0163-769X/03/$20.00/0
*Printed in U.S.A.*

Endocrine Reviews 24(2):183–217

doi: 10.1210/er.2001-0025

# Androgens and Coronary Artery Disease

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**A significant and independent association between endogenous testosterone (T) levels and coronary events in men and women has not been confirmed in large prospective studies, although cross-sectional data have suggested coronary heart disease can be associated with low T in men. Hypoandrogenemia in men and hyperandrogenemia in women are associated with visceral obesity; insulin resistance; low high-density lipoprotein (HDL) cholesterol (HDL-C); and elevated triglycerides, low-density lipoprotein cholesterol, and plasminogen activator type 1. These gender differences and confounders render the precise role of endogenous T in atherosclerosis unclear. Observational studies do not support the hypothesis that dehydroepiandrosterone sulfate deficiency is a risk factor for coronary artery disease.**

**The effects of exogenous T on cardiovascular mortality or morbidity have not been extensively investigated in prospective controlled studies; preliminary data suggest there may be short-term improvements in electrocardiographic changes in men with coronary artery disease. In the majority of animal experiments, exogenous T exerts either neutral or beneficial effects on the development of atherosclerosis. Exogenous androgens induce both apparently beneficial and deleterious effects on cardiovascular risk factors by decreasing serum levels of HDL-C, plasminogen activator type 1 (apparently deleterious), lipoprotein (a), fibrinogen, insulin, leptin, and visceral fat mass (apparently beneficial) in men as well as women. However, androgen-induced declines in circulating HDL-C should not automatically be assumed to be proatherogenic, because these declines may instead reflect accelerated reverse cholesterol transport. Supraphysiological concentrations of T stimulate vasorelaxation; but at physiological concentrations, beneficial, neutral, and detrimental effects on vascular reactivity have been observed. T exerts proatherogenic effects on macrophage function by facilitating the uptake of modified lipoproteins and an antiatherogenic effect by stimulating efflux of cellular cholesterol to HDL.**

**In conclusion, the inconsistent data, which can only be partly explained by differences in dose and source of androgens, militate against a meaningful assessment of the net effect of T on atherosclerosis. Based on current evidence, the therapeutic use of T in men need not be restricted by concerns regarding cardiovascular side effects. Available data also do not justify the uncontrolled use of T or dehydroepiandrosterone for the prevention or treatment of coronary heart disease. (*Endocrine Reviews* 24: 183–217, 2003)**



Abbreviations: AAS, Anabolic-androgenic steroid; apo, apolipoprotein; BMI, body mass index; CAD, coronary heart (artery) disease; CE, cholesterol ester; CETP, CE transfer protein; CI, confidence interval; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; EC, endothelial cell; ECG, electrocardiogram; ER, estrogen receptor; FFA, free fatty acids; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HL, hepatic lipase; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; Lp(a), lipoprotein (a); MI, myocardial infarction; NCEH, neutral cholesterol esterase; NO, nitric oxide; OR, odds ratios; PAI-1, plasminogen activator type 1; PCOS, polycystic ovarian syndrome; SMC, smooth muscle cell; SR-B1, scavenger receptor B1; T, testosterone; VLDL, very LDL; WHR, waist-hip ratio.

## I. Introduction

ANDROGEN REPLACEMENT THERAPY has been used for over 60 yr to treat, with proven efficacy and safety, a relatively small number (estimated to be <0.5% of adult male population) of patients with male hypogonadal disorders and/or failure of sexual development. However, in

the last 10 yr, evidence has accumulated to support a wider therapeutic role of androgens for nonclassical indications (1). These include male contraception; aplastic anemia; and sarcopenic, osteopenic, and depressive states frequently associated with an expanding variety of chronic systemic conditions (characterized by reduced circulating testosterone, T) such as AIDS, rheumatoid arthritis, chronic renal failure, chronic obstructive airways disease, and physiological aging. Androgens are also being investigated as an additional component of hormone replacement therapy, in conjunction with estrogens, in postmenopausal women, especially in those who have had bilateral oophorectomy (2). We are poised on the threshold of witnessing a greatly expanded population of patients of all ages who may potentially benefit from the biological actions of T or related androgens.

Despite substantial reductions in mortality over the past 30 yr, heart disease remains the leading cause of death, claiming a total of 6.3 million lives worldwide in 1990. Ischemic heart disease, fifth in the rank order of disabilities in 1990, is predicted to become the leading global cause of disease burden by 2020 (3). It is well known that the age-adjusted morbidity and mortality rates from coronary heart disease (CAD) are 2.5- to 4.5-fold higher in men than in women and that the gender gap narrows after the menopause (4). The lifetime risk of CAD at the age of 40 yr is 1 in 2 for men and 1 in 3 for women (5). This male preponderance is remarkably consistent across 52 countries with hugely divergent rates of CAD mortality and lifestyles (6). The universality of gender disparity makes it likely that there is an intrinsic sexual dimorphism in susceptibility to CAD that may involve genetic, hormonal, lifestyle, or aging factors. The most popular explanation for this male preponderance in CAD is that adult male levels of T are proatherogenic, and/or there is a lack of the cardioprotective effects of estrogens in men. With the prospects of much wider therapeutic applications of androgens (for nonclassical indications), especially in the older age groups, an important clinical question is whether androgen treatment might increase the risk or severity of CAD. Being the most common cause of mortality and morbidity in men, even a tiny increase in the risk of CAD will not only negate any personal therapeutic benefits from androgen treatment but will also impose an unacceptable extra burden on healthcare resources. This concern has become a major safety issue for androgen therapy.

The aim of this review is to summarize disparate and often conflicting data from a variety of disciplines into a global assessment of the relationship between androgens and CAD. It is based on MEDLINE searches up to April 30, 2002, using the following keywords: androgens, testosterone, dehydroepiandrosterone (DHEA), oestrogens (estrogens), androgen receptor, oestrogen (estrogen) receptor (ER), aromatase, 5$\alpha$ reductase, polycystic ovary syndrome, hypogonadism, or hyperandrogenism in combination with cardiovascular disease, coronary heart (artery) disease, atherosclerosis, arteriosclerosis, diabetes mellitus, obesity, lipids, lipoproteins, hemostasis, coagulation, vascular reactivity, macrophage, endothelium, endothelial cell (EC), smooth muscle cell (SMC), or platelets. Only full published papers, but not conference abstracts, were included. No minimum criteria for inclusion of individual studies have been imposed; the intention was to achieve comprehensive literature coverage. The relative merits and limitations of quoted information will be critically discussed in the text.

## II. The Gender Difference in Coronary Artery Disease

Male gender is one of the classic risk factors for CAD (7), and average life expectancy is some 8 yr less in males than females. Androgens or the lack of estrogens have traditionally been regarded as the proximate cause underlying this male disadvantage. However, the consistent 2.5–4.5:1 sex ratio in CAD across many countries compared with the ethnic/geographic disparity, with a 5- to 10-fold higher CAD mortality rates in eastern and northern Europe than in southern Europe and Japan (8), suggests that the gender effect is not as important as other risk factors that act on both men and women (Fig. 1). The narrowing of the gender gap after middle age, associated with a relative deceleration of CAD deaths in men and an absence of acceleration of CAD deaths perimenopausally in women, would also argue against a prime role for sex hormones in the pathogenesis of CAD (9). Nonhormonal factors may play a predominant part in the gender disparity in CAD. Interactions between a multiple genetic and environmental/lifestyle factors are important in the pathogenesis of atherosclerosis (10). Thus, uncommon genetic polymorphisms are responsible for a low background prevalence of CAD in both men and women. In addition, common genetic polymorphisms interact with classic risk factors to negate the protective genetic effects or enhance the deleterious actions of environmental or lifestyle variables (10). The gender-specific expression of candidate genes may involve diverse mechanisms ranging from *in utero* sex hormone imprinting on gender-specific behavior patterns and distribution of visceral body fat to vascular and myocardial structural and functional adaptation to aging, pressure overload, and disease (11). Gender differences are detectable in vascular endothelial functions (12, 13), lipid loading in human monocyte-derived macrophages (14), and abdominal visceral fat deposition (15). These mechanisms/factors will be discussed in more detail in the ensuing sections.

## III. Relationships between Serum Levels of T and CAD—Observational Studies

This section updates and modifies the excellent review of Alexandersen *et al.* (16), which was based on studies published between January 1982 and June 1995 that investigated the relationship between androgens (T and DHEA) and CAD in males. Because of the increased interest in DHEA since then, T and DHEA are dealt with separately in the present review. In perusing the clinical literature on this subject, it is clear that the reported endpoints for CAD were extremely variable [mortality, morbidity such as myocardial infarction (MI), angina, angiography, electrocardiogram (ECG), ultrasound, or postmortem-based diagnosis or unspecified cardiac events], study populations were heterogeneous, and selection criteria nonuniform. Types of study ranged from

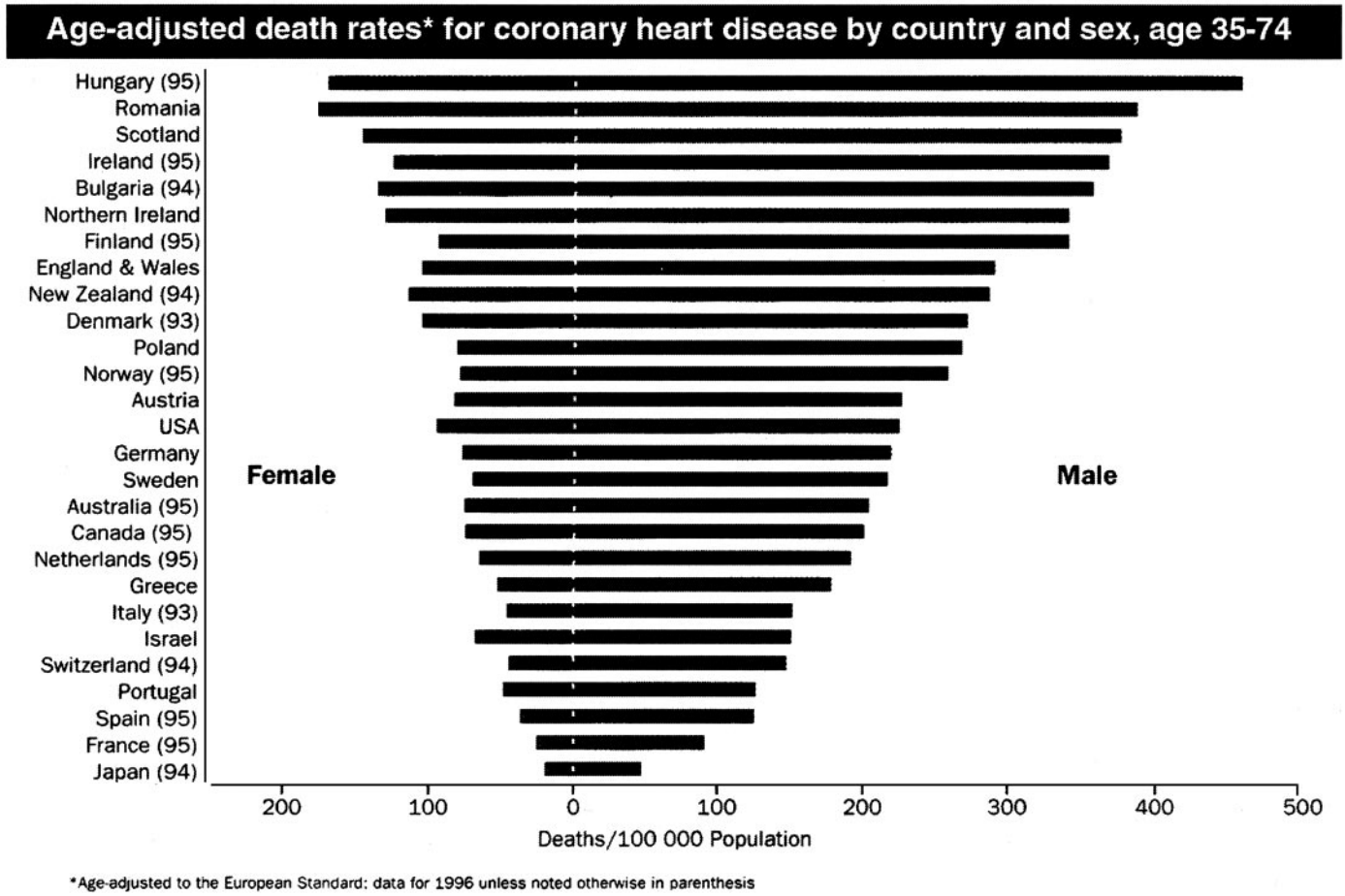


FIG. 1. Age-adjusted mortality rates for CAD by country and sex (age 35–74 yr). Note the much higher difference in mortality between countries than between genders. A woman living in Scotland has a higher chance of dying from CAD than a man living in France (430).

cross-sectional/case-control and prospective nested case-control to longitudinal cohorts. Many studies were too small to draw valid conclusion, and adjustment had not always been made for confounders. Moreover, cross-sectional/case-control observational studies are subject to survivor bias (subjects with extreme levels of hormones have died), behavior change (*e.g.*, diet and lifestyle) and medical interventions (*e.g.*, medications) after diagnosis, and to the possibility that chronic illnesses including CAD lower serum levels of T (17). Studies of endogenous T may be further confounded by the diurnal variation (highest in the early morning) in circulating levels in younger but not older men (18) and an artifactual upward shift in assayed concentration of T due to a progressive alteration in frozen serum samples with time of storage (19). We have taken heed of these deficiencies of observational (especially cross-sectional) studies, and only those with adequate methodologies in terms of design, statistical power, hormone sampling/measurement, and allowance for confounders will be considered when drawing our overall conclusions. Studies will be summarized by their positive (higher androgens in cases), null, or negative (lower androgen levels in cases) relationships, together with information on study design, number of subjects, and the different diagnostic endpoints, to enable the reader to gain an impression of the power, validity, and quality of each study. When available, odds ratios (OR) with 95% confidence interval (CI) will be provided to give additional indications of the quality of individual studies and the adequacy of statistical power.

### *A. T and CAD in men*

Table 1 summarizes 39 studies (19–57) of the relationships between circulating T and CAD in men.

