lipoprotein, LDL, and HDL subclass levels and reduces tHcy, fibrinogen, and plasminogen activator inhibitor-1 concentrations without increasing markers of thrombotic risk (119). Estrogens may particularly limit lipid accumulation in the presence of an intact endothelium, which synthesizes and releases NO, which in turn relaxes the vessel wall and inhibits platelet aggregation, leukocyte adhesion to endothelium, vascular smooth muscle cell migration and growth, and LDL-cholesterol oxidation, *i.e.* estrogens prevent atherosclerosis (121). Estrogen decreases tHcy levels in women by direct or indirect mechanisms (119).

So-called “experiments of nature” have revealed the importance of estrogens to male cardiovascular functioning. A point mutation in exon 9 of the gene encoding aromatase (CYP19) is associated with an inability to convert androgen to estrogen (141). Estrogen insensitivity caused by a disruptive mutation in the estrogen-receptor gene has also been described (142). The absence of a functional estrogen receptor appears to be associated with structural abnormalities of the coronary vasculature and an impaired flow-mediated endothelium-dependent peripheral vasodilatation (143, 144). Furthermore, glucose intolerance, hyperinsulinemia, and lipid abnormalities are present in patients with either estrogen deficiency (aromatase deficiency) or estrogen resistance (estrogen receptor mutation) (145) (Table 3).

Adrenal androgens. Several plausible mechanisms for a beneficial effect of DHEAS on cardiovascular morbidity or mortality have been postulated; these include prevention of platelet aggregation (146), inhibition of macrophage accumulation in the intima as well as proliferation of smooth muscle cells from the media into the intima (147), interference with arterial uptake of cholesterol (148), suppression of superoxide radical formation (149), conversion of DHEA to estrogen (150), and binding of DHEA metabolite (androstenediol) to vacant estrogen receptors, and enhanced estrogen-like effects in men (151).

Clinical implications and conclusions

The cross-sectional and prospective studies reviewed in this article suggest that natural circulating androgens and estrogens have a neutral or beneficial effect on CVD in men. As stated before, firm conclusions about the relation between endogenous sex hormones and male CVD cannot be drawn. Many studies have been carried out in selected populations generally involving small numbers. Moreover, few studies investigated physiological levels of sex hormones, and not all studies adjusted their outcome for age and other cardiovascular risk factors.

With respect to the potential of sex hormone supplement-

tation in elderly men, existing data do not suggest that this is associated with a high risk of adverse events with an unacceptable risk (152, 153). The principal issues surrounding androgen administration include an increased risk of prostate carcinoma, precipitation of benign prostatic hyperplasia, an increased hematocrit, sleep apnea, gynecomastia, and water retention (153, 154). The scientific basis for these concerns is scarce. The literature available about the association between androgens and prostate cancer indicates that caution has to be taken with supraphysiological levels of androgens (152). Moreover, concerns about the effect of supplemental T on cardiac mass and blood pressure, especially when supraphysiological concentrations are reached, need to be carefully considered (46, 137). Studies of men undergoing T replacement should include prospective measurements of hematocrit, prostate-specific antigen, and cardiac mass.

Taking the results of the reviewed data and the possible side effects of androgens into account, we would, for the time being, advise against androgen therapy unless male patients have both the presence of clinical symptoms (155) and reduced (bioavailable) T levels (in combination with high LH levels), indicating hypogonadism. When previously mentioned conditions are fulfilled, clinicians could consider measuring the androgen status of men with a high cardiovascular risk profile. It is, however, too early to consider low serum levels of androgens as an important risk factor for CVD. Furthermore, in today's clinical and research settings, there is a lack of consistency in the term "androgen status" (6). Morley *et al.* (155) developed and validated a questionnaire for androgen deficiency in aging males that may be useful in identifying males in need for hormonal measurements for hypogonadism.

There is a need for studies of the possible benefits of high levels of endogenous androgens in the prevention of heart disease in men, especially large-scale prospective studies and randomized trials involving men with low serum levels of androgens, to firmly establish their role in the prevention and treatment of CVD.

Acknowledgments

Received April 8, 2003. Accepted August 1, 2003.

Address all correspondence and requests for reprints to: Yvonne T. van der Schouw, Ph.D., Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, P.O. Box 85500, D01.335, 3508 GA Utrecht, The Netherlands. E-mail: y.t.vanderschouw@jc.azu.nl.

This work was supported by the International Health Foundation, Geneva, Switzerland.

References

- Ferrera PC, Putnam DL, Verdile VP 1997 Anabolic steroid use as the possible precipitant of dilated cardiomyopathy. *Cardiology* 88:218–220
- English KM, Steeds RP, Jones TH, Diver MJ, Channer KS 2000 Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: a randomized, double-blind, placebo-controlled study. *Circulation* 102:1906–1911
- Ong PJ, Patrizi G, Chong WC, Webb CM, Hayward CS, Collins P 2000 Testosterone enhances flow-mediated brachial artery reactivity in men with coronary artery disease. *Am J Cardiol* 85:269–272
- Vermeulen A 1996 Declining androgens with age: an overview. In: Oddens BJ, Vermeulen A, eds. *Androgens and the aging male*. New York: The Parthenon Publishing Group; 3–14
- Braunstein GD 1994 Testes. In: Greenspan FS, Baxter JD, eds. *Basic, clinical endocrinology*. 4th ed. East Norwalk, CT: Appleton, Lange; 403–433
- Klee GG, Heser DW 2000 Techniques to measure testosterone in the elderly. *Mayo Clin Proc* 75(Suppl):S19–S25
- Thijssen JHH 2002 Laboratory tests in the endocrine evaluation of aging males. In: Lunenfeld B, Gooren LJ, eds. *Textbook of men's health*. 1st ed. New York: Parthenon Publishing Group; 44–50
- Winters SJ, Kelley DE, Goodpaster B 1998 The analog free testosterone assay: are the results in men clinically useful? *Clin Chem* 44:2178–2182
- Morley JE, Patrick P, Perry III HM 2002 Evaluation of assays available to measure free testosterone. *Metabolism* 51:554–559
- Vermeulen A, Verdonck G 1992 Representativeness of a single point plasma testosterone level for the long term hormonal milieu in men. *J Clin Endocrinol Metab* 74:939–942
- Heller RF, Wheeler MJ, Micallef J, Miller NE, Lewis B 1983 Relationship of high density lipoprotein cholesterol with total and free testosterone and sex hormone binding globulin. *Acta Endocrinol (Copenh)* 104:253–256
- Gyllenberg J, Rasmussen SL, Borch-Johnsen K, Heitmann BL, Skakkebaek NE, Juul A 2001 Cardiovascular risk factors in men: the role of gonadal steroids and sex hormone-binding globulin. *Metabolism* 50:882–888
- Hak AE, Witteman JC, de Jong FH, Geerlings MI, Hofman A, Pols HA 2002 Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *J Clin Endocrinol Metab* 87:3632–3639
- Lichtenstein MJ, Yarnell JW, Elwood PC, Beswick AD, Sweetnam PM, Marks V, Teale D, Riad-Fahmy D 1987 Sex hormones, insulin, lipids, and prevalent ischemic heart disease. *Am J Epidemiol* 126:647–657
- Van Pottelbergh I, Braeckman D, De Baquer D, De Backer G, Kaufman JM 2003 Differential contribution of testosterone and estradiol in the determination of cholesterol and lipoprotein profile in healthy middle-aged men. *Atherosclerosis* 166:95–102
- Tchernof A, Labrie F, Belanger A, Prud'homme D, Bouchard C, Tremblay A, Nadeau A, Despres JP 1997 Relationships between endogenous steroid hormone, sex hormone-binding globulin and lipoprotein levels in men: contribution of visceral obesity, insulin levels and other metabolic variables. *Atherosclerosis* 133:235–244
- Haffner SM, Mykkanen L, Valdez RA, Katz MS 1993 Relationship of sex hormones to lipids and lipoproteins in nondiabetic men. *J Clin Endocrinol Metab* 77:1610–1615
- Gutai J, LaPorte R, Kuller L, Dai W, Falvo Gerard L, Caggiula A 1981 Plasma testosterone, high density lipoprotein cholesterol and other lipoprotein fractions. *Am J Cardiol* 48:897–902
- Dai WS, Gutai JP, Kuller LH, LaPorte RE, Falvo Gerard L, Caggiula A 1984 Relation between plasma high-density lipoprotein cholesterol and sex hormone concentrations in men. *Am J Cardiol* 53:1259–1263
- Hamalainen E, Adlercreutz H, Ehnholm C, Puska P 1986 Relationships of serum lipoproteins and apoproteins to sex hormones and to the binding capacity of sex hormone binding globulin in healthy Finnish men. *Metabolism* 35:535–541
- Duell PB, Bierman EL 1990 The relationship between sex hormones and high-density lipoprotein cholesterol levels in healthy adult men. *Arch Intern Med* 150:2317–2320
- Khaw KT, Barrett Connor E 1991 Endogenous sex hormones, high density lipoprotein cholesterol, and other lipoprotein fractions in men. *Arterioscler Thromb* 11:489–494
- Zmuda JM, Cauley JA, Kriska A, Glynn NW, Gutai JP, Kuller LH 1997 Longitudinal relation between endogenous testosterone and cardiovascular disease risk factors in middle-aged men. A 13 year follow-up of former multiple risk factor intervention trial participants. *Am J Epidemiol* 146:609–617
- Haffner SM 1996 Androgens in relation to cardiovascular disease and insulin resistance in aging men. In: Oddens BJ, Vermeulen A, eds. *Androgens and the aging male*. New York: The Parthenon Publishing Group; 68–72
- Haffner SM, Mykkanen L, Gruber KK, Rainwater DL, Laakso M 1994 Lack of association between sex hormones and Lp(a) concentrations in American and Finnish men. *Arterioscler Thromb* 14:19–24
- Albers JJ, Taggart HM, Applebaum Bowden D, Haffner S, Chesnut CH3, Hazzard WR 1984 Reduction of lecithin-cholesterol acyltransferase, apolipoprotein D and the Lp(a) lipoprotein with the anabolic steroid stanozolol. *Biochim Biophys Acta* 795:293–296
- Seidell JC, Bjorntorp P, Sjostrom L, Kvist H, Sannerstedt R 1990 Visceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels. *Metabolism* 39:897–901
- Marin P, Arver S 1998 Androgens and abdominal obesity. *Baillieres Clin Endocrinol Metab* 12:441–451
- Haffner SM 1996 Sex hormone-binding protein, hyperinsulinemia, insulin resistance and noninsulin-dependent diabetes. *Horm Res* 45:233–237
- Goodman-Gruen D, Barrett-Connor E 2000 Sex differences in the association of endogenous sex hormone levels and glucose tolerance status in older men and women. *Diabetes Care* 23:912–918
- Abate N, Haffner SM, Garg A, Peshock RM, Grundy SM 2002 Sex steroid hormones, upper body obesity, and insulin resistance. *J Clin Endocrinol Metab* 87:4522–4527
- Haffner SM, Valdez RA, Mykkanen L, Stern MP, Katz MS 1994 Decreased testosterone and dehydroepiandrosterone sulfate concentrations are associ-