*1. Cross-sectional clinical studies.* Thirty-two cross-sectional studies (20–42, 44, 47, 49, 50, 53–57) are summarized in Table 1. Sixteen studies found lower levels of T in patients with CAD compared with healthy controls. Sixteen showed no difference in T levels between cases and controls. None suggested high levels of T were associated with CAD. It is important to reemphasize the limitations of these studies. For example, the largest study, the Caerphilly Heart Study with 2512 men (51), showed a modest reduction in T in survivors of MI. The association, however, became insignificant when adjusted for plasma insulin and triglycerides. In the second largest study, with 1709 community-dwelling subjects from the Massachusetts Male Aging Study (55), the clinical endpoint was self-reported treated heart disease, which predicts CAD (MI and angina) with 75% accuracy but was not dif-

TABLE 1. Relationships between circulating T levels and CAD in men

| First author, year (Ref.) | n | Study type | Hormone | Endpoint | Relationship OR |
|---|---|---|---|---|---|
| Mendoza, 1983 (20) | 52 | Cross-sectional | T | MI, angio | Negative |
| Barth, 1983 (21) | 20 | Cross-sectional | T | CAD, angio | Negative |
| Hromadova, 1985 (22) | 67 | Cross-sectional | T | Coronary findings, angio | Negative |
| Breier, 1985 (23) | 139 | Cross-sectional | T | CAD, angio | Negative |
| Aksut, 1986 (24) | 54 | Cross-sectional | T | MI, angina | Negative |
| Sewdarsen, 1986 (25) | 56 | Cross-sectional | T, free T | MI | Negative |
| Chute, 1987 (26) | 146 | Cross-sectional | T, free T | CAD, angio | Negative |
| Hämäläinen, 1987 (27) | 57 | Cross-sectional | T, free T | CHD, angio | Negative |
| **Lichtenstein, 1987 (28)** | **2512** | **Cross-sectional** | **T** | **IHD** | **Negative** |
| Swartz, 1987 (29) | 71 | Cross-sectional | T | MI | Negative |
| Sewdarsen, 1988 (30) | 20 | Cross-sectional | T | MI, angio | Negative |
| **Sewdarsen, 1990 (31)** | **224** | **Cross-sectional** | **T** | **MI** | **Negative** |
| Rice, 1993 (32) | 272 | Cross-sectional | T, free T | MI | Negative |
| Phillips, 1994 (33) | 55 | Cross-sectional | T, free T | CAD, Angio | Negative |
| Zhao, 1998 (34) | 201 | Cross-sectional | T | CAD | Negative |
| English, 2000 (35) | 90 | Cross-sectional | T, free T, bio T | CAD, angio | Negative |
| Luria, 1982 (36) | 50 | Cross-sectional | T | MI | Null |
| Labropoulos, 1982 (37) | 144 | Cross-sectional | T | MI | Null |
| Zumoff, 1982 (38) | 117 | Cross-sectional | T | MI, CAD | Null |
| Phillips, 1983 (39) | 122 | Cross-sectional | T | CHD | Null |
| Heller, 1983 (40) | 295 | Cross-sectional | T | CHD | Null |
| Small, 1985 (41) | 100 | Cross-sectional | T | IHD | Null |
| Franzen, 1986 (42) | 92 | Cross-sectional | T | MI | Null |
| Baumann, 1988 (44) | 58 | Cross-sectional | T | Atherosclerosis | Null |
| Slowinska-Srzednicka, 1989 (47) | 108 | Cross-sectional | T | MI, Angio | Null |
| Cengiz, 1991 (49) | 55 | Cross-sectional | T | MI, angina | Null |
| Hauner, 1991 (50) | 274 | Cross-sectional | T | CAD, angio | Null |
| Mitchell, 1994 (53) | 98 | Cross-sectional | T, free T | MI | Null |
| Marquez-Vidal, 1995 (54) | 116 | Cross-sectional | T | MI | Null |
| **Feldman, 1998 (55)** | **1709** | **Cross-sectional** | **T, free T** | **Heart disease** | **Null 0.8** |
| Kabakci, 1999 (56) | 337 | Cross-sectional | T, free T | CAD, angio | Null |
| Schuler-Lüttmann, 2000 (57) | 189 | Cross-sectional | T, free T index | CAD, angio | Null |
| **Cauley, 1987 (43)** | **163, 163** | **Nested case-control 6–8 yr** | **T, free T** | **MI** | **Null 1.1 (0.7–1.9)** |
| **Barrett-Connor, 1988 (45)** | **1009** | **Prospective cohort 12 yr** | **T** | **IHD** | **Null 1.1 (0.8–1.3)** |
| **Phillips, 1988 (46)** | **96, 96** | **Nested case-control 19–20 yr** | **T** | **MI** | **Null** |
| **Contoreggi, 1990 (48)** | **46, 124** | **Nested case-control 9.5 yr** | **T** | **CAD** | **Null** |
| **Yarnell, 1993 (51)** | **2512** | **Prospective cohort 5 yr** | **T** | **CHD** | **Null 1.1 (0.9–1.3)** |
| **Hautanen, 1994 (52)** | **62, 97** | **Nested case-control 5 yr** | **T** | **Cardiac endpoints** | **Null** |
| **Harman, 2001 (19)** | **890** | **Prospective cohort 31 yr** | **T, free T index** | **CAD** | **Null** |

Negative relationship indicates lower T levels in patients with CAD compared to controls, and a null relationship indicates no difference between cases and controls. For prospective cohort or nested case-control studies, the number of cases (first n) and controls (second n) and duration under study are listed. Highlighted in *bold* are the most important studies in terms of adequacy of design, statistical power, and allowance for confounding factors.

Free T, Unbound T measured by equilibrium dialysis or analog assay; free T index, unbound T derived from total T and SHBG; bio T, bioavailable (non-SHBG-bound) T; angio, coronary angiography; IHD, ischemic heart disease.

ferentiated from congestive heart failure. This study, however, did rule out any potential confounding effects of cardiac medications including vasodilators, antihypertensives, and lipid-lowering agents. The latter is important because the effective lowering of circulating cholesterol may reduce the substrate for steroidogenesis, and high-dose simvastatin was confirmed to lower total and free T after 12-wk treatment (58). Phillips *et al.* (33) demonstrated a significant dose-dependent negative relationship between free T (measured with the analog assay) and the degree of coronary arterial occlusion in 55 men undergoing angiography who had not previously had MI. The authors suggested, overenthusiastically in our view, that low circulating T might be a risk marker for coronary atherosclerosis. It is also of interest that, in a few studies in which both T and dehydroepiandrosterone sulfate (DHEAS) were measured (see *Section VIII*), T showed no difference between cases and controls, whereas DHEAS was decreased (47, 53, 55, 57), suggesting that different mechanisms, probably not mediated by the androgen receptor, may underlie the potential relationships between these two hormones and CAD.

*2. Prospective cohort or nested case-control studies.* Table 1 also summarizes the seven non-cross-sectional studies (19, 43, 45, 46, 48, 51, 52). None of these studies showed T to have any significant relationship or predictive value for incident CAD.

The three prospective cohort studies followed 1009 Californian (Rancho Bernardo) men aged 40–79 yr over a 12-yr period (45), 2512 men aged 45–59 yr in the United Kingdom (Caerphilly) for a 5-yr period (51), and 890 largely middle-class and 87% Caucasian (Baltimore) men aged 53.8 ± 16 yr for a period up to 31 yr (19). There was no correlation between baseline T levels and subsequent development of fatal or nonfatal CAD, stroke, or heart failure after adjusting for relevant confounders. Despite the concern that only a single hormone measurement at recruitment was undertaken and possible storage artifact, the relatively large size and long follow-up period of these three cohort studies go a long way toward confirming that T is not an independent risk factor for CAD in men.

In the four nested case-control studies, baseline T levels in cases of CAD and matched controls from the Honolulu Heart Program (43), Multiple Risk Factors Interventional Trial (46), Baltimore Longitudinal Study of Ageing (Ref. 48, the earlier and shorter version of Ref. 19), and the Helsinki Heart Study (52) did not predict CAD events during observation periods of 6–8, 19–20, 9.5, and 5 yr, respectively.

In summary, the seven prospective studies provide a consistent and convincing data set that shows the lack of a relationship between circulating T and incident or existing CAD in men. There is a suggestion, only from cross-sectional studies, that patients with CAD may have lower T levels; the nature of this relationship is unclear. None of the 39 studies in the literature showed a positive relationship between T and CAD to suggest that high levels of this androgen may be a risk factor.

### *B. T and CAD in women*

There are relatively few studies that investigated the relationship between endogenous levels of androgens and CAD in women (Table 2A). Age-adjusted concentrations of T, bioavailable T, and androstenedione did not differ significantly in 651 postmenopausal women, from the Rancho Bernardo study, with and without a history of heart disease at baseline and did not predict cardiovascular death or death from ischemic heart disease during the subsequent 19 yr (59). In contrast, in a cross-sectional angiographic study of 109 postmenopausal women with chest pain, serum levels of free T were correlated with the maximum percentage reduction of the luminal diameter of coronary arteries. This correlation was independent of age, body mass index (BMI), systolic blood pressure, smoking, or levels of cholesterol, insulin, and estradiol (60). However, higher free T and androstenedione within the physiological range had also been correlated with less carotid artery atherosclerosis in premenopausal and postmenopausal women (61).

*1. Polycystic ovarian syndrome (PCOS).* Indirect evidence for the atherogenicity of androgens in women comes from clinical observational studies in women with PCOS. Much has been written recently about the potentially increased CAD risk in patients with PCOS (62–70). This is based on cross-sectional data that consistently showed a strong obesity-independent cluster of cardiovascular risk factors including insulin resistance, dyslipidemia, and impaired fibrinolysis in patients with PCOS. This has given rise to the view that the chronically abnormal hormonal and metabolic milieu in PCOS, starting from adolescence, may predispose these women to premature atherosclerosis. Based on calculated risk profiles, women with PCOS were predicted to have a relative risk for MI of 7.4:1 (71).

Wild *et al.* (72) assessed the waist-hip ratio (WHR) and previous history of symptomatic androgen excess (hirsutism and acne) in 102 consecutive women undergoing cardiac catheterization. A positive correlation between angiographic evidence of coronary artery disease and clinical evidence of hyperandrogenism was found (Table 2B). In a combined

TABLE 2. Relationships between circulating T levels and CAD in women (A) and between PCOS and CAD in women (B)

| First author, year (Ref.) | n | Study type | Hormone (A)/phenotype (B) | Endpoint | Relationship OR |
|---|---|---|---|---|---|
| A | | | | | |
| Phillips, 1997 (60) | 109 | Cross-sectional | Free T | Coronary Angio | Positive |
| Bernini, 1999 (61) | 101 | Cross-sectional | Free T, A | CIMT | Negative |
| **Barrett-Connor, 1995 (59)** | **651** | **Prospective cohort 19 yr** | **T, bio T, A** | **CVD mortality** | **Null 1.0 (0.99–1.03)** |
| B | | | | | |
| Wild, 1990 (72) | 102 | Cross-sectional | Hirsutism/acne | Coronary Angio | Positive |
| Birdsall, 1997 (73) | 143 | Cross-sectional | Pelvic USS, PCOS | Coronary Angio | Positive |
| Guzick, 1996 (74) | 16, 16 | Cross-sectional | PCOS, T | CIMT | Positive |
| Talbott, 2000 (75) | 47, 60 | Cross-sectional | PCOS, T | CIMT | Positive |
| Cibula, 2000 (76) | 28, 752 | Cross-sectional | PCOS[a] | Various CAD | Positive |
| Christian, 2000 (77) | 32, 52 | Cross-sectional | PCOS | Coronary calcification | Positive |
| Mather, 2000 (78) | 18, 19 | Cross-sectional | PCOS, T | Vascular responses | Null |
| Pierpoint, 1998 (79) | 786 | Historical prospective cohort | PCOS | CVD mortality | Null SMR 1.4 (0.8–2.4) |
| Wild, 2000 (80) | 319 | Historical prospective cohort | PCOS | CVD | Null 1.2 (0.5–2.6) |
| Elting, 2001 (81) | 346 | Retrospective clinic survey | PCOS | Cardiac complaints | Null |

T, Total T; free T, unbound T measured by equilibrium dialysis or analog assay; bio T, bioavailable (non-SHBG-bound) T, including albumin-bound fraction; A, androstenedione; CIMT, carotid artery intima-media thickness; USS, ultrasound; angio, angiography; SMR, standardized mortality.

Highlighted in *bold* is the most important study in terms of adequacy of design, statistical power, and allowance for confounding factors.

[a] Patient who had ovarian wedge resection.

angiography and pelvic ultrasound study of 143 women aged 60 yr or less who were referred because of chest pain or valvular heart disease, the presence of polycystic ovaries (in 42% of patients) was associated with an increased number of stenosed coronary arteries (73). Moreover, the presence of CAD and a family history of MI as well as elevated levels of insulin and triglycerides and lower levels of high-density lipoprotein (HDL)-cholesterol (HDL-C) were independent predictors of polycystic ovaries. The prevalence of CAD (history of chest pain, MI, angioplasty, or coronary artery bypass grafts) was found to be significantly higher in 28 women (45–59 yr old) who had undergone ovarian wedge resection over 18 yr ago compared with 752 aged-matched controls (76). The low response rate in the cases (<50%) and the uncertain diagnosis of CAD based on history of possible angina or MI make the data in this study difficult to interpret. In cross-sectional studies using B-mode ultrasound, significantly increased carotid artery intima-media thickness was found in patients with PCOS compared with age-matched controls (74, 75). This was not entirely explained by BMI, fat distribution, and other risk factors and may be regarded as evidence in support of subclinical premature atherosclerosis in middle-aged (>45 yr) women independently related to the increased T in PCOS. Similarly, a recent study (77) demonstrated an increased prevalence of coronary artery calcification (which correlates with atherosclerosis) in 32 premenopausal (30–45 yr old) women with PCOS compared with 52 controls using electron beam computed tomography. These three studies employed noninvasive markers of early atherosclerosis to demonstrate an excessive risk for subclinical cardiovascular disease in relatively young PCOS patients. The data require confirmation with larger numbers and prospective follow-up. However, despite marked differences in glucose/insulin ratio and free androgen index in 18 healthy, obese, young women (32.7 ± 1.9 yr) with PCOS, insulin resistance, hyperandrogenism, and endothelium-dependent and -independent vascular responses were normal compared with age-matched controls (78).

In terms of actual cardiovascular disease events associated with PCOS, there is information from only two long-term longitudinal studies. Mortality and morbidity over an average 30 yr in 786 of 1028 women (over 45 yr of age) diagnosed to have PCOS on histopathological and hospital in-patient diagnostic records between 1930 and 1979, most of whom underwent ovarian wedge resection, were compared retrospectively with 1060 age-matched control women. Despite the significantly increased diabetes, hypertension, cholesterol, and nonfatal cerebrovascular disease, the standardized mortality ratio for CAD of 1.4 (95% CI, 0.8–2.4) and OR for a history of CAD of 1.2 (95% CI, 0.5–2.6) were not significantly raised (79, 80). In a recent Dutch cohort of 346 nonobese patients aged 30.3–55.7 yr diagnosed to have PCOS in a specialized clinic 12 yr (range 1.2–31.6) previously, the prevalence of cardiac complaints (serious heart disease or cardiac arrest) ascertained by telephone questionnaire was not significantly different from that in 8950 age-matched females in the general population, despite the higher prevalence of both diabetes and hypertension (81). This suggests that previous estimates of CAD risk in PCOS may have been somewhat excessive. However, both these studies suffer from methodological drawbacks such as underascertainment of PCOS (79, 80) and the relative young age of the smaller cohort (81).

Endogenous T is unlikely to have a causal or protective role for CAD in women. On the other hand, there is little doubt that PCOS patients (younger women of reproductive age) have an adverse risk profile for cardiovascular disease. However, whether this leads to increased, premature heart disease and, if so, whether this is causally related to chronic hyperandrogenemia *per se*, as opposed to associated variables, remain unresolved questions. Nevertheless, it is important not to dismiss the possibility of an association between PCOS and CAD events (probably independent of T). Given the high prevalence of PCOS in the female population, this should remain a high priority target for future research.

## IV. Relationships between Serum Levels of T and CAD—Interventional Clinical Studies

### *A. Endogenous androgen deprivation*

A frequently cited study (82) compared the life span of 297 castrated inmates with 735 intact inmates (white males) in a single state institution for the mentally retarded in Kansas between 1895 and 1950. The reasons for castration were unclear. Castrated males lived an average of 13.6 yr longer than intact controls. However, the excess mortality in intact inmates was due to infections with no difference in cardiovascular disease mortality between the two groups. The authors concluded that postpubertal castration did not decrease the frequency of deaths due to cardiovascular disease. In a historical review (83), the life span of 50 castrated singers (prepubertal castrates) born between 1581 and 1858 in Europe was 65.5 ± 13.8 yr compared with 64.3 ± 14.1 yr in 50 noncastrated singers. In another historical survey of castration, Wilson and Roehrborn (84) also concluded that there are no valid data indicating that castration has any effect on life span of men. Doubts about ascertainment accuracy and the small size of these historical studies make it difficult to draw clear conclusions. The findings are, however, consistent with findings from cross-gender sex-hormone treatment in 816 male-to-female transsexuals aged 18–86 yr (mean, 41 yr; Ref. 85). Administration of ethinylestradiol (100 μg/d) and cyproterone acetate (100 mg/d) for 7734 patient-years was not associated with any significant difference in cardiovascular mortality or morbidity compared with the general male population, despite a 20-fold increase in venous thromboembolic complications.

### *B. Androgen excess from anabolic steroid abuse*

Excessive T exposure in men is uncommon in clinical practice. However, anabolic-androgenic steroid (AAS) abuse in the general population is said to have reached epidemic proportion, with over 1 million current and former users in the United States alone (86–88). In two reviews of the literature covering a 12-yr period from 1987–1998 (89, 90), there was a total of 17 case reports of cardiovascular events in young male body builders using supraphysiological doses of AAS. Invariably, multiple preparations seldom pre-

scribed in clinical practice, including oral 17$\alpha$-alkylated androgens, are used in combination simultaneously. There are 11 documented cases of acute MI, 4 cardiomyopathy, and 2 strokes. It is not possible to draw firm scientific conclusions from these sporadic case reports, especially when the baseline denominator information on prevalence and extent of exposure is shrouded in uncertainty and secrecy. But with the vast increase in abuse since the 1960s (86, 87, 89), there is no clear evidence for an epidemic of cardiovascular events among likely users and ex-users of AAS. A formal case-control study of AAS abuse in younger men presenting with acute MI has not been performed. Nevertheless, it has been suggested that dose-dependent androgen-induced vasospasm, platelet aggregation, activation of coagulation cascade, atherogenic lipid profiles [increased low-density lipoprotein (LDL)-cholesterol (LDL-C) and decreased HDL-C], and abnormal left ventricular function and hypertrophy are relevant mechanisms precipitating sudden cardiac deaths in young power athletes and body builders (90). It must be emphasized that pathological data from men abusing exotic AASs in doses several orders of magnitude higher than those prescribed in the clinical setting should not be extrapolated to the legitimate medical therapeutic use of approved T preparations or indeed to androgen physiology.

### *C. Exogenous T treatment in men with CAD*

There are 17 reports in the literature documenting the effects of therapeutic doses of T in men with CAD. All showed some improvement or beneficial effects. The early studies from the 1940s are of historical interest only because of the small number of patients included and the uncontrolled observations (91–101).

Webb and colleagues (102, 103) showed that a single iv bolus of 2.3 mg of T increased the time to 1-mm ST-segment depression on ECG by 66 sec (15–117, $P < 0.016$) in 14 men with CAD and low plasma T. The plasma T increased from 5.2–117 nmol/liter, indicating that this is a pharmacological action on the coronary vasculature. These direct acute pharmacological effects of T have been further studied during coronary angiography. Webb and colleagues (102, 103) infused T over 3 min into the coronary arteries of 13 men with established CAD during coronary angiography at doses of $10^{-7}$ to $10^{-10}$ mol/liter (8 $\mu$mol/liter to 8 nmol/liter). Coronary vessel diameter increased by 3.1–4.5% at the three higher doses but not at the physiological dose of $10^{-10}$ mol/liter. Coronary artery blood flow increased by 12–17.4% at all four doses of T. These effects were mediated by endothelium-independent and nongenomic mechanisms. This is the first demonstration of a direct vasodilatory action of T on coronary arteries *in vivo* in human males. These results have been confirmed by a similar study (104) in 14 men with established CAD. Intravenous infusion of 2.5 mg of T prolonged time to 1-mm ST depression from 471–579 sec and increased total exercise time from 541–631 sec. Whether the acute vasodilatory action of T at pharmacological doses translates into physiological therapy remains to be determined (also see *Section VII.B*).

Jaffe (105) reported the first randomized placebo-controlled double-blind study investigating the effects of T cypionate (200 mg im weekly) in 50 men with positive exercise ECG (n = 25 in each group). The sum of ST-segment depression in leads II, V4, V5, and V6 immediately 2, 4, and 6 min after the standard two-step exercise test (16 measurements in all) decreased by 32% and 51% from baseline after 4 and 8 wk in the active group with no change in the placebo group. There was no mention of any symptomatic improvement. Wu and Weng (106) reported another randomized placebo-controlled crossover (but not double-blinded) study in 62 elderly men with CAD treated with oral T undecanoate or placebo for 4 wk. T increased from 17–27 nmol/liter on T undecanoate (120–40 mg daily). The response categories were established by the Chinese Ministry of Public Health and denoted as very effective, effective, ineffective, worsened, and total efficacy but were not defined further. Both subjective symptom scores and resting ECG were improved in 69% and 75% of subjects, respectively, after 4 wk of treatment. In a recent study, English *et al.* (107) investigated the effects of a physiological dose of transdermal T (5 mg daily) for 12 wk in 50 patients with symptomatic CAD in a double-blind randomized placebo-controlled add-on trial. Plasma T increased from 13.6–22.3 and 18.6 nmol/liter after 4 and 12 wk of T treatment. The time to 1-mm ST-segment depression increased from 309–343 at wk 4 and 361 sec at wk 12 in the treated and from 266–284 at wk 6 and 292 sec at wk 12 in the placebo group ($P < 0.02$, treated *vs.* placebo).

These preliminary data suggest short-term improvements in ECG changes of CAD after (maximum of 12 wk) T supplement. Whether there are real symptomatic or functional benefits or decreased mortality in the long term remain important but unanswered questions.

### *D. Exogenous T treatment in women*

The possible physiological roles of androgens in women may include increasing libido, energy, bone mineral density, muscle mass, and strength, but the data to support these possible roles are currently limited and not entirely convincing (108). Although hypopituitary (109) and bilaterally ovariectomized females (110) are undoubtedly androgen deficient, circulating T is only minimally lower after the natural menopause because ovarian secretion is maintained (111, 108). Nevertheless, there is increasing interest in the use of T as part of postmenopausal hormone replacement therapy, in particular to improve reported impaired sexual function (2, 112). Whether the concurrent use of T will impact the effects of estrogen hormone replacement therapy on the cardiovascular system is currently unknown. In a 20-yr (1975–1994) retrospective survey of the Amsterdam Gender Dysphoria Clinic (85), 293 female-to-male transsexuals aged 17–70 yr (mean, 34 yr) were treated for 2 months to 41 yr (total exposure of 2418 patient-years) with oral T undecanoate (160 mg daily) or T (Sustanon; 250 mg im every 2 wk). There was no excess of cardiovascular mortality (all cause) or morbidity compared with the general female Dutch population. However, there is currently insufficient evidence to exclude harmful cardiovascular effects of T treatment in women.

In summary, interventional studies to decrease endogenous T or administration of T generally do not suggest a causal relationship between T exposure and the develop-

ment of CAD. Although some preliminary information hints at possible beneficial effects on myocardial ischemia, prospective controlled data on cardiovascular disease endpoints (MI, angina, mortality) from large-scale interventional studies using physiological doses of androgens are currently lacking.

## V. Relationships between Serum Levels of T and CAD—Animal Studies

The influence of androgens on the development and progression of experimentally induced atherosclerosis has been investigated in six animal models with diet- or injury-induced atherosclerosis and in two genetic atherosclerosis-susceptible mouse models (Refs. 113–122 and Table 3). Larsen *et al.* (114) investigated the effects of im T enanthate in castrated male rabbits and found no difference in the cholesterol content of abdominal aorta lesion after 17 wk. A similar negative result was obtained with the anabolic steroid stanozolol (115). Bruck *et al.* (118) demonstrated gender-specific effects of T and estradiol in castrated male and female rabbits fed an atherogenic diet. After 12 wk, aortic arch atheroma formation was significantly inhibited by im estradiol valerate (1 mg/kg·wk) in females but not in males, by T enanthate (25 mg/kg·wk) in males but not in females, and by combined estradiol and T administration in both sexes. Interestingly, T treatment in female rabbits increased plaque sizes, but estradiol had no effect in male rabbits. The authors concluded that the antiatherogenic effects of sex steroids involve gender-specific mechanisms and are independent of changes in plasma lipids. T did not have any effect on the myointimal proliferation response to balloon injury of the carotid artery *in vivo* in either male or female intact or gonadectomized rats, whereas estradiol inhibited this response in both sexes (117). Alexandersen *et al.* (119) showed that castration *per se* in male rabbits resulted in a doubling of aortic atherosclerosis compared with sham-operated controls, suggesting that endogenous T has an antiatherogenic effect. This can be reversed by oral T undecanoate (80 mg daily) or DHEA (500 mg daily) via a lipid-dependent mech-

TABLE 3. Relationship between androgens and atherosclerosis in animals fed atherogenic cholesterol-enriched diets or after vessel injury

| First author, year (Ref.) | Model | n | Duration | Hormone | Endpoints | Effect on atherosclerosis |
|---|---|---|---|---|---|---|
| Toda, 1984 (113) | Male chicks | 24 | 7 wk | T | Aortic atherosclerosis | Increase |
| Larsen, 1993 (114) | Male odx rabbits | 36 | 17 wk | T | Abdominal aorta cholesterol | Null |
| Adams, 1995 (116) | Female ovx monkeys | 64 | 24 months | T | Coronary artery plaque size | Increase[a] |
| Chen, 1996 (117) | Male odx rats<br>Female ovx rats | 30<br>23 | 14 d | T & E2 | Myointimal proliferation after balloon injury of carotids | T null, E2 decreased in both sexes |
| Bruck, 1997 (118) | Male odx rabbits<br>Female ovx rabbits | 32<br>32 | 12 wk | T & E2 | Aortic plaque size | T decreases in male, E2 decreases in female, T increases in female |
| Alexandersen, 1999 (119) | Male odx rabbits | 100 | 30 wk | T | Aortic atherosclerosis | Decrease |
| Elhage, 1997 (121) | Male apoE−/− odx mice[b]<br>Female apoE−/− ovx mice[b] | 70<br>70 | 8 wk | T & E2 | Aortic fatty streak lesions | Castration null, T and E2 decrease in both sexes |
| von Dehn, 2001 (122) | Male apoE−/− mice[b]<br>Female apoE−/− mice[b] | 19<br>19 | 8 wk | Cetrorelix[c], T | Aortic fatty streak lesions | Cetrorelix[c] decreases in both sexes, T increases in male, T decreases in female |
| Nathan, 2001 (122a) | Male LDLR−/− mice[d] | 6–11 | 8 wk | Orchidectomy, T & E2, aromatase inhibitor | Aortic fatty streak lesions | Castration increases, T & E2 decrease but reversed by aromatase inhibitor |
| Fogelberg, 1990 (115) | Male rabbits | 17 | 12 wk | Stanozolol | Aortic atherosclerosis | Null |
| Obasanjo, 1996 (120) | Female ovx monkeys | 52 | 12–24 months | Nandrolone | Coronary plaque & lumen size | Increase[e] |
| Gordon, 1988 (405) | Male rabbits | 34 | 12 wk | DHEA | Aortic atherosclerosis following balloon-induced intimal injury | Decrease |
| Arad, 1989 (406) | Male rabbits | 15 | 8 wk | DHEA | Aortic fatty streak | Decrease |
| Eich, 1993 (407) | Male rabbits heterotopic cardiac transplants | 48 | 5 wk | DHEA | Graft atherosclerosis | Decrease |
| Alexandersen, 1999 (119) | Male odx rabbits | 100 | 30 wk | DHEA | Aortic atherosclerosis | Decrease |
| Hayashi, 2000 (408) | Female ovx rabbits | 48 | 10 wk | DHEA | Aortic atherosclerosis | Decrease |

ovx, Ovariectomized; odx, orchidectomized; E2, estradiol.
[a] T reversed atherosclerosis-related impairment of endothelium-dependent vasodilation response, *i.e.*, functional benefit.
[b] apoE−/− mice, apoE-deficient knockout mice.
[c] Cetrorelix, GnRH antagonist.
[d] LDLR−/− mice, LDL-receptor-deficient knockout mice.
[e] Nandrolone treatment for 12 months increased coronary artery lumen size despite increased atherosclerotic plaque size.

anism. In addition, im T enanthate (25 mg twice weekly), which raised circulating T levels by 10-fold, decreased aortic atherosclerosis by lipid-independent mechanisms. This suggests that androgens in pharmacological doses may exert antiatherogenic effects on the vasculature. In contrast, treatment of male chicks with T resulted in a dose-dependent increase in aortic atherosclerosis (113). Similarly, in female ovariectomized cynomolgus monkeys fed an atherogenic diet for 24 months, the extent of coronary atherosclerosis was doubled with loss of compensatory remodeling of the arterial lumen in the T-treated group compared with the intact and untreated ovariectomized controls (116). These effects were independent of various risk factors including lipids. However, the acetylcholine-induced atherosclerosis-related coronary artery vasoconstriction was reversed by T treatment. Thus, despite the adverse pathomorphological changes in the arterial wall, functional parameters of the endothelium nevertheless improved upon treatment with T (116). It should also be pointed out that the SILASTIC-brand (Dow Corning Corp., Midland, MI) T implants used failed to maintain T levels (0.6 nmol/liter) in the adult male physiological range in the ovariectomized animals. These results may therefore be more relevant to atherogenesis in androgenized females, *e.g.*, PCOS, rather than males. With the same experimental model and design, Obasanjo *et al.* (120) showed that coronary artery atherosclerosis was significantly increased by the AAS nandrolone for 2 yr compared with the intact sham-operated group ($P < 0.05$) but not with the ovariectomized placebo group. The groups administered nandrolone had significantly larger arteries than the other two groups. Lumen area was significantly larger in the group given nandrolone for 1 yr (deferred start by 12 months) compared with all other groups ($P < 0.05$). Remodeling of the vessel wall and lumen could possibly counterbalance the increased plaque size. In view of these inconsistent results and the major gender-specific action of T (118), it should be emphasized that data obtained on experimentally induced atherosclerosis in female animals should not be extrapolated to males. To date, no experimental studies have been performed to investigate the effects of androgens on the mechanisms underlying atherosclerosis in male monkeys.

Three studies investigated the effect of castration and exogenous T on atherosclerosis in atherosclerosis-susceptible genetically engineered mice. In the study by Elhage *et al.* (121), castration had no effect on atherosclerosis of either male or female mice. Application of 7.5-mg T pellets increased T serum levels from undetectable to 1.3 ng/ml in females and from 0.5–1.7 ng/ml T in males. Compared with intact and castrated control animals, application of T significantly decreased serum levels of cholesterol and inhibited the development of fatty streak lesions in the sinus aortae by about 30% in both sexes. In the study by von Dehn *et al.* (122), the animals received either 100 $\mu$g of the GnRH antagonist Cetrorelix every 48 h or a 35-mg implant of T. Suppression of T led to a decrease in atherosclerosis in both the sinus aortae and the ascending aorta despite increases of cholesterol in male and decreases of HDL-C in female mice. Treatment with T increased serum levels to 6.1 ng/ml in male mice and to small but significant increases of cholesterol levels and atherosclerotic lesions in male mice. Despite an increase of T levels to 10.1 ng/ml, female mice showed no change in lipids and fewer atherosclerotic lesions. In LDL-receptor-deficient male mice (122a), both castration and the aromatase inhibitor anastrazole increased the extent of fatty streak lesions in the aortic arch compared with control mice. Lesion formation was attenuated by treatment of orchidectomized animals with either T or estradiol. The atheroprotective effect of T was abolished by the simultaneous application of anastrazole. These results suggest that T attenuates early atherogenesis by being aromatized to estrogens (see *Section IX*). The discrepancy between these three studies may partly be explained by the different dosages of T and gender-specific actions. The effects of T on early atherogenesis were not explained by changes in lipid levels in any of these three studies.