- ated with increased insulin and glucose concentrations in nondiabetic men. *Metabolism* 43:599–603
33. Pasquali R, Casimirri F, De Iasio R, Mesini P, Boschi S, Chierici R, Flamia R, Biscotti M, Vicennati V 1995 Insulin regulates testosterone and sex hormone-binding globulin concentrations in adult normal weight and obese men. *J Clin Endocrinol Metab* 80:654–658
 34. Stellato RK, Feldman HA, Hamdy O, Horton ES, McKinlay JB 2000 Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. *Diabetes Care* 23:490–494
 35. Simon D, Charles MA, Nahoul K, Orssaud G, Kremiski J, Hully V, Joubert E, Papoz L, Eschwege E 1997 Association between plasma total testosterone and cardiovascular risk factors in healthy adult men: the Telecom Study. *J Clin Endocrinol Metab* 82:682–685
 36. Oh JY, Barrett-Connor E, Wedick NM, Wingard DL 2002 Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. *Diabetes Care* 25:55–60
 37. Livingstone C, Collison M 2002 Sex steroids and insulin resistance. *Clin Sci (Lond)* 102:151–166
 38. Fogari R, Zoppi A, Preti P, Rinaldi A, Marasi G, Vanasia A, Mugellini A 2002 Sexual activity and plasma testosterone levels in hypertensive males. *Am J Hypertens* 15:217–221
 39. Phillips GB, Jing TY, Resnick LM, Barbagallo M, Laragh JH, Sealey JE 1993 Sex hormones and hemostatic risk factors for coronary heart disease in men with hypertension. *J Hypertens* 11:699–702
 40. Khaw KT, Barrett-Connor E 1988 Blood pressure and endogenous testosterone in men: an inverse relationship. *J Hypertens* 6:329–332
 41. Turner HE, Wass JAH 1997 Gonadal function in men with chronic illness. *Clin Endocrinol (Oxf)* 47:379–401
 42. Phillips GB, Jing TY, Laragh JH, Sealey JE 1995 Serum sex hormone levels and renin-sodium profile in men with hypertension. *Am J Hypertens* 8:626–629
 43. Reckelhoff JF, Granger JP 1999 Role of androgens in mediating hypertension and renal injury. *Clin Exp Pharmacol Physiol* 26:127–131
 44. Hautanen A, Manttari M, Manninen V, Tenkanen L, Huttunen JK, Frick MH, Adlercreutz H 1994 Adrenal androgens and testosterone as coronary risk factors in the Helsinki Heart Study. *Atherosclerosis* 105:191–200
 45. Schunkert H, Hense HW, Andus T, Riegger GA, Straub RH 1999 Relation between dehydroepiandrosterone sulfate and blood pressure levels in a population-based sample. *Am J Hypertens* 12:1140–1143
 46. Dubey RK, Oparil S, Imthurn B, Jackson EK 2002 Sex hormones and hypertension. *Cardiovasc Res* 53:688–708
 47. De Pergola G, De Mitrio V, Sciaraffia M, Pannaciuoli N, Minenna A, Giorgino F, Petronelli M, Laudadio E, Giorgino R 1997 Lower androgenicity is associated with higher plasma levels of prothrombotic factors irrespective of age, obesity, body fat distribution, and related metabolic parameters in men. *Metabolism* 46:1287–1293
 48. Glueck CJ, Glueck HI, Stroop D, Speirs J, Hamer T, Tracy T 1993 Endogenous testosterone, fibrinolysis, and coronary heart disease risk in hyperlipidemic men. *J Lab Clin Med* 122:412–420
 49. Winther O 1965 Testosterone and blood fibrinolytic activity. *Lancet* 1:823
 50. Fearnley GR, Chakrabarti R 1962 Increase of blood fibrinolytic activity by testosterone. *Lancet* ii:128–132
 51. Giltay EJ, Hoogeveen EK, Elbers JM, Gooren LJ, Asscheman H, Stehouwer CD 1998 Effects of sex steroids on plasma total homocysteine levels: a study in transsexual males and females. *J Clin Endocrinol Metab* 83:550–553