In summary, various animal models have highlighted the existence of many different mechanisms in the evolution of atherosclerosis that can potentially be influenced by androgens. The inconsistent and conflicting results from these *in vivo* studies reflect the complexity of pathogenesis, the sexually dimorphic response to atherogenic triggers, as well as the gender-specific response to sex steroids.

## VI. Effects of T on Cardiovascular Risk Factors

The effects of T on cardiovascular risk factors are contradictory depending on whether associations with endogenous T or effects of exogenous T have been investigated.

### *A. Associations between endogenous T and cardiovascular risk factors: role of adipose tissue and insulin*

Several cross-sectional population studies found statistically significant correlations between plasma levels of T and various cardiovascular risk factors that appear to be profoundly influenced by the interrelationships between T, adipose tissue, and insulin action. Furthermore, T showed opposite relationships with risk factors in men and women.

*1. Observations in men.* In men, plasma T levels showed positive correlations with HDL-C and inverse correlations with triglycerides, total cholesterol, LDL-C, fibrinogen, and plasminogen activator type 1 (PAI-1; Refs. 33, 51, 123–130). However, T levels have even stronger inverse correlations with BMI; waist circumference; WHR; amount of visceral fat; and serum levels of leptin, insulin, and free fatty acids (FFA). After adjustment for these anthropometric, radiological, or biochemical measures of obesity and insulin resistance, the correlations of the cardiovascular risk factors with T but not with visceral fat or insulin lost their statistical significance (131–133). Likewise, in a case-control study of 50 men who were matched by age and ethnic background but segregated by T levels, hypoandrogenemia was associated with significantly higher BMI, WHR, higher systolic blood pressure, higher fasting and 2-h glucose and insulin levels, and higher levels of total cholesterol, LDL-C, triglycerides, and apolipoprotein (apo)B as well as with lower levels of HDL-C and apoA-I. After adjustment for BMI and WHR, only the negative correlations of T with insulin and triglycerides remained statistically significant (134). These findings indicate that low T in men is a component of a plurimetabolic syndrome, which is characterized by obesity, type 2 diabetes

mellitus, hypertension, hypertriglyceridemia, low HDL-C, and a procoagulatory and antifibrinolytic state.

What comes first, hypotestosteronemia, obesity, or insulin resistance? On the one hand, morbidly obese and insulin-resistant men frequently have low serum levels of T (132, 135) that increase upon weight loss (136, 137). Estradiol levels show the opposite changes to T, with obesity and weight loss. It has therefore been suggested that obesity causes hypotestosteronemia by increased aromatization of T to estradiol in the adipose tissue (Fig. 2). In agreement with an important role of hyperinsulinemia as an etiological factor of hypotestosteronemia in obese men is the negative regulatory effect of insulin on the production of SHBG (138) and the inverse correlation between serum concentrations of insulin and SHBG (139). Also supporting a role of insulin in the determination of T levels in men, in one study infusion of insulin during euglycemic clamp increased T levels in obese men, but not in lean men (140). On the other hand, hypogonadal men are frequently obese with increased levels of leptin and insulin (140–145). Body weight, leptin levels, and insulin levels decrease upon substitution of T in hypogonadal men (146–148). Treatment of eugonadal obese men with T led to a decrease of visceral fat mass and, in parallel, improved insulin sensitivity and corrected dyslipidemia (149–151). In the opposite experiment, suppression of T by the GnRH antagonist cetrorelix increased serum levels of leptin and insulin (152). These latter data indicate that, in men, the dominant action in the bidirectional relationship between T

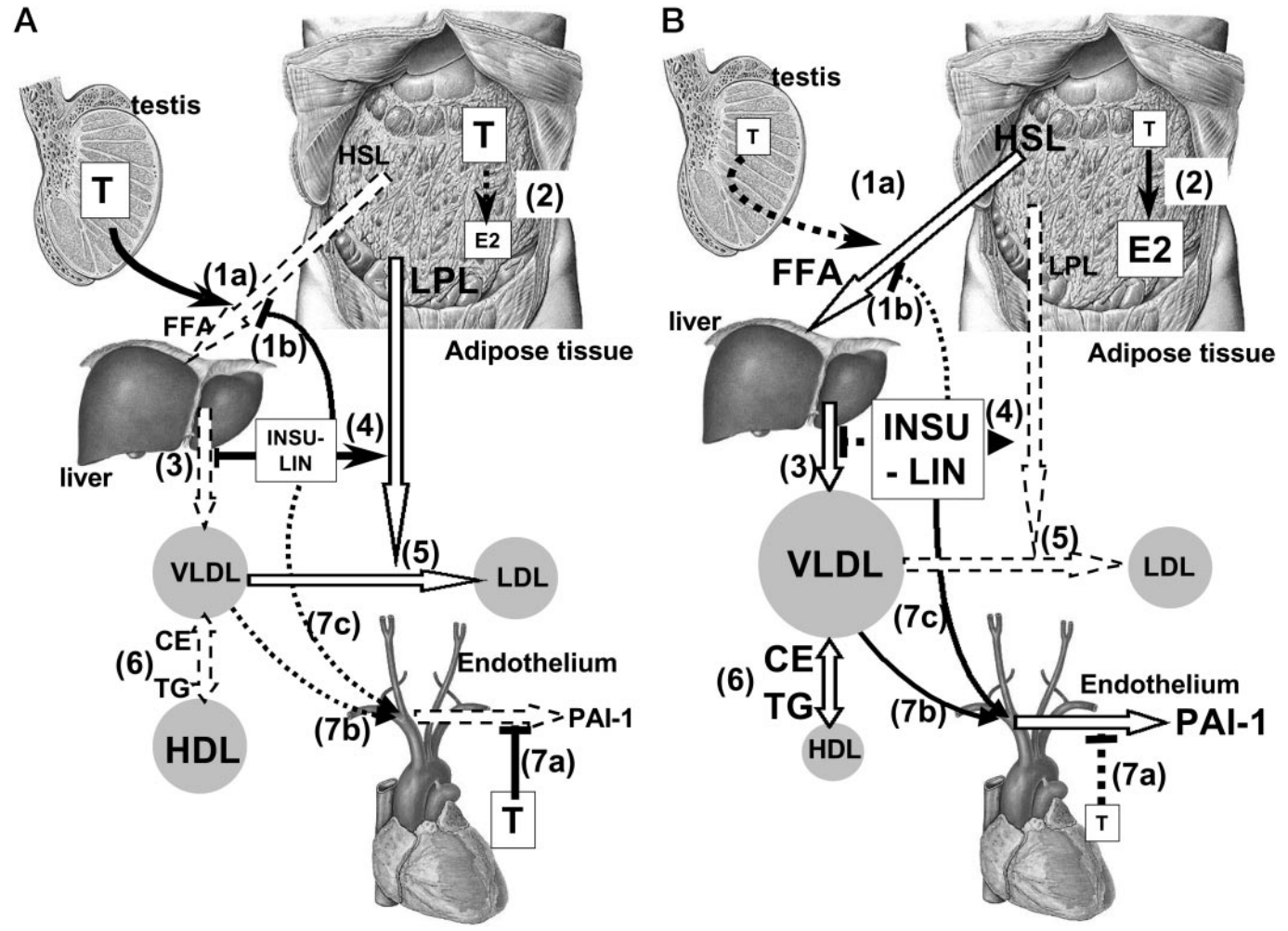


FIG. 2. Model of metabolic effects of T in eugonadal nonobese (A) and hypogonadal obese (B) men. T activates hormone sensitive lipase (HSL) in adipocytes and thereby decreases body fat mass (1a). This implies little aromatization of T into estradiol (2), *i.e.*, facilitates the maintenance of normal T levels in nonobese men (A). Hydrolysis of body fat by HSL produces FFA, which stimulate hepatic very (V)LDL production (3a). However, this hypertriglyceridemic effect is balanced by improved insulin sensitivity in lean individuals with the result of reduced FFA release from adipocytes (1b), inhibited VLDL production (2b), and stimulated secretion of lipoprotein lipase (LPL) by the adipose tissue (3). Normal VLDL production and regular lipolysis of VLDL (and chylomicrons) by LPL (4) lead to normotriglyceridemia and, via low cholesterol ester (CE) transfer protein (CETP)-mediated exchange of CEs and triglycerides between VLDL and HDL (6), to normal HDL-C levels. Taken together, normal T levels, low insulin levels, and normotriglyceridemia help to suppress PAI-1 production in the endothelium (7). In hypogonadal men (B), low T levels impair lipolysis in adipocytes and favor obesity (1a). Enhanced aromatization of T into estradiol in obese men (2) further decreases T levels. Obesity causes insulin resistance with the result of increased FFA release from adipocytes (1b), disinhibited VLDL production (3), and decreased LPL secretion (4). Both increased VLDL secretion (3) and decreased lipolysis of triglyceride-rich lipoproteins (5) cause hypertriglyceridemia, which stimulates the CETP-mediated removal of CEs from HDL and thereby causes low HDL-C (6). Finally, hypotestosteronemia (7a), hypertriglyceridemia (7b), and hyperinsulinemia (7c) stimulate the production of PAI-1 in endothelial cells.

and insulin is that T reduces fat mass, especially in the abdomen, and improves insulin action (Fig. 2). Mediated by the androgen receptor in adipocytes, and further up-regulated by T, androgens activate the expression of β-adrenergic receptors, adenylate cyclase, protein kinase A, and hormone-sensitive lipase (153, 154). As a result, T stimulates lipolysis and thereby reduces fat storage in adipocytes (Fig. 2). Androgens elicit an antiadipogenic effect in preadipocytes *in vitro*, whereas estrogens behave as proadipogenic hormones, effects that are related to changes in the expression of the IGF receptor (androgens and estrogens) and peroxisome proliferator-activated receptor γ2 expression (estrogens; Ref. 155). This may explain the reduction of fat mass after androgen treatment (132).

*2. Observations in women.* Women present the opposite relationships between endogenous androgens and obesity, insulin, and cardiovascular risk factors. In cross-sectional studies, serum levels of T were found to have significant positive correlations with BMI and leptin levels (153, 154, 156, 157). Low serum levels of SHBG, which are an indirect measure of female hyperandrogenism, were associated with high BMI and WHR as well as with high serum levels of leptin and insulin and low serum levels of HDL-C (131, 158). Moreover, in a large prospective study, 20% of women with SHBG levels below the fifth percentile developed type 2 diabetes mellitus during the 12-yr follow-up period (159). Thus, hyperandrogenemia in women, rather than hypoandrogenemia in men, is associated with insulin resistance and diabetes mellitus. In agreement with this, hyperandrogenic women with PCOS frequently present with hypercholesterolemia, low HDL-C, hypertriglyceridemia, elevated fibrinogen and PAI-1, and a family history of diabetes mellitus (160–169). In a retrospective study, Dahlgren *et al.* (164) observed that the adverse cardiovascular risk profile of women with PCOS is also maintained after menopause.

Because many women with PCOS are overweight, and most, if not all, are insulin resistant, it is a matter of debate whether the dyslipidemic and procoagulatory states in women with PCOS are secondary to obesity and insulin resistance (160, 162, 167, 168, 170, 171) or whether hyperandrogenemia itself contributes to obesity, insulin resistance, and hyperinsulinemia (71, 132, 153, 154, 172–179). On the one hand, insulin sensitivity appears to play an important role for the pathogenesis of hyperandrogenemia in PCOS. Insulin stimulates androgen synthesis in the ovaries via its cognate receptor and the inositolglycan pathway (Ref. 180 and Fig. 3). Because the ovaries remain sensitive to insulin when other tissues such as fat and muscle are resistant, hyperinsulinemia can augment the LH- and ACTH-dependent hyperandrogenism in insulin-resistant women with PCOS (Ref. 181 and Fig. 3). In support of this, treatment of insulin resistance in women with PCOS with metformin or the insulin-sensitizer troglitazone significantly decreased serum levels of insulin as well as T, independently of BMI or gonadotropin levels (182–185). Concomitantly, plasma levels of HDL-C increased, and plasma levels of PAI-1 decreased (181–183). In contrast, short-term lowering of ovarian androgens by laparoscopic ovarian cautery did not alter insulin or lipid levels (186).

On the other hand, lowering androgen levels with GnRH agonists (187) and androgen receptor blockade (188) in hyperandrogenic women improved insulin sensitivity and lipid profile (189). The magnitude of these changes, however, is less than that usually encountered in PCOS. Supraphysiological doses of exogenous T administered to genetic females for gender reassignment therapy (153, 154, 190, 191) or to female cynomolgus monkeys (116) increased BMI and the mass of both visceral fat and muscle and decreased insulin sensitivity. Nandrolone treatment in obese postmenopausal women produced a gain in visceral fat and a relatively greater loss of sc fat (192). Methyltestosterone administration (5 mg three times daily for 10–12 d) to young nonobese women with regular menstrual cycles reduced glucose uptake during hyperglycemic and euglycemic clamp studies (193). Experiments in rats and marmoset monkeys showed evidence for androgen imprinting. Transient intrauterine or perinatal exposure to T predisposed female animals to central adiposity and insulin resistance in adult life (194, 195). Thus, there may be a vicious circle in which early androgen excess may contribute to insulin resistance in adult women with PCOS in whom hyperinsulinism aggravates the hyperandrogenism and the associated clinical phenotype (Fig. 3). A further hypothesis linking hyperandrogenism and insulin resistance is the concurrent dysregulation of cytochrome P450c17α action (leading to excessive androgen synthesis) and insulin receptor function by excessive serine phosphorylation or decreased chironinositol (65, 196, 197). Whatever the likely etiology(s), defective insulin action (independent of obesity) is thought to be the root cause of the metabolic disarray (198, 199) in PCOS.

In summary, the observational studies do not allow any clear conclusions on the role of T in determining cardiovascular risks because the associations between serum levels of T and cardiovascular risk factors are in opposite directions for men and women. These gender-specific correlations are also confounded by the bidirectional relationships between T, adipose tissue, and insulin sensitivity. However, the weight of current experimental evidence would suggest that low endogenous T may be the driving etiological factor for obesity, insulin resistance, and the occurrence of multiple cardiovascular risk factors in men, whereas in women defective insulin action appears to be critical for the development of the hyperandrogenemia associated with polycystic ovaries.

### *B. Effects of puberty on cardiovascular risk factors*

Longitudinal studies were used to study the effect of puberty and hence endogenous sex hormones on cardiovascular risk factors in children. Prepubertal boys and girls do not differ significantly in their serum lipid and lipoprotein levels. In contrast to girls, in whom levels of HDL-C and LDL-C change little with puberty, sexually maturing boys experience a decrease in HDL-C and increases in LDL-C and triglycerides (200). However, these changes may not reflect effects of sex hormones only because they are confounded by other endocrine changes, for example in the GH/IGF-I axis, which also regulate lipoprotein metabolism.

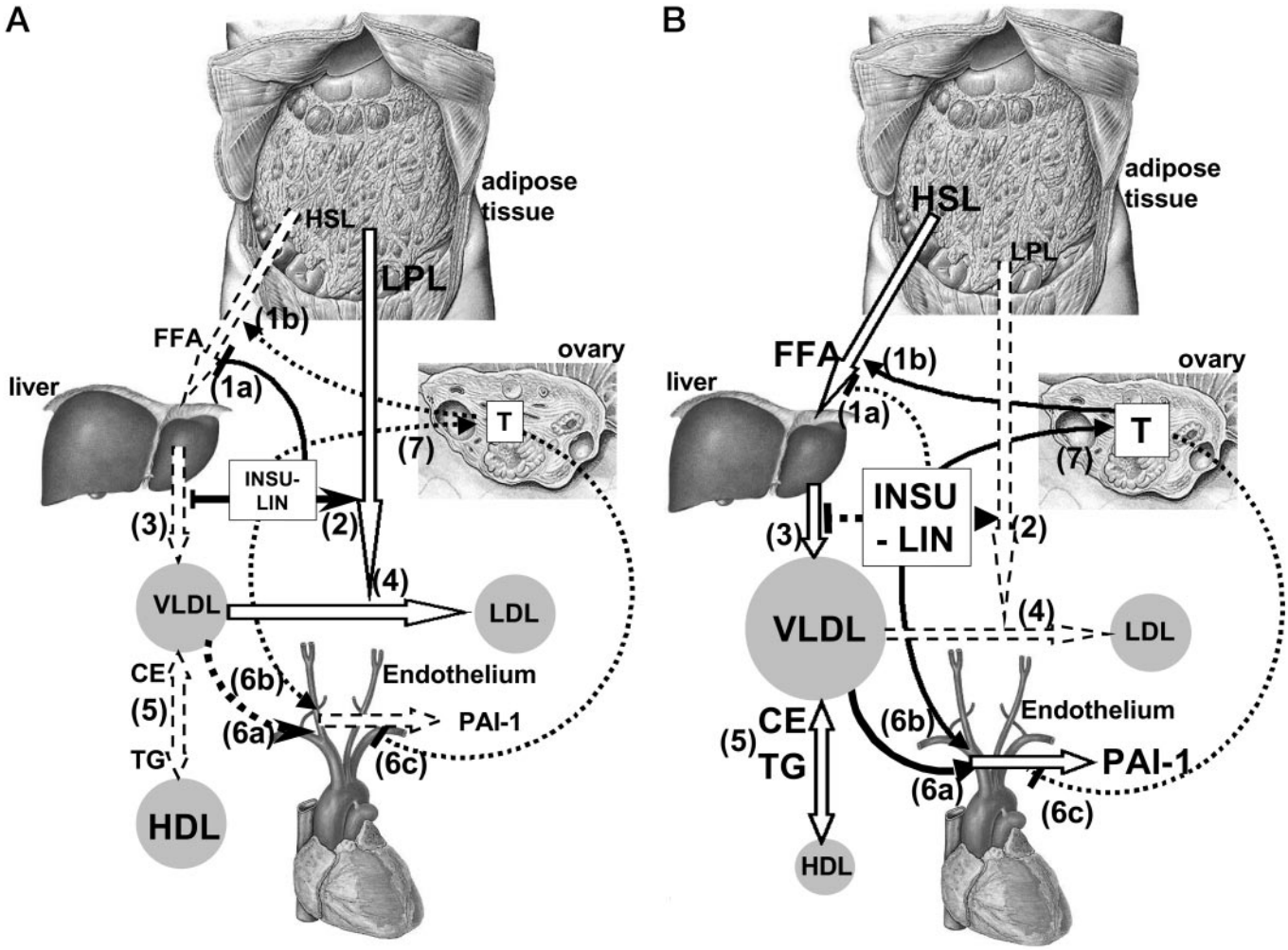


FIG. 3. Model of metabolic effects of T in women. In nonobese women with normal insulin sensitivity (A), adipocytes release limited amounts of FFA (1) and regular amounts of lipoprotein lipase (LPL; 2). VLDLs are secreted at regular amounts by the liver (3) and properly hydrolyzed by LPL (4). Normotriglyceridemia is associated with a low exchange of triglycerides and CE between HDL and VLDL (5) so that HDL-C levels stay normal. Normotriglyceridemia (6a) and low insulin levels (6b) inhibit the release of PAI-1 from endothelial cells. Low insulin levels also limit the ovarian production of T (7). Low T levels support insulin in suppressing FFA release from adipocytes (1b), and thereby hepatic VLDL production, as well as in inhibiting PAI-1 release from endothelial cells (6c). Obese women, by contrast (B), have insulin resistance and hyperinsulinemia. In the adipose tissue, insulin resistance increases the production of FFA (1) and inhibits LPL secretion (2). Enhanced hepatic VLDL secretion (3) and low LPL activity (4) cause hypertriglyceridemia and, indirectly via enhanced CE/triglycerides exchange between VLDL and HDL (5), low HDL-C. Hypertriglyceridemia (6a) and hyperinsulinemia (6b) both stimulate PAI-1 secretion from endothelial cells. Hyperinsulinemia also stimulates the production of T in the ovary (7). Hyperandrogenemia then aggravates the detrimental effects of insulin resistance on FFA release from adipocytes (1b) and thereby on hepatic VLDL production.

*C. Effects of exogenous T on cardiovascular risk factors*

In clinical studies, the effects of exogenous T on cardiovascular risk factors differed considerably depending on the dose, route of administration, and duration of treatment, as well as the age, gender, and conditions of the recipients (Table 4). The most consistent findings were decreases in plasma levels of HDL-C, lipoprotein(a) [Lp(a)] and fibrinogen, which are accompanied by much less prominent declines of LDL-C and triglycerides.

*1. HDL-C.* Administration of AASs to either men or women were consistently found to cause substantial reductions of HDL-C (184–188), which, in the extreme, leads to the virtual absence of circulating HDL. Likewise, administration of supraphysiological dosages of T to healthy eugonadal men in contraceptive studies (201–205), especially when combined with synthetic progestins (206–210), as well as treatment of women with premenstrual syndrome or hormone replacement therapy for postmenopausal women with regimens that contain either T or androgenic steroids, led to a decrease in HDL-C (211–215). Castration or suppression of endogenous T in patients with prostate cancer or treatment with GnRH antagonists in experimental studies was found to increase HDL-C by about 20% (152, 216–223). The effect of GnRH antagonists can be prevented by coadministration of T (224). Taken together, these data indicate that T exerts profound effects on HDL metabolism. These effects are most marked on the large HDL subclass (*i.e.*, $HDL_2$), which is devoid of apoA-II (*i.e.*, LpA-I; Refs. 152, 219, and 225–227).

Substitution of T in hypogonadal men or in elderly men

with low to normal T or elevated gonadotropins led to minor or no decrease in HDL-C (Table 4 and Refs. 148, 200, 202–204, 221, 225, 226, and 228–256). In a recent meta-analysis of 19 studies published between 1987 and 1999, Whitsel *et al.* (257) calculated that im administration of an average dosage of 179 ± 13 mg of T ester every 16 ± 1 d for 6 ± 1 months was associated with a decrease of 2–5 mg/dl HDL-C. The older the treated men and the longer the treatment, this decrease of HDL-C appeared to become less prominent. T substitution for up to 3 yr in men over the age of 50 yr did not produce any consistent changes in circulating lipid levels (247, 258). Moreover, an international multicenter male contraception study found a significant decrease in HDL-C in non-Chinese but not in Chinese volunteers (259). Transdermal application of T or dihydrotestosterone also exerted less effect on HDL-C than im application (237, 248–250).

Lowering of HDL-C by T is considered to increase cardiovascular risks because HDL-C exerts several potentially antiatherogenic actions. However, in transgenic animal models, only increases of HDL-C induced by apoA-I overproduction, but not by inhibition of HDL catabolism, were consistently found to prevent atherosclerosis (260). Therefore, the mechanism of HDL modification and, by inference, changes in metabolism of HDL-C rather than changes in levels of HDL-C *per se* appear to determine the (anti-)atherogenicity of HDL (260, 261). Unfortunately, the mechanism and target genes by which T regulates HDL metabolism are not well understood at present. Figure 4 summarizes important steps in HDL metabolism. The production rate of HDL is determined by the hepatic and, to lesser degree, intestinal synthesis of apoA-1, the main protein constituent of HDL (262). The effects of T on apoA-1 production in man are not known. In mice, T increases the synthesis of apoA-1 (263), which at first sight is counter to the HDL-lowering effect of exogenous T. However, in mice, as opposed to man, HDL-C is increased by T and decreased by estradiol (121). At the catabolic site, two genes are likely to be regulated by T, namely hepatic lipase (HL) and scavenger receptor B1 (SR-B1). Regulated by corticotropin and gonadotropins, SR-B1 mediates the selective uptake of HDL lipids into hepatocytes and steroidogenic cells including Sertoli and Leydig cells of the testes as well as cholesterol efflux from peripheral cells including macrophages (260, 264). T up-regulates SR-B1 in the human hepatocyte cell line HepG2 and in macrophages and thereby stimulates selective cholesterol uptake and cholesterol efflux, respectively (264a). HL hydrolyzes phospholipids on the surface of HDL, thereby facilitating the selective uptake of HDL core lipids by SR-B1 (260, 227). The activity of HL in postheparin plasma is increased after administration of exogenous T (225, 226, 229, 238, 265) and slightly decreased by suppression of T after GnRH antagonist treatment (152). However, castration of male rats did not cause significant changes in postheparin plasma activity of HL or in HL mRNA levels in the liver. Subsequent substitution of T raised HL activity without changing HL mRNA expression (266). This raises the possibility that T does not directly regulate the HL gene. In agreement with this, we did not observe any change of HL activity in the supernatants of HepG2 cells that were incubated with T (264a). The increase in both SR-B1 and HL activities is consistent with the HDL-lowering effect of T. Up-regulation of HL and SR-BI also explains why T induces the most prominent changes in HDL subclasses $HDL_2$ and LpA-I, because these particles are preferred substrates of HL and SR-BI over small $HDL_3$ and apoA-II-containing HDL. Interestingly, in transgenic mice, overexpression of HL caused a dramatic fall in HDL-C but inhibited rather than enhanced atherosclerosis (260, 264, 227). This again demonstrates the difficulty in extrapolating the HDL-lowering effect of T to increased cardiovascular risk.

*2. Lp(a).* Lp(a) has striking structural homology to plasminogen but no fibrinolytic activity. Lp(a) resembles LDL because of the presence of one molecule, apoB-100, and by its high content of cholesteryl esters. Lp(a) differs from LDL by the disulfide bridge binding of apoB to a glycoprotein termed apo(a). Lp(a) levels vary considerably in the population between 0 and 300 mg/dl with a frequency distribution that is skewed to lower concentrations. Most of the interindividual variability in Lp(a) levels is determined through variation in the apo(a) gene (267). Of special importance is a size polymorphism. A genetically determined variable number of kringle-IV-repeats within apo(a) is inversely correlated with Lp(a) levels. Results of many case-control studies and most prospective population studies demonstrated that Lp(a) levels higher than 30 mg/dl are an independent risk factor for coronary, cerebrovascular, and peripheral atherosclerotic vessel diseases, especially if the high Lp(a) level coexists with other cardiovascular risk factors (268, 269). Interestingly, in some previous studies, elevated Lp(a) was also found to increase the risk for venous thromboembolic disease, habitual abortion, and preeclampsia, especially if coinciding with other thrombophilic risk factors (270–272).

Lp(a) levels are generally assumed to remain stable throughout life. However, estrogens, progestins, GH, and $T_4$ can lower Lp(a) levels (273, 274). Likewise, administration of R to orchidectomized patients with prostate cancer (275, 276), as well as administration of supraphysiological doses of T enanthate to healthy men, decreased serum levels of Lp(a) significantly by 25–59% (203, 218, 236, 252, 277). Lp(a) levels were increased by 40–60% in controls and in patients in whom endogenous T was suppressed by treatment with the GnRH antagonist cetrorelix or the GnRH agonist buserelin (219, 275, 276, 278, 279). The Lp(a)-lowering effect of T is independent of estradiol because Lp(a) levels were also lowered when T was administered in combination with an aromatase inhibitor, testolactone (277). Animal studies have also provided evidence for the involvement of T in the regulation of Lp(a) levels. Frazer *et al.* (280) observed that Lp(a) levels decrease in male, but not in female, apo(a)-transgenic mice after sexual maturation. Castration of male animals restored initial Lp(a) levels, which decreased again upon application of dihydrotestosterone. However, it is also important to note that, similar to the changes observed in HDL-C, treatment of hypogonadal men with physiological dosages of T did not cause large changes in Lp(a) levels (Table 4).

It is not known how T regulates Lp(a). Turnover studies have shown that Lp(a) levels are mainly determined by production. The majority of newly synthesized apo(a) is degraded intracellularly before secretion. The larger the apo(a)

TABLE 4. Change in lipids in hypogonadal men receiving T replacement (A) and change in lipids in eugonadal men receiving T treatment (B)

| First author/year (Ref.) | Study design | Patients | Mode of treatment | Duration | Δ LDL-C | Δ HDL-C | Δ TG | Δ Other risk factors |
|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | |
| Valdemarsson, 1987 (228) | Open label | 10 hypogonadal men | 250 mg TE im/3 or 4 wk | 9 months | −6% | 0 | −14% | |
| Kirkland, 1987 (200) | Open label | 14 hypogonadal boys | 100 or 200 mg TE im/month | 3 months | n.d. | −14% | n.d. | |
| Sorva, 1988 (229) | Open label | 13 hypophysectomized men | 100 mg TE im/2 wk | 1 month | −4% | −8% | −11% | apoA-I: −11% |
| Jones, 1989 (230) | Open label | 10 Klinefelter men | TE implant im | 4 wk | +19% | −11% | −4% | apoA-I: +8% |
| Hromadova, 1989 (251) | Open label | 30 sterile men | 100 mg methyltestosterone/d | 30 d | +?? | −??% | +??% | |
| Hana, 1991 (234) | Open label | 9 hypogonadal men | 100 mg TI im/wk | 24 months | 24%* | +19% | −30% | |
| Tenover, 1992 (235) | Placebo-controlled | 13 hypogonadal elderly men | 100 mg TE im/wk | 3 months | −15%* | −4% | −16% | apoA-I: −11% |
| Bhasin, 1992 (246) | Open label | 10 hypogonadal men | Microcapsulated T im | 12 wk | −7% | −16% | +14% | apoA-I: −24%<br>apoB: −14% |
| Morley, 1993 (239) | Open label | 8 hypogonadal elderly men | 200 mg TE im/2 wk | 3 months | n.d. | 0 | n.d. | |
| Salehian, 1995 (244) | Randomized | 63 hypogonadal men | 200 mg TE im/3 wk or 2.5 mg T sublingual/d or 5 mg T sublingual/d | 2 months | n.d. | 10%<br>−8%<br>−10$ | n.d. | |
| Brodsky, 1996 (253) | Open label | 5 hypogonadal men | 3 mg TC per kg body mass im every 2 wk | 6 months | +5% | −18% | +3% | |
| Katznelson, 1996 (254) | Open label | 29 hypogonadal men | 100 mg TE or TC im/wk | 6 months | −6% | −10% | −20% | |
| Ozata, 1996 (252) | Open label | 9 hypogonadal men | 250 mg TE im/3 wk | 3 months | +9% | +6% | 0 | |
| Zgliczynski, 1996 (240) | Open label | 22 hypogonadal men | 200 mg TE im/2 wk | 1 yr | −18%[a] | −9%[b] | −20% | |
| Arslanian, 1997 (255) | Open label | 7 boys with delayed puberty | 50 mg TE im/2 wk | 4 wk | −14% | −20%[c] | −9% | |
| Tripathy, 1998 (241) | Open label | 10 hypogonadal men | 200 mg TE im/wk | 12 wk | −41% | +5% | −25% | |
| Tan, 1998 (225) | Open label | 11 hypogonadal men | 250 mg TE im/4 wk | 12 wk | −2% | −1% | −12% | apoA-I: −10%[b]<br>Lp(a): −2% |
| Rabijewski, 1998 (256) | Open label | 30 hypogonadal men | 200 mg TE im/2 wk | 12 months | −16% | −4% | n.d. | |