 52. Zmuda JM, Bausserman LL, Maceroni D, Thompson PD 1997 The effect of supraphysiologic doses of testosterone on fasting total homocysteine levels in normal men. *Atherosclerosis* 130:199–202
 53. Ridker PM, Hennekens CH, Rifai N, Buring JE, Manson JE 1999 Hormone replacement therapy and increased plasma concentration of C-reactive protein. *Circulation* 100:713–716
 54. Ng MK, Liu PY, Williams AJ, Nakhla S, Ly LP, Handelsman DJ, Celermajer DS 2002 Prospective study of effect of androgens on serum inflammatory markers in men. *Arterioscler Thromb Vasc Biol* 22:1136–1141
 55. Singh AB, Hsia S, Alaupovic P, Sinha-Hikim I, Woodhouse L, Buchanan TA, Shen R, Bross R, Berman N, Bhasin S 2002 The effects of varying doses of T on insulin sensitivity, plasma lipids, apolipoproteins, and C-reactive protein in healthy young men. *J Clin Endocrinol Metab* 87:136–143
 56. Alexandersen P, Haarbo J, Christiansen C 1996 The relationship of natural androgens to coronary heart disease in males: a review. *Atherosclerosis* 125:1–13
 57. Wu SZ, Weng XZ 1993 Therapeutic effects of an androgenic preparation on myocardial ischemia and cardiac function in 62 elderly male coronary heart disease patients. *Chin Med J (Engl)* 106:415–418
 58. Barrett-Connor E, Khaw KT 1988 Endogenous sex hormones and cardiovascular disease in men. A prospective population-based study. *Circulation* 78:539–545
 59. Barrett-Connor E, Khaw KT, Yen SS 1986 A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N Engl J Med* 315:1519–1524
 60. Cauley JA, Gutai JP, Kuller LH, Dai WS 1987 Usefulness of sex steroid hormone levels in predicting coronary artery disease in men. *Am J Cardiol* 60:771–777
 61. Newcomer LM, Manson JE, Barbieri RL, Hennekens CH, Stampfer MJ 1994 Dehydroepiandrosterone sulfate and the risk of myocardial infarction in US male physicians: a prospective study. *Am J Epidemiol* 140:870–875
 62. Phillips GB, Yano K, Stemmermann GN 1988 Serum sex hormone levels and myocardial infarction in the Honolulu Heart Program. Pitfalls in prospective studies on sex hormones. *J Clin Epidemiol* 41:1151–1156
 63. Contoreggi CS, Blackman MR, Andres R, Muller DC, Lakatta EG, Fleg JL, Harman SM 1990 Plasma levels of estradiol, testosterone, and DHEAS do not predict risk of coronary artery disease in men. *J Androl* 11:460–470
 64. Yarnell JW, Beswick AD, Sweetnam PM, Riad Fahmy D 1993 Endogenous sex hormones and ischemic heart disease in men. The Caerphilly prospective study. *Arterioscler Thromb* 13:517–520
 65. LaCroix AZ, Yano K, Reed DM 1992 Dehydroepiandrosterone sulfate, incidence of myocardial infarction, and extent of atherosclerosis in men. *Circulation* 86:1529–1535
 66. Breier C, Drexel H, Lisch HJ, Muhlberger V, Herold M, Knapp E, Braunsteiner H 1985 Essential role of post-heparin lipoprotein lipase activity and of plasma testosterone in coronary artery disease. *Lancet* 1:1242–1244
 67. Small M, Lowe GD, Beastall GH, Beattie JM, McEachern M, Hutton I, Lorimer AR, Forbes CD 1985 Serum oestradiol and ischaemic heart disease—relationship with myocardial infarction but not coronary atheroma or haemostasis. *Q J Med* 57:775–782
 68. Zumoff B, Troxler RG, O'Connor J, Rosenfeld RS, Kream J, Levin J, Hickman JR, Sloan AM, Walker W, Cook RL, Fukushima DK 1982 Abnormal hormone levels in men with coronary artery disease. *Arteriosclerosis* 2:58–67