TABLE 4. *Continued*

| First author/year (Ref.) | Study design | Patients | Mode of treatment | Duration | Δ LDL-C | Δ HDL-C | Δ TG | Δ Other risk factors |
|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | |
| Jockenhövel, 1999[d] (232) | Open label | 12 hypogonadal men<br>13 hypogonadal men<br>15 hypogonadal men<br>15 hypogonadal men | 100 mg mesterolone po/d<br>or 160 mg TU po/d<br>or 250 mg TE im/3 wk<br>or 1200 mg testosterone sc/d | 17 wk | −6%<br>+9%<br>+6%<br>−9% | −6%<br>−16%<br>−8%<br>−11% | −12%<br>−8%<br>+5%<br>0 | |
| Tan, 1999 (226) | Open label | 10 hypogonadal men | 4 mg transdermal T/d | 3 months | −10% | −11%[c] | +3% | apoA-I: −7% |
| Wang, 2000 (148) | Randomized | 227 hypogonadal men | 6 mg T/d scrotal patch *vs.* gel with 50 mg or 100 mg T/d | 180 d | n.s. | n.s. | n.s. | |
| Snyder, 2000 (237) | Placebo-controlled double-blind | 108 hypogonadal elderly men | 6 mg transdermal (scrotal) T/d | 3 yr | 0 | −2% | −3% | apoA-I: −3%<br>apoB: −5% |
| Howell, 2001 (248) | Placebo-controlled | 35 hypogonadal men | 2.5 mg transdermal T/d | 1 yr | −11%[b] | 0 | +26% | |
| Dobs, 2001 (249) | Open label | 20 hypogonadal men | 2–2.5 mg transdermal T/d | 1 yr | −3% | −9%[c] | +16%[b] | |
| Ly, 2001 (250) | Placebo-controlled | 33 hypogonadal men | 70 mg transdermal DHT/d | 3 months | −11%[b] | 0 | −10% | |
| B | | | | | | | | |
| Thompson, 1989 (231) | Cross-over | 11 eugonadal men | 200 mg TE im/wk | 6 wk | −16%[b] | −9%[b] | +13% | |
| Friedl, 1990 (233) | Randomized | 18 eugonadal men | 280 mg TE im/wk without<br>or with 250 mg testolactone/d or 20 mg methyltestosterone | 12 wk | n.d. | −4%<br>−16%<br>−33% | +10%<br>+20%<br>+40% | apoA-I: −12%<br>0<br>−40% |
| Zmuda, 1993 (238) | Cross-over | 14 eugonadal men | 200 mg TE im/wk,<br>250 mg testolactone per day,<br>or both | 3 wk | +2%<br>−3%<br>−1% | −15%[b]<br>−4%<br>−20%[b] | +17%<br>−13%<br>+3% | |
| Bagatell, 1994 (202) | Open label | 19 eugonadal men | 200 mg TE im/wk | 20 wk | −6%[b] | −15%[b] | +5% | apoA-I: −8% |
| Meriggiola, 1995 (204) | Open label | 36 eugonadal men | 200 mg TE im/wk | 1 yr | −8% | −16% | n.d. | |
| Anderson, 1995 (203) | Open label | 63 eugonadal men | 200 mg TE im/wk | 1 yr | n.s. | −13%[a] | n.s. | |
| Wu, 1996 (259) | Open label | 189 non-Chinese men<br>82 Chinese men | 200 mg TE im/wk<br>200 mg TE im/wk | 1 yr<br>1 yr | n.s. | −14%[a]<br>−2% | +10%<br>(all) | |
| Marcovina, 1996 (236) | Open label | 19 eugonadal men | 200 mg TE im/wk | 20 wk | −8% | −14%[b] | +13% | Lp(a): −22%[c] |
| Grinspoon, 2000 (242) | Placebo-controlled | 54 men with AIDS | 200 mg TE im/wk | 12 wk | −6% | −8%[b] | −25% | |
| Bhasin, 2001 (243) | Randomized | 61 eugonadal men with suppressed T | 25, 50, 125, 300, or 600 mg TE im/wk | 20 wk | n.d. | Dose-dependent + 10–20%[a] | n.d. | |
| Uyanik, 1997 (245) | Placebo-controlled | 37 eugonadal men | 120 mg TU po per day | 2 months | −25%[c] | +3% | −4% | apoA-I: −15%<br>apoB: +12% |

Δ LDL-C, Change in plasma LDL-C from pretreatment baseline; Δ HDL-C, change in plasma HDL-C from pretreatment baseline; Δ TG, change in plasma triglycerides from pretreatment baseline; TE, T enanthate; TC, T cypionate; TU, T undecanoate; DHT, dihydrotestosterone; TI, T isobutyrate; n.d., not done.

[a] $P < 0.001$.

[b] $P < 0.05$.

[c] $P < 0.01$.

[d] Changes were compared to follow-up; baseline (screening) results are implausibly extreme and different from follow-up.

isoforms, the more they are degraded intracellularly and, hence, the less is secreted. Interestingly, estradiol decreases Lp(a) production, but it is not known whether estradiol regulates the transcription of the apo(a) gene or the posttranslational processing of apo(a) (267, 281).

It is also not known whether changes in Lp(a) induced by T will affect cardiovascular risk. Interestingly, however, in the Heart and Estrogen/Progestin Replacement Study (HERS; Ref. 282), postmenopausal hormone replacement therapy prevented coronary events only in those women who had elevated Lp(a) at baseline and experienced a decrease of Lp(a) levels by treatment with conjugated equine estradiol and medroxyprogesterone.

*3. The hemostatic system.* In agreement with an important role of thrombus formation in the pathogenesis of acute coronary events and stroke, prospective studies have identified various hemostatic variables as cardiovascular risk factors (283). The risk of MI increases with plasma levels of the thrombogenic factors fibrinogen and factor VII, as well as with plasma levels of the fibrinolysis inhibitor PAI-1 or tissue plasminogen activator antigen, which represents the inactivated form (283). Platelet aggregability is another important factor that determines thrombogenicity and, thereby, cardiovascular risk. T was shown to regulate plasma levels of fibrinogen and PAI-1. Administration of supraphysiological dosages of T to 32 healthy men participating in a trial of male contraception led to a sustained decrease of fibrinogen by 15–20% over 52 wk of treatment (284). In this study the doubling of T levels initially also led to significant decreases of PAI-1, protein S, and protein C, as well as to increases of antithrombin III and β-thromboglobulin. Likewise, PAI-1 was decreased in men who received the anabolic androgen stanozolol (285). Suppression of T in patients with prostate cancer or benign prostate hypertrophy, however, by treatment with the nonsteroidal antiandrogen casodex or the GnRH agonist leuprolide exerted no significant effects on plasma fibrinogen levels (220, 221). In women, treatment of endometriosis with the weak androgen danozolol, as well as postmenopausal hormone replacement therapy with tibolone, led to significant decreases of fibrinogen and PAI-1 levels (286–288). In

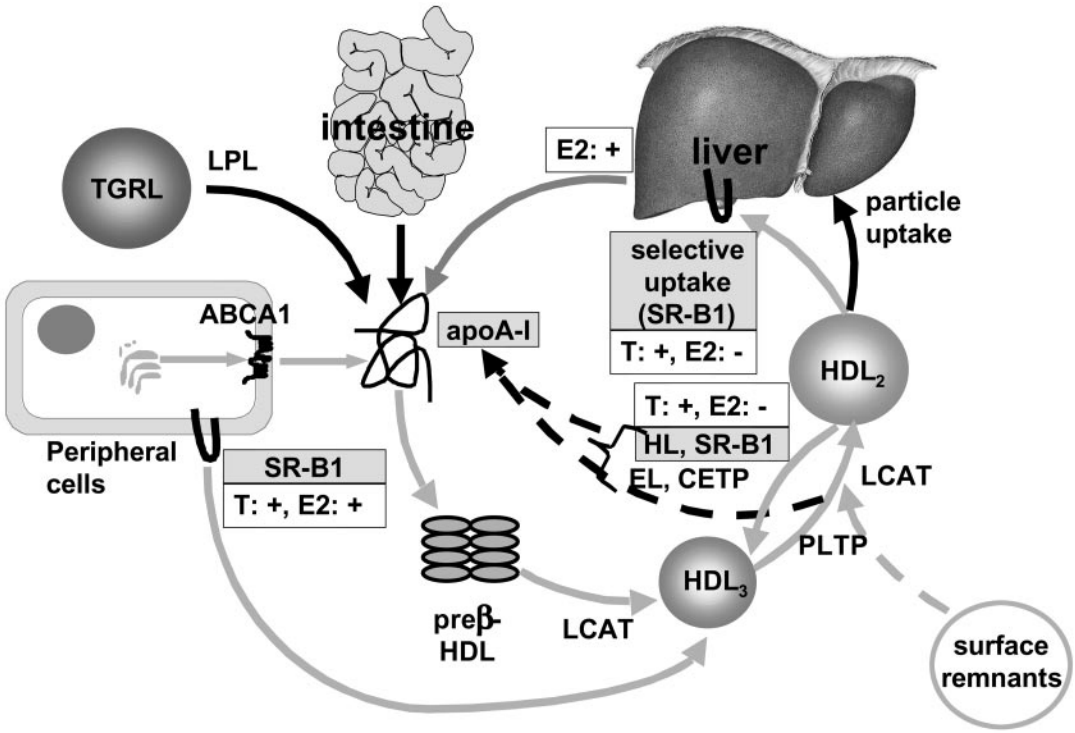


FIG. 4. Pathways of HDL metabolism and regulation by T and estradiol (E2). Mature $HDL_3$ and $HDL_2$ are generated from lipid-free apoA-I or lipid-poor pre$\beta_1$-HDL as the precursors. These precursors are produced as nascent HDL by the liver or intestine or are released from lipolyzed VLDL and chlyomicrons, or by interconversion of $HDL_3$ and $HDL_2$. ATP-binding cassette 1 (ABCA1)-mediated lipid efflux from cells is important for initial lipidation; lecithin cholesterol acyl transferase (LCAT)-mediated esterification of cholesterol generates spherical particles, which continue to grow upon ongoing cholesterol esterification, and phospholipid transfer protein (PLTP)-mediated particle fusion and surface remnant transfer. These mature HDL particles also continue to accept cellular cholesterol by processes that are facilitated by SR-BI and LCAT. Larger $HDL_2$ are converted into smaller $HDL_3$ upon CETP-mediated export of CEs from HDL onto apoB-containing lipoproteins, SR-B1-mediated selective uptake of CEs into liver and steroidogenic organs, and HL-mediated hydrolysis of phospholipids. HDL lipids are catabolized either separately from HDL proteins, *i.e.*, by selective uptake or via CETP-transfer, or together with HDL proteins, *i.e.*, via uptake through as-yet unknown HDL receptors or apoE receptors. Both the conversion of $HDL_2$ into $HDL_3$ and the PLTP-mediated conversion of $HDL_3$ into $HDL_2$ liberate lipid-free or poorly lipidated apoA-I, which is either reused for the formation of mature HDL or is filtrated into the kidney. *Gray arrows* represent lipid transfer processes, and *black arrows* represent protein transfer processes. The hepatic expression and activity of both HL and SR-B1 was shown to be up-regulated by T and down-regulated by estradiol. In addition estradiol up-regulates the hepatic expression and secretion of apoA-I. These actions of T and estradiol are in good agreement with their lowering and increasing effect on HDL-C, respectively. In addition, both T and estradiol stimulate SR-BI expression in macrophages and thereby cholesterol efflux from these cells onto lipidated HDL. [Modified from Refs. 260 and 261.]

agreement with the lowering effects of T on PAI-1, T inhibited the secretion of PAI-1 from bovine aortic ECs *in vitro* (289). Taken together, the current data indicate that T lowers fibrinogen and PAI-1. However, these anticoagulatory and profibrinolytic effects may be opposed by proaggregatory effects on platelets because high dosages of androgens were found to decrease cyclooxygenase activity and thereby increase platelet aggregability (288, 290).

In conclusion, exogenous T exerts significant dose-dependent effects on several risk factors, some of which at first sight appear beneficial, namely lowering of Lp(a), insulin, fibrinogen, and PAI-1, whereas lowering of HDL-C is considered adverse. The metabolic effects of T are very prominent if supraphysiological dosages or synthetic androgens are used but appear to be rather subtle in the setting of hormone replacement of hypogonadal men. It is also important to emphasize that the mechanism by which T reduces circulating HDL-C may actually confer protection from, rather than promotion of, atherosclerosis. Given that several moderately expressed risk factors interact with one another in a nonlinear fashion, it is difficult to predict the net effect of exogenous T on an individual's cardiovascular risk, even by the use of algorithms or scoring systems that take into consideration multiple risk factors simultaneously (291, 292).

## VII. Effects of T on Cells of the Arterial Wall and Vascular Function

Atherosclerosis is a chronic process developing over decades. It is initiated by an injury to the endothelium via physical (shear) stress and exposure to atherogenic lipids and toxins, such as those contained in tobacco smoke, or infectious agents (293–295). The dysfunctional endothelium is impaired in its abilities to serve as a barrier against atherogenic lipoproteins, to regulate vascular tone by the production of nitric oxide (NO) and other vasoactive molecules, and to prevent thrombosis. Activated ECs also express selectins, adhesion molecules, and integrins to which circulating monocytes and T lymphocytes bind before they transmigrate into the subendothelial space. There, the monocytes differentiate to macrophages and ingest lipoproteins that have permeated the endothelium and become modified within the arterial wall, by oxidation, for example. The uptake of modified lipoproteins (oxidized LDL in particular) by macrophages leads to the formation of large foam cells. These, together with T lymphocytes, release inflammatory mediators that stimulate the proliferation and migration of SMCs. All three cell types together form the so-called fatty streak. All stages of lesion formation up to fatty streak development are probably reversible, especially with lipid-lowering drugs. If the fatty streak does not regress, however, it progresses to the incompletely reversible fibrofatty lesion and, ultimately, to the fibrous or cell-rich full-blown atherosclerotic plaque. In this complicated lesion, activated macrophages, macrophage-derived foam cells, T lymphocytes, and mast cells surround a necrotic and sometimes partially calcified lipid-rich core. These lesions can narrow the lumen of the coronary artery and thereby interfere with myocardial perfusion. Clinically, these lesions will cause stable angina pectoris. Some of these plaques have a very thin fibrous cap and can rupture and expose tissue factor, collagen, and lipids to the circulating blood. This local procoagulant surface will induce platelet aggregation and intravascular coagulation. The thrombi thus formed may become incorporated into the plaque, or may disseminate to occlude the vessel, resulting in infarction of the downstream myocardium (293–295).

Stimulated by the gender difference in risk for premature atherosclerosis, research on effects of sex hormones on vascular function and vascular cell biology has only started recently. Efforts so far however have focused predominantly on estrogens on the premise that they are cardioprotective. (11, 296, 297).

### *A. Vascular expression of sex hormone receptors and T converting enzymes*

A direct genomic effect of T on vascular function requires the expression of the androgen receptor in vascular cells. Receptor binding assays, *in situ* hybridization, RT-PCR, and immunohistochemical studies have demonstrated androgen receptor gene expression in the arterial wall of rabbits, dogs, monkeys, and men (298, 299), as well as in cultivated vascular SMCs, ECs, macrophages, megakaryocytes, and platelets (Fig. 5 and Refs. 14 and 300–302). Interestingly, human monocyte-derived macrophages were found to express the androgen receptor in a gender-specific manner. Macrophages of male donors exhibit a 4-fold higher expression of the androgen receptor than macrophages of female donors (14). In isolated rings of de-endothelialized rabbit aorta, T was found to stimulate the expression of the androgen receptor and to inhibit neointimal plaque formation, indicating autoregulatory effects of T (299).

T may also exert vascular effects indirectly, *i.e.*, after conversion to estradiol. In agreement with this concept, vascular ECs and SMCs as well as macrophages and platelets were found to express aromatase and 17$\beta$-hydroxysteroid dehydrogenase (303–305), so that estradiol can be produced locally within the arterial wall from circulating precursors. Indeed, labeling experiments confirmed that vascular ECs and SMCs can synthesize estradiol from both T and DHEA (303, 304, 306). ER$\alpha$, ER$\beta$, as well as a membrane ER are expressed in EC, SMC, macrophages, and platelets as well as coronary arteries of monkey and man (307–317). The extraglandular and intravascular production of estrogens (from local aromatization of circulating androgens) may therefore play a role in male cardiovascular physiology and pathophysiology (see *Section IX*).

There is increasing evidence that steroid hormones including T are also ligands of plasma membrane steroid receptors and can modulate cell membrane channels, *e.g.*, ATP-sensitive, voltage-dependent, and calcium-activated potassium channels (318, 319, 320). In particular, the effects of supraphysiological doses of T on vasoreactivity have been assigned to nongenomic modes of actions. Finally, T was shown to regulate macrophage function by nongenomic effects via a G protein-coupled, agonist-sequesterable plasma membrane receptor that initiates calcium- and 1,4,5-trisphosphate-signaling pathways (321, 322).

In summary, vascular cells contain steroid hormone re-

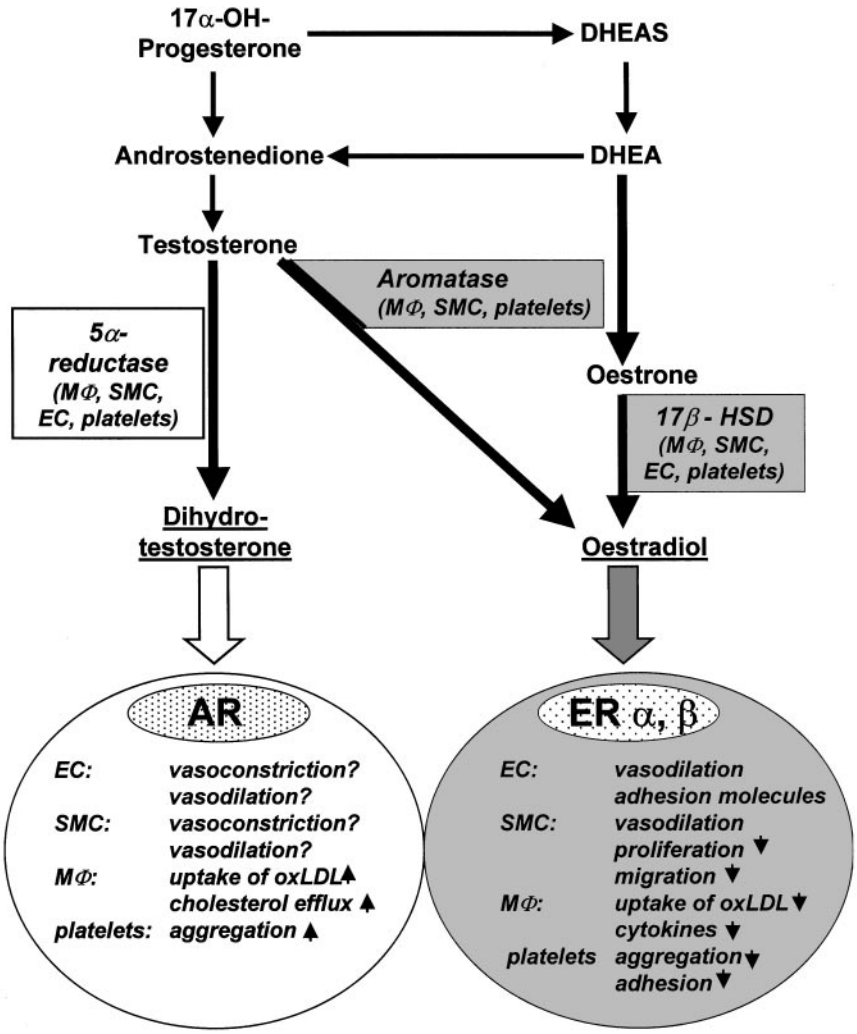


FIG. 5. Metabolism and modes of action of sex steroids in vascular cells. In various cells of the vascular wall, T can exert direct effects either by activation of the androgen receptor (AR) or by nongenomic effects on plasma membrane receptors and channels. However, the expression of aromatase and 17β-hydroxysteroid dehydrogenase in SMCs, ECs, and macrophages (MΦ) opens the possibility of local conversion of T (and DHEA) into estradiol. Both the classic ERα and the alternative ERβ are expressed by various vascular cells, so that T can also modulate vascular physiology indirectly via local estradiol production.

ceptors and converting enzymes needed for the genomic effects of T and estradiol as well as for the local production of estradiol. T can therefore regulate vascular physiology and contribute to the pathogenesis of atherosclerosis both directly and indirectly via estradiol. Furthermore, the vascular effects of supraphysiological doses of T appear to be mediated independently of nuclear sex hormone receptors.

### *B. Effects of T on vascular reactivity*

An early hallmark of atherosclerosis is decreased vascular responsiveness to various hormonal stimuli either due to endothelial dysfunction or due to endothelium-independent disturbances in vascular SMC physiology. As a result, decreased vasodilation and enhanced vasoconstriction can lead to vasospasm and angina pectoris. Moreover, endothelial dysfunction also contributes to coronary events by promoting plaque rupture and thrombosis (323–326). In contrast to the many clinical studies and experimental studies documenting the protective effects of estrogens on vascular function (for reviews, see Refs. 11, 274, 295, 314, and 327–331), relatively little and contradictory data are available on the effects of T on vascular reactivity. T can induce vasodilation or vasoconstriction via endothelium-dependent or endothelium-independent mechanisms and by genomic or nongenomic modes of action (Table 5). The diversity of these findings appears to be due to differences in species, gender, concomitant disease, and, most importantly, dosage of T.

In two case-control studies (Table 5), male-to-female transsexuals receiving high-dose estrogens had greater endothelium-dependent vasodilation than male controls (332, 333). However, these studies may have monitored the effects of estradiol application rather than of T removal. Indicative of an adverse effect of T, nitrate-induced and, hence, endothelium-independent dilation of the brachial arteries was significantly reduced in female-to-male transsexuals taking high-dose androgens (334). Moreover, in another case-control study, patients with prostate cancer, who were deprived of endogenous androgens either surgically or pharmacologically, had a greater flow-induced (*i.e.*, endothelium-dependent) dilation of brachial arteries than controls, who were healthy men or men with nonprostate cancers. The endothelium-independent vasodilation by nitroglycerin did not differ between the groups (335). In a group of 110 healthy men, we have observed a positive association between the number of CAG repeats in exon 1 of the androgen receptor gene and endothelium-dependent as well as endothelial-

TABLE 5. Effects of testosterone on vasoreactivity

| First author, year (Ref.) | Species | Artery | Study type | Dose[a] | Effect | Role of endothelium | Vasodilation |
|---|---|---|---|---|---|---|---|
| McCrohon, 1997 (332) | Human (M-to-F transsexual) | Brachial | Case-control | 0 | ? | Dependent | Increased |
| Herman, 1997 (335) | Human (M castrated) | Brachial | Case-control | 0 | ? | Dependent | Increased |
| New, 1997 (333) | Human (F-to-M transsexual) | Brachial | Case-control | nmol | ? | Dependent & independent | Decreased |
| McCredie, 1998 (334) | Human (F-to-M transsexual) | Brachial | Case-control | nmol | ? | Dependent & independent | Decreased |
| Webb, 1999 (103) | Human (M) | Coronary | Intervention | nmol | Direct | Independent | Increased |
| Rosano, 1999 (104) | Human (M) | Coronary | Intervention | μmol<br>nmol | Direct | ? | Increased<br>No effect |
| Ong, 2000 (337) | Human (M) | Brachial | Intervention | μmol<br>nmol | Direct | Dependent | Increased<br>No effect |
| Zitzmann, 2001 (336)[b] | Human (M) | Brachial | Association | Endo | ? | Dependent & independent | Decreased |
| Warboys, 2001 (338) | Human (F) | Brachial | Intervention | nmol | Direct | Dependent & independent | Increased |
| Adams, 1995 (116) | Monkey (F) | Coronary | *In vivo* | nmol | Direct | Dependent | Increased |
| Chou, 1996 (339) | Dog (M & F) | Coronary | *In vivo* | μmol | Direct | Dependent | Increased |
| Farhat, 1995 (341) | Pig (M & F) | Coronary | *Ex vivo* | μmol | Direct | Dependent & independent | Increased |
| Quan, 1999 (344) | Pig (M & F) | Coronary | *Ex vivo* | μmol | Direct | Independent | Increased |
| Teoh, 2000 (343) | | | | nmol | Indirect | | Decreased |
| Yue, 1995 (342) | Rabbit (M & F) | Coronary | *Ex vivo* | μmol | Direct | Dependent & independent | Increased |
| Ceballos, 1999 (345) | Rat | Coronary | *Ex vivo* | nmol | Indirect | Dependent | Decreased |
| Costarella, 1996 (340) | Rat (M) | Aorta | *Ex vivo* | μmol | Direct | Dependent & independent | Increased |
| Hutchison, 1997 (346) | Rabbit | Aorta | *Ex vivo* | nmol | Indirect | Dependent | Decreased |
| Geary, 2000 (347) | Rat (M) | Cerebral | *Ex vivo* | μmol | Direct | Dependent | Increased |

M, Male; F, female.
[a] Change in serum levels: 0, castration; endo, endogenous T level; nmol, nanomolar; μmol, micromolar.
[b] Association with CAG repeat polymorphism in the androgen receptor, *i.e.*, T sensitivity.

independent vasodilatation. Thus, the greater the sensitivity to T, the less brachial arteries dilate in response to either flow or nitrate (336).

In contrast to these case-control or cross-sectional studies, acute interventional studies with iv administration of T to male patients with CAD revealed apparently beneficial vasodilatory effects of T (Refs. 103, 104, and 337 and Table 5; also see *Section IV.C*). However, the extremely high doses employed question the specificity and physiological relevance of these findings. In postmenopausal women, T plus estradiol improved endothelial-dependent (flow-mediated) and nonendothelial-dependent (GTN) brachial artery vasodilatation for 6 wk in an uncontrolled study (338).

*In vivo* studies in monkeys and dogs of both sexes as well as most *in vitro* studies with animal vessels suggest that T exerts beneficial effects on vascular reactivity. After T treatment for 2 yr in ovariectomized female cynomolgus monkeys, intracoronary injections of acetylcholine caused significant endothelium-dependent vasodilation in treated but not in untreated animals. In contrast, endothelium-independent vasodilation in response to nitroglycerin occurred normally in both groups (116). In dogs, T induced vasodilation of coronary arteries by endothelium-dependent and -independent mechanisms (339). *In vitro* studies with isolated rings of coronary arteries and/or aortas from rats, rabbits, and pigs also found that, in both sexes, T improved both endothelium-dependent and/or endothelium-independent vascular responsiveness (340–342). Again, it must be emphasized that all these studies employed supraphysiological to pharmacological doses of T in the micromolar range. Teoh *et al.* (343) observed a direct vasodilatory effect of T on porcine coronary artery rings at micromolar concentrations but no direct effect at nanomolar dosages. In contrast, a physiological dose of T inhibited the vasodilatory effects of bradykinin and calcium ionophores (343, 344). Similarly, T inhibited the adenosine-mediated vasodilation of rat coronary arteries (345) and impaired endothelium-dependent relaxation of aortic rings from rabbits that were either made hypercholesterolemic or exposed to tobacco smoke (346).

The cellular and molecular mechanisms by which T regulates vascular tone are not well understood. Evidence for and against endothelium-dependent or endothelium-independent mechanisms has been found (Table 5). Results of some studies suggest the involvement of endothelial NO (116, 318, 339–341, 347). In dog coronary arteries, rat aorta, and rat cerebral arteries, the NO synthase inhibitor $N^G$-monomethyl-L-arginine prevented T-induced vasodilation (339, 340, 347). However, in another *in vitro* study, $N^G$-monomethyl-L-arginine had no effect on T-induced vasodilation of rabbit aortas and coronary arteries (342). In agreement with the latter, *in vitro* expression of NO synthase in human aortic ECs was stimulated by estradiol but not by T (348). The involvement of prostaglandins is suggested by the observation that T increases the response of coronary arteries to prostaglandin $F_{2\alpha}$ (341, 349) and by the finding that dihydrotestosterone increases the density of thromboxane receptors in smooth muscle cells of rats and guinea pigs (350). However, in some *in vivo* and *in vitro* animal studies, pre-

treatment with the prostaglandin synthesis inhibitor indomethacin had no effect on T-induced vasodilation, so that the role of eicosanoids in mediating the actions of T on the arterial wall is still controversial (318, 339, 342, 343).

It is also unclear whether T regulates vasoreactivity by genomic or nongenomic effects or both. The association of endothelium-dependent and -independent vasoreactivity with the CAG repeat polymorphism in the androgen receptor provides some indirect evidence for the importance of genomic T effects on vascular ECs and SMCs (336). Moreover, T may exert its effects on vasoactivity after conversion into estradiol and activation of the ERs. However, neither the aromatase inhibitor aminoglutethimide nor the ER antagonist ICI 182,780 prevented the T-induced vasodilation (339, 342). In view of several short-term clinical studies that found vasodilatory effects of estradiol in postmenopausal women (314), this observation is surprising at first sight. However, it is in agreement with several long-term studies that did not verify the vasodilatory effect of estradiol (351–353). Two observations also indicate that T, especially in supraphysiological doses, modulates vascular tone via nongenomic modes of action. First, the androgen receptor antagonists, flutamide or cyproterone acetate, did not inhibit the effects of T on rabbit or pig coronary arteries (342, 343). Second, barium chloride attenuated the T-induced vasorelaxation of rabbit aortas and coronary arteries, indicating that T modulates the opening of potassium channels in vascular SMCs (342).

In summary, T modulates vasoreactivity by both endothelium-dependent and -independent mechanisms as well as by genomic and nongenomic modes of action. Physiological concentrations of T appear to restrict vasodilatation by activation of the androgen receptor. In contrast, supraphysiological or pharmacological doses of T seem to potentiate arterial vasodilatation through nongenomic actions.

### *C. Effects of T on macrophage functions*

Increased uptake of oxidatively modified lipoproteins via type A scavenger receptors leads to the intracellular accumulation of cholesteryl esters in macrophages and thereby to foam cell formation (293, 354–356). Estradiol inhibits oxidation of LDL both in the presence and absence of cells including macrophages (297). In contrast, T increases the oxidation of LDL by placental macrophages *in vitro* (357). Moreover, dihydrotestosterone dose-dependently stimulates the uptake of acetylated LDL by scavenger receptor type A and intracellular cholesteryl ester accumulation in macrophages (14). In addition to the higher expression of the androgen receptor in male donors, this effect was only seen in macrophages of male but not female donors. The stimulatory effect of dihydrotestosterone was blocked by the androgen receptor antagonist hydroxyflutamide (14).