 69. Herrington DM, Gordon GB, Achuff SC, Trejo JF, Weisman HF, Kwiterovich PO, Pearson TA 1990 Plasma dehydroepiandrosterone and dehydroepiandrosterone sulfate in patients undergoing diagnostic coronary angiography [corrected and republished with original paging, article originally printed in *J Am Coll Cardiol* 1990 16:862–870]. *J Am Coll Cardiol* 16:862–870
 70. Sewdarsen M, Vythilingum S, Jialal I, Desai RK, Becker P 1990 Abnormalities in sex hormones are a risk factor for premature manifestation of coronary artery disease in South African Indian men. *Atherosclerosis* 83:111–117
 71. Sewdarsen M, Jialal I, Naidu RK 1988 The low plasma testosterone levels of young Indian infarct survivors are not due to a primary testicular defect. *Postgrad Med J* 64:264–266
 72. Barth JD, Jansen H, Hugenholtz PG, Birkenhager JC 1983 Post-heparin lipases, lipids and related hormones in men undergoing coronary arteriography to assess atherosclerosis. *Atherosclerosis* 48:235–241
 73. Swartz CM, Young MA 1987 Low serum testosterone and myocardial infarction in geriatric male inpatients. *J Am Geriatr Soc* 35:39–44
 74. Slowinska-Szednicka J, Zgliczynski S, Ciswicka-Sznajderman M, Szednicki M, Soszynski P, Biernacka M, Woroszyńska M, Ruzyllo W, Sadowski Z 1989 Decreased plasma dehydroepiandrosterone sulfate and dihydrotestosterone concentrations in young men after myocardial infarction. *Atherosclerosis* 79:197–203
 75. Gray A, Feldman HA, McKinlay JB, Longcope C 1991 Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. *J Clin Endocrinol Metab* 73:1016–1025
 76. Phillips GB, Pinkernell BH, Jing TY 1994 The association of hypotestosteronemia with coronary artery disease in men. *Arterioscler Thromb* 14:701–706
 77. Phillips GB, Castelli WP, Abbott RD, McNamara PM 1983 Association of hyperestrogenemia and coronary heart disease in men in the Framingham cohort. *Am J Med* 74:863–869
 78. Luria MH, Johnson MW, Pego R, Seuc CA, Manubens SJ, Wieland MR, Wieland RG 1982 Relationship between sex hormones, myocardial infarction, and occlusive coronary disease. *Arch Intern Med* 142:42–44
 79. Chute CG, Baron JA, Plymate SR, Kiel DP, Pavia AT, Lozner EC, O'Keefe T, MacDonald GJ 1987 Sex hormones and coronary artery disease. *Am J Med [Erratum (1988) 85:129]* 83:853–859
 80. Sewdarsen M, Jialal I, Vythilingum S, Desai R 1986 Sex hormone levels in young Indian patients with myocardial infarction. *Arteriosclerosis* 6:418–421
 81. Mitchell LE, Sprecher DL, Borecki IB, Rice T, Laskarzewski PM, Rao DC 1994 Evidence for an association between dehydroepiandrosterone sulfate and nonfatal, premature myocardial infarction in males. *Circulation* 89:89–93
 82. Hromadova M, Hacik T, Riecanek I 1985 Concentration of lipid, apoprotein-B and testosterone in patients with coronarographic findings. *Klin Wochenschr* 63:1071–1074
 83. Aksut SV, Aksut G, Karamehmetoglu A, Oram E 1986 The determination of serum estradiol, testosterone and progesterone in acute myocardial infarction. *Jpn Heart J* 27:825–837
 84. Mendoza SG, Zerpa A, Carrasco H, Colmenares O, Rangal A, Gartside PS, Kshyap ML 1983 Estradiol, testosterone, apolipoproteins, lipoprotein cholesterol, and lipolytic enzymes in men with premature myocardial infarction and angiographically assessed coronary occlusion. *Artery* 12:1–23
 85. Hamalainen E, Tikkanen H, Harkonen M, Naveri H, Adlercreutz H 1987 Serum lipoproteins, sex hormones and sex hormone binding globulin in middle-aged men of different physical fitness and risk of coronary heart disease. *Atherosclerosis* 67:155–162

86. Rice T, Sprecher DL, Borecki IB, Mitchell LE, Laskarzewski PM, Rao DC 1993 Cincinnati myocardial infarction and hormone family study: family resemblance for testosterone in random and MI families. *Am J Med Genet* 47:542–549
87. Labropoulos B, Velonakis E, Oekonomakos P, Laskaris J, Katsimades D 1982 Serum sex hormones in patients with coronary disease and their relationship to known factors causing atherosclerosis. *Cardiology* 69:98–103
88. Franzen J, Fex G 1986 Low serum apolipoprotein A-I in acute myocardial infarction survivors with normal HDL cholesterol. *Atherosclerosis* 59:37–42
89. Baumann G, Reza P, Chatterton R, Green D, Krumlovsky F 1988 Plasma estrogens, androgens, and von Willebrand factor in men on chronic hemodialysis. *Int J Artif Organs* 11:449–453
90. Cengiz K, Alvir M, Dindar U 1991 Serum creatine phosphokinase, lactic dehydrogenase, estradiol, progesterone and testosterone levels in male patients with acute myocardial infarction and unstable angina pectoris. *Mater Med Pol* 23:195–198
91. Hauner H, Stangl K, Burger K, Busch U, Blomer H, Pfeiffer EF 1991 Sex hormone concentrations in men with angiographically assessed coronary artery disease—relationship to obesity and body fat distribution. *Klin Wochenschr* 69:664–668
92. Marques-Vidal P, Sie P, Cambou JP, Chap H, Perret B 1995 Relationships of plasminogen activator inhibitor activity and lipoprotein(a) with insulin, testosterone, 17 β -estradiol, and testosterone binding globulin in myocardial infarction patients and healthy controls. *J Clin Endocrinol Metab* 80:1794–1798
93. Feldman HA, Johannes CB, McKinlay JB, Longcope C 1998 Low dehydroepiandrosterone sulfate and heart disease in middle-aged men: cross-sectional results from the Massachusetts Male Aging Study. *Ann Epidemiol* 8:217–228
94. Feldman HA, Johannes CB, Araujo AB, Mohr BA, Longcope C, McKinlay JB 2001 Low dehydroepiandrosterone and ischemic heart disease in middle-aged men: prospective results from the Massachusetts Male Aging Study. *Am J Epidemiol* 153:79–89
95. Trivedi DP, Khaw KT 2001 Dehydroepiandrosterone sulfate and mortality in elderly men and women. *J Clin Endocrinol Metab* 86:4171–4177
96. English KM, Mandour O, Steeds RP, Diver MJ, Jones TH, Channer KS 2000 Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms. *Eur Heart J* 21:890–894
97. Phillips GB, Pinkernell BH, Jing TY 1996 The association of hyperestrogenemia with coronary thrombosis in men. *Arterioscler Thromb Vasc Biol* 16:1383–1387
98. Zhao SP, Li XP 1998 The association of low plasma testosterone level with coronary artery disease in Chinese men. *Int J Cardiol* 63:161–164
99. Ishihara F, Hiramatsu K, Shigematsu S, Aizawa T, Niwa A, Takasu N, Yamada T, Matsuo K 1992 Role of adrenal androgens in the development of arteriosclerosis as judged by pulse wave velocity and calcification of the aorta. *Cardiology* 80:332–338
100. Kiechl S, Willeit J, Bonora E, Schwarz S, Xu Q 2000 No association between dehydroepiandrosterone sulfate and development of atherosclerosis in a prospective population study (Bruneck study). *Arterioscler Thromb Vasc Biol* 20:1094–1100
101. van den Beld AW, Bots ML, Pols HAP, Janssen JAMJL, Lamberts SW, Grobbee DE 2003 Endogenous hormones and carotid atherosclerosis in elderly men. *Am J Epidemiol* 157:25–31
102. Celemajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE 1992 Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 340:1111–1115
103. Woolam GL, Schnur PL, Vallbona C, Hoff HE 1962 The pulse wave velocity as an early indicator of atherosclerosis in diabetic subjects. *Circulation* 25:533–539
104. Price JF, Lee AJ, Fowkes FG 1997 Steroid sex hormones and peripheral arterial disease in the Edinburgh Artery Study. *Steroids* 62:789–794
105. Katznelson L, Finkelstein JS, Schoenfeld DA, Rosenthal DI, Anderson EJ, Klibanski A 1996 Increase in bone density and lean body mass during testosterone administration in men with acquired hypogonadism. *J Clin Endocrinol Metab* 81:4358–4365
106. Walker TC 1942 Use of testosterone propionate and estrogenic substance in treatment of essential hypertension, angina pectoris and peripheral vascular disease. *J Clin Endocrinol* 2:560–568
107. Hamm L 1942 Testosterone propionate in the treatment of angina pectoris. *J Clin Endocrinol* 2:325–328
108. Sigler LH, Tulgan J 1943 Treatment of angina pectoris by testosterone propionate. *NY State J Med* 43:1424–1428
109. Lesser MA 1946 Testosterone propionate therapy in one hundred cases of angina pectoris. *J Clin Endocrinol* 6:549–557
110. Levine SA, Likoff WB 1943 The therapeutic value of testosterone propionate in angina pectoris. *N Engl J Med* 229:770–772
111. Rosano GM, Leonardo F, Pagnotta P, Pelliccia F, Panina G, Cerquetani E, della Monica PL, Bonfigli B, Volpe M, Chierchia SL 1999 Acute anti-ischemic effect of testosterone in men with coronary artery disease. *Circulation* 99:1666–1670
112. Thompson PD, Ahlberg AW, Moyna NM, Duncan B, Ferraro-Borgida M, White CM, McGill CC, Heller GV 2002 Effect of intravenous testosterone on myocardial ischemia in men with coronary artery disease. *Am Heart J* 143:249–256
113. Dockery F, Rajkumar C, Agarwal S, Waxman J, Bulpitt CJ 2000 Androgen deprivation in males is associated with decreased central arterial compliance and reduced central systolic blood pressure. *J Hum Hypertens* 14:395–397
114. Smith JC, Bennett S, Evans LM, Kynaston HG, Parmar M, Mason MD, Cockcroft JR, Scanlon MF, Davies JS 2001 The effects of induced hypogonadism on arterial stiffness, body composition, and metabolic parameters in males with prostate cancer. *J Clin Endocrinol Metab* 86:4261–4267
115. Herman SM, Robinson JT, McCredie RJ, Adams MR, Boyer MJ, Celemajer DS 1997 Androgen deprivation is associated with enhanced endothelium-dependent dilatation in adult men. *Arterioscler Thromb Vasc Biol* 17:2004–2009
116. McCredie RJ, McCrohon JA, Turner L, Griffiths KA, Handelsman DJ, Celemajer DS 1998 Vascular reactivity is impaired in genetic females taking high-dose androgens. *J Am Coll Cardiol* 32:1331–1335
117. Rockhold RW 1993 Cardiovascular toxicity of anabolic steroids. *Annu Rev Pharmacol Toxicol* 33:497–520
118. Price JF, Leng GC 2000 Steroid sex hormones for lower limb atherosclerosis. *Cochrane Database Syst Rev* CD000188
119. Giri S, Thompson PD, Taxel P, Contois JH, Otvos J, Allen R, Ens G, Wu AH, Waters DD 1998 Oral estrogen improves serum lipids, homocysteine and fibrinolysis in elderly men. *Atherosclerosis* 137:359–366
120. Henriksson P, Angelin B, Berglund L 1992 Hormonal regulation of serum Lp (a) levels. Opposite effects after estrogen treatment and orchidectomy in males with prostatic carcinoma. *J Clin Invest* 89:1166–1171
121. New G, Timmins KL, Duffy SJ, Tran BT, O'Brien RC, Harper RW, Meredith IT 1997 Long-term estrogen therapy improves vascular function in male to female transsexuals. *J Am Coll Cardiol* 29:1437–1444
122. The Coronary Drug Project 1970 Initial findings leading to modifications of its research protocol. *JAMA* 214:1303–1313
123. Tikkanen MJ, Nikkila EA, Kuusi T, Sipinen SU 1982 High density lipoprotein-2 and hepatic lipase: reciprocal changes produced by estrogen and norgestrel. *J Clin Endocrinol Metab* 54:1113–1117
124. Holmang A, Bjorntorp P 1992 The effects of testosterone on insulin sensitivity in male rats. *Acta Physiol Scand* 146:505–510
125. Lin AL, McGill Jr HC, Shain SA 1982 Hormone receptors of the baboon cardiovascular system. Biochemical characterization of aortic and myocardial cytoplasmic progesterone receptors. *Circ Res* 50:610–616
126. Marsh JD, Lehmann MH, Ritchie RH, Gwathmey JK, Green GE, Schiebinger RJ 1998 Androgen receptors mediate hypertrophy in cardiac myocytes. *Circulation* 98:256–261
127. Horwitz KB, Horwitz LD 1982 Canine vascular tissues are targets for androgens, estrogens, progestins, and glucocorticoids. *J Clin Invest* 69:750–758
128. Khetawat G, Faraday N, Nealen ML, Vijayan KV, Bolton E, Noga SJ, Bray PF 2000 Human megakaryocytes and platelets contain the estrogen receptor β and androgen receptor (AR): testosterone regulates AR expression. *Receptor* 95:2289–2296
129. Hanke H, Lenz C, Hess B, Spindler KD, Weidemann W 2001 Effect of testosterone on plaque development and androgen receptor expression in the arterial vessel wall. *Circulation* 103:1382–1385
130. Chou TM, Sudhir K, Hutchison SJ, Ko E, Amidon TM, Collins P, Chatterjee K 1996 Testosterone induces dilation of canine coronary conductance and resistance arteries *in vivo*. *Circulation* 94:2614–2619
131. Yue P, Chatterjee K, Beale C, Poole-Wilson PA, Collins P 1995 Testosterone relaxes rabbit coronary arteries and aorta. *Circulation* 91:1154–1160
132. Webb CM, McNeill JG, Hayward CS, de Zeigler D, Collins P 1999 Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. *Circulation* 100:1690–1696
133. Deenadayalu VP, White RE, Stallone JN, Gao X, Garcia AJ 2001 Testosterone relaxes coronary arteries by opening the large-conductance, calcium-activated potassium channel. *Am J Physiol Heart Circ Physiol* 281:H1720–H1727
134. English KM, Jones RD, Jones TH, Morice AH, Channer KS 2002 Testosterone acts as a coronary vasodilator by a calcium antagonistic action. *J Endocrinol Invest* 25:455–458
135. Ceballos G, Figueroa L, Rubio I, Gallo G, Garcia A, Martinez A, Yanez R, Perez J, Morato T, Chamorro G 1999 Acute and nongenomic effects of testosterone on isolated and perfused rat heart. *J Cardiovasc Pharmacol* 33:691–697
136. Ajayi AA, Mathur R, Halushka PV 1995 Testosterone increases human platelet thromboxane A2 receptor density and aggregation responses. *Circulation* 91:2742–2747
137. Hayward CS, Webb CM, Collins P 2001 Effect of sex hormones on cardiac mass. *Lancet* 357:1354–1356
138. Nathan L, Shi W, Dinh H, Mukherjee TK, Wang X, Lusic AJ, Chaudhuri G 2001 Testosterone inhibits early atherogenesis by conversion to estradiol: critical role of aromatase. *Proc Natl Acad Sci USA* 98:3589–3593
139. Simpson ER, Davis SR 2001 Minireview: aromatase and the regulation of estrogen biosynthesis—some new perspectives. *Endocrinology* 142:4589–4594
140. Collins P 2001 Vascular effects of hormones. *Maturitas* 38:45–50
141. Bilezikian JP, Morishima A, Bell J, Grumbach MM 1998 Increased bone

- mass as a result of estrogen therapy in a man with aromatase deficiency. *N Engl J Med* 339:599–603
142. **Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS** 1994 Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med* 331:1056–1061
 143. **Sudhir K, Chou TM, Chatterjee K, Smith EP, Williams TC, Kane JP, Malloy MJ, Korach KS, Rubanyi GM** 1997 Premature coronary artery disease associated with a disruptive mutation in the estrogen receptor gene in a man. *Circulation* 96:3774–3777
 144. **Sudhir K, Chou TM, Messina LM, Hutchison SJ, Korach KS, Chatterjee K, Rubanyi GM** 1997 Endothelial dysfunction in a man with disruptive mutation in oestrogen-receptor gene. *Lancet* 349:1146–1147
 145. **MacGillivray MH, Morishima A, Conte F, Grumbach M, Smith EP** 1998 Pediatric endocrinology update: an overview. The essential roles of estrogens in pubertal growth, epiphyseal fusion and bone turnover: lessons from mutations in the genes for aromatase and the estrogen receptor. *Horm Res* 49(Suppl 1):2–8
 146. **Jesse R, Nestler J, Eich D, Hess M** 1991 Dehydroepiandrosterone *in vivo* and *in vitro* inhibits platelet aggregation. *J Am Coll Cardiol* 17:376A
 147. **Dworkin CR, Gorman SD, Pashko LL, Cristofalo VJ, Schwartz AG** 1986 Inhibition of growth of HeLa and WI-38 cells by dehydroepiandrosterone and its reversal by ribo- and deoxyribonucleosides. *Life Sci* 38:1451–1457
 148. **Leszczynski DE, Schafer RM, Perkins EG, Jerrell JP, Kummerow FA** 1989 Esterification of dehydroepiandrosterone by human plasma HDL. *Biochim Biophys Acta* 1014:90–97
 149. **Whitcomb JM, Schwartz AG** 1985 Dehydroepiandrosterone and 16 α -B-epiandrosterone inhibit 12-O-tetradecanoylphorbol-13-acetate stimulation of superoxide radical production by human polymorphonuclear leukocytes. *Carcinogenesis* 6:333–335
 150. **Hayashi T, Esaki T, Muto E, Kano H, Asai Y, Thakur NK, Sumi D, Jayachandran M, Iguchi A** 2000 Dehydroepiandrosterone retards atherosclerosis formation through its conversion to estrogen: the possible role of nitric oxide. *Arterioscler Thromb Vasc Biol* 20:782–792
 151. **Ebeling P, Koivisto VA** 1994 Physiological importance of dehydroepiandrosterone. *Lancet* 343:1479–1481
 152. **Eaton NE, Reeves GK, Appleby PN, Key TJ** 1999 Endogenous sex hormones and prostate cancer: a quantitative review of prospective studies. *Br J Cancer* 80:930–934
 153. **Bhasin S, Bagatell CJ, Bremner WJ, Plymate SR, Tenover JL, Korenman SG, Nieschlag E** 1998 Issues in testosterone replacement in older men. *J Clin Endocrinol Metab* 83:3435–3448
 154. **Morley JE** 2001 Andropause: is it time for the geriatrician to treat it? *J Gerontol A Biol Sci Med Sci* 56:M263–M265
 155. **Morley JE, Charlton E, Patrick P, Kaiser FE, Cadeau F, McCready D, Perry HM** 2000 Validation of a screening questionnaire for androgen deficiency in aging males. *Metabolism* 49:1239–1242