After internalization, oxidized LDL is transported via endosomes to lysosomes for degradation. Cholesteryl esters are hydrolyzed by lysosomal acid lipase. The liberated cholesterol leaves the lysosome membrane to be re-esterified by the microsomal enzyme lecithin:cholesterol acyltransferase to form cholesteryl esters that can be stored in the cytosol, giving the foamy appearance of lipid-laden macrophages (356). The transport of cholesterol from lysosomes to the site of re-esterification is inhibited *in vitro* by various steroids with an oxo-group at the C17 or C20 position such as progesterone, pregnenolone, and androstenedione. 17-Hydroxysteroids including T were less effective (358). Cytosolic cholesteryl esters can be hydrolyzed by neutral cholesterol esterase (NCEH), which is activated by cAMP. In adipose tissue of female rats, NCEH is more active than in adipose tissue of male rats. Moreover, exogenous estradiol increases NCEH activity in male rats and in female rats that have been ovariectomized. *In vitro,* estradiol but not T increased the activity of NCEH in the murine macrophage cell line J774, probably by increasing the activity of a cAMP-dependent protein kinase A (297, 359).

Nonhepatic and nonsteroidogenic cells such as macrophages cannot metabolize cholesterol and, therefore, can only dispose of excess cholesterol by secretion. Hence, cholesterol efflux from cells is central to the regulation of the cellular cholesterol homeostasis. Nonspecific and passive (*i.e.*, aqueous diffusion) as well as specific and active processes (*i.e.*, receptor-mediated) are involved. To date, two plasma membrane proteins are known to facilitate cholesterol efflux (Fig. 4). Interaction of the SR-B1 with mature lipid-containing HDL is thought to facilitate cholesterol efflux by reorganizing the distribution of cholesterol within bilayer plasma membrane. The ATP binding cassette transporter A1 mediates phospholipid and cholesterol efflux to extracellular lipid-free apolipoproteins by translocating these lipids from intracellular compartments to the plasma membrane and/or by forming a pore within the plasma membrane, through which the lipids are secreted (260). We have found that T up-regulates the expression of the SR-B1 in human monocyte-derived macrophages, thereby stimulating HDL-induced cholesterol efflux. No effect of T was seen on the expression of the ATP binding cassette transporter A1 (264a). Interestingly, macrophage SR-B1 has also been shown to be stimulated by estradiol (360). However, the stimulatory effect of T on cholesterol efflux from macrophages was inhibited by flutamide so that T appears to regulate SR-B1 directly rather than indirectly via estradiol.

Activated macrophages produce various cytokines including chemotactic protein 1, IL-1$\beta$ and IL-10, and TNF$\alpha$, as well as growth factors such as platelet-derived growth factor 1. These bioactive molecules induce or inhibit various processes that contribute to atherosclerosis, *e.g.*, recruitment of macrophages into the vascular wall and SMC proliferation and migration (293, 294, 326). Effects of T on the production of cytokines and growth factors have not been studied in foam cell macrophage models but only in unstimulated or lipopolysaccharide-stimulated macrophages. Whether these results are also valid for macrophages in the arterial wall is not known For example, estradiol but not T inhibited the migration of monocytes in response to chemotactic protein 1. IL-1$\beta$ production in rat testicular macrophages and hamster peritoneal macrophages was also independent of T (297, 361, 362). In J774 macrophages, T exerted potentially antiinflammatory effects by stimulating IL-10 synthesis and inhibiting the production of TNF$\alpha$ and NO (363).

In summary, T appears to modulate lipid transport mechanisms of macrophages that favor both lipid accumulation

(uptake of modified LDL via scavenger receptor type A) and lipid secretion (cholesterol efflux via SR-B1). These regulatory effects are at least partially exerted via the androgen receptor.

### *D. Effects of T on arterial smooth muscle functions*

In addition to regulating vascular tone (see *Section VII.B* for details), arterial SMCs play an important role in atherosclerosis by proliferation, migration, and matrix production (293, 326, 364). These processes have both negative and positive implications for the clinical course of atherosclerosis by causing stenoses and stabilizing plaques, respectively. In contrast to estradiol, T was found not to affect proliferation and migration of SMCs (365, 366). Moreover, the protection of female rabbits by estradiol, but not male rabbits by T, from atherosclerosis was associated with decreased incorporation of 5′-bromo-2′-deoxyuridine into DNA of neointimal cells, an *in vivo* marker of arterial SMC proliferation (118). Finally, the effect of T to attenuate early atherosclerosis of LDL-receptor-deficient mice is prevented by inhibition of aromatase, indicating that locally produced estradiol rather than T is important for the modulation of plaque growth and stabilization (122a).

### *E. Effects of T on platelet functions*

Aggregation of platelets is a prerequisite for thrombus formation and, hence, a critical step in acute coronary events. Administration of T cypionate to eugonadal men led to enhanced *ex vivo* platelet aggregation in response to the thromboxane analog I-BOP but not in response to thrombin (367). T increases the expression of the androgen receptor in a megakaryocyte cell line, as well as in platelets (368, 369). The androgen receptor antagonist flutamide inhibited the stimulatory effect of T on thromboxane receptor expression (368, 369), suggesting that the effect is mediated via the androgen receptor.

## VIII. DHEA(S) and CAD in Men and Women

DHEA and its sulfate DHEAS are weak but highly abundant adrenal androgens that show a progressive age-related decline in both men and women from the third decade onward (370, 371). There is a growing body of opinion suggesting that DHEA supplementation may be beneficial to the elderly in a variety of physiological functions including the prevention of cardiovascular disease (355–375). It is implied that, against an androgenic milieu in men, DHEA acts as a prohormone for metabolites with predominantly estrogenic effects and antiatherogenic actions (373). The concept that DHEA protects against atherosclerosis was first put forward by Kask in 1959 (376).

Many clinical studies in men have attempted to demonstrate a correlation between serum DHEAS levels with different CAD endpoints, including the extent of atherosclerosis assessed by autopsy, coronary angiography, carotid vessel thickness/pulse wave, aortic calcification, and clinical disease states including angina, MI, and mortality (Table 6). These studies have shown either an inverse (Refs. 47, 53, 55, 61, and 377–383, which are mostly cross-sectional), null (33, 48, 50, 57, 60, 379, 383–388), or positive (38, 52, 389) relationship between DHEAS levels and CAD (Table 6). Interpretation of these observational studies is hampered by the same methodological shortcomings that have been outlined for T (see *Section III*).

In men, all but three of the nested case-control or prospective cohort studies showed no association between DHEAS levels and incident CAD (Table 6A). In the Helsinki Heart Study of middle-aged dyslipidemic men, higher DHEAS levels were associated with an increased risk of CAD (52). In the Honolulu Heart Study of 6000 men of Japanese descent followed for 18 yr (379), low DHEA was associated with fatal but not nonfatal CAD. In the Rancho Bernardo cohort study, a preliminary report of 242 men also showed a negative relationship between DHEAS and CAD mortality (377). However, in the full analysis of the same study on 942 men over 19 yr (382), there was only a modest negative relationship between DHEAS and those that survived their cardiac events but none with CAD mortality. Low DHEAS levels appear to be associated with increased mortality from all causes of death in men over the age of 50 (379, 382, 384), giving rise to the notion that this is a nonspecific marker of poor health and of lack of adaptive capacity to acute illnesses or a secondary phenomenon consequent upon various diseases of aging such as malignancies and heart failure (371, 386, 390). Moreover, the postulated relationship between DHEA deficiency and CAD is not ecologically or gender consistent. Thus, DHEAS levels vary greatly between different male populations (379). Japanese men living in Japan have the lowest levels of DHEAS in any study population, but they also have one of the lowest rates of coronary artery disease. The reverse is true for American men in California. Similarly, women have lower DHEAS levels than men—yet they have lower incidences of CAD.

The preliminary Rancho Bernardo analysis on 30 CAD deaths during a 12-yr follow-up of 289 postmenopausal women 60–79 yr of age revealed a positive association between serum levels of DHEAS and cardiovascular and CAD mortality (Ref. 389 and Table 6B). However, in the subsequent report of the 19-yr follow-up of the full cohort of 942 Rancho Bernardo women, cardiovascular and CAD mortality were not associated with serum DHEA levels at baseline (Ref. 391 and Table 6B). This lack of association was confirmed by five other shorter prospective studies (384, 385–388) and one cross-sectional study (60). In contrast, a negative relationship between DHEAS and CAD and atherosclerosis has been documented in cross-sectional studies in younger women (378, 380, 392, 61).

Taken together, data from observational studies on DHEAS do not support the hypothesis that DHEAS deficiency is a risk factor for CAD fatalities or that DHEA may confer an antiatherogenic action in men or women. Low DHEA may be a nonspecific marker for ill health in general.

Interventional studies with DHEA have only been of short duration, and no data are available on the putative effects of DHEA on CAD (393–401, 403, 404). Any effects on cardiovascular risk factors appear to be marginal. In postmenopausal women, application of DHEA results in a slight reduction of HDL-C (396). Female patients with Addison's

TABLE 6. Relationships between circulating HEA and DHEAS levels and CAD in men (A) and women (B)

| First author, year (Ref.) | n (age yr) | Study type | Hormone | Endpoint | Relationship OR |
|---|---|---|---|---|---|
| A | | | | | |
| Zumoff, 1982 (38) | 38, 79 (21–85) | Cross-sectional | DHEA, DHEAS | CAD, angio | Positive |
| Slowinska-Srzednicka, 1989 (47) | 108 (26–40) | Cross-sectional | DHEAS | MI, angio | Negative |
| Herrington, 1990 (378) | 101 (<50) | Cross-sectional | DHEA, DHEAS | CAD, angio | Negative |
| Ishihara, 1992 (380)[b] | 69 (15–83) | Cross-sectional | DHEA, DHEAS | Aortic calcific, pulse wave | Negative |
| Mitchell, 1994 (53)[b] | 98 (<56) | Cross-sectional | DHEAS | MI | Negative |
| Herrington, 1995 (381) | 206 & 61 (none) | Cross-sectional | DHEA, DHEAS | Angio, graft vasculopathy | Negative |
| Feldman, 1998 (55)[b] | 1709 (40–70) | Cross-sectional | DHEAS | Heart disease | Negative 0.6 (0.5–0.8) |
| Hauner, 1991 (50) | 274 (30–74) | Cross-sectional | DHEAS | CAD, angio | Null |
| Phillips, 1994 (33) | 55 (39–89) | Cross-sectional | DHEAS | Angio | Null |
| Schuler-Lüttmann, 2000 (57) | 189 (<70) | Cross-sectional | DHEAS | CAD, angio | Null[a] |
| **Barrett-Connor, 1986 (377)[b]** | **242 (50–79)** | **Prospective cohort 12 yr** | **DHEAS** | **CAD mortality** | **Negative 0.6** |
| **Contoreggi, 1990 (48)[b]** | **46, 124 (41–92)** | **Nested case-control 9.5 yr** | **DHEAS** | **CAD** | **Null** |
| **Lacroix, 1992 (379)[b]** | **238, 476 (48–71)** | **Nested case-control 18 yr** | **DHEAS** | **MI, autopsy** | **Negative[c] 0.5 (0.2–1.1)** |
| **Lacroix, 1992 (379)[b]** | **238, 476 (48–71)** | **Nested case-control 18 yr** | **DHEAS** | **CAD, MI** | **Null[d]** |
| **Newcomer, 1994 (383)[b]** | **157, 169 (40–84)** | **Nested Case-control 28 months** | **DHEAS** | **MI** | **Null 1.0 (0.4–2.6)** |
| **Barrett-Connor, 1995 (382)[b]** | **942 (65.2)** | **Prospective cohort 19 yr** | **DHEAS** | **CAD deaths** | **Null** |
| **Barrett-Connor, 1995 (382)[b]** | **942 (65.2)** | **Prospective cohort 19 yr** | **DHEAS** | **CAD survivors** | **Negative 0.9** |
| **Berr, 1996 (384)[b]** | **266 (66–>80)** | **Prospective cohort 4 yr** | **DHEAS** | **Cardiovascular deaths** | **Null[e]** |
| **Jansson, 1998 (385)** | **42, 53 (<70)** | **Nested case-control (survivors) 1 yr** | **DHEAS** | **Reinfarction & CAD deaths** | **Null** |
| **Tilvis, 1999 (386)[b]** | **571 (75–85)** | **Prospective cohort 5 yr** | **DHEAS** | **CVD deaths** | **Null** |
| **Kiechl, 2000 (387)[b]** | **371 (40–79)** | **Prospective cohort 5 yr** | **DHEAS** | **CVD, CIMT** | **Null 1.1 (0.9–1.4)** |
| **Trevedi, 2001 (388)[b]** | **963 (65–7)** | **Prospective cohort 7.4 yr** | **DHEAS** | **CVD mortality** | **Null 0.6 (0.3–1.3)** |
| **Hautenen, 1994 (52)** | **62, 97 (48)** | **Nested case-control 5 yr** | **DHEAS** | **MI, cardiac deaths** | **Positive 2.0 (1.0–4.9)** |
| B | | | | | |
| Herrington, 1990 (378) | 103 (<50) | Cross-sectional | DHEA, DHEAS | CAD, angio | Negative |
| Ishihara, 1992 (380) | 119 (16–80) | Cross-sectional | DHEAS | Aortic pulse wave, calcif | Negative |
| Slowinska-Srzednicka, 1995 (392) | 35 (35–47) | Cross-sectional | DHEAS | Coronary Angio, ETT | Negative |
| Bernini, 1999 (61)[b] | 101 (21–73) | Cross-sectional | DHEAS | CIMT | Negative |
| Phillips, 1997 (60) | 109 (68.9 ± 1.0) | Cross-sectional | DHEAS | Coronary Angio | Null |
| **Barrett-Connor, 1987 (389)[b]** | **289 (60–79)** | **Prospective cohort 12 yr** | **DHEAS** | **CAD mortality** | **Positive 1.5** |
| **Barrett-Connor, 1995 (391)[b]** | **942 (30–88)** | **Prospective cohort 19 yr** | **DHEAS** | **CAD mortality** | **Null 0.9 (0.9–1.2)** |
| **Berr, 1996 (384)[b]** | **356 (66–>80)** | **Prospective cohort 4 yr** | **DHEAS** | **Cardiovascular mortality** | **Null** |
| **Jansson, 1998 (385)** | **42, 53 (<70)** | **Case-control (survivors) 1 yr** | **DHEAS** | **Reinfarction & CAD deaths** | **Null** |
| **Tilvis, 1999 (386)[b]** | **571 (75–85)** | **Prospective cohort 5 yr** | **DHEAS** | **CVD deaths** | **Null U-shaped** |
| **Kiechl, 2000 (387)[b]** | **496 (40–79)** | **Prospective cohort 5 yr** | **DHEAS** | **CVD, CIMT** | **Null 1.0 (0.9–1.2)** |
| **Trevedi, 2001 (388)[b]** | **1171 (65–76)** | **Prospective cohort 7.4 yr** | **DHEAS** | **CVD mortality** | **Null 1.0 (0.4–2.5)** |

CVD, Cardiovascular disease; CIMT, carotid intima-media thickness ultrasound; Angio, coronary angiography.

Negative relationship indicates lower DHEA(S) levels in patients with CAD compared to controls, positive relationship indicates higher DHEA(S) levels in CAD, and a null relationship indicates no difference between cases and controls.

For prospective cohort or nested case-control studies, the number of cases (first n) and controls (second n) and duration under study.

Highlighted in *bold* are the most important studies in terms of adequacy of design, statistical power, and allowance for confounding factors.

[a] Negative only upon univariate analysis, null upon multivariate analysis.

[b] Population sample rather than patients.

[c] Fatal cases.

[d] Nonfatal cases.

disease administered oral DHEA, 50 mg daily for 3–4 months, showed either no change (401) or a decrease in total and HDL-C (398), whereas a relatively greater increase in T was induced by DHEA bioconversion. In men aged 60–84 yr, DHEA, 100 mg daily for 3 months, decreased total and HDL-C (403), but this was not confirmed in a larger study (404).

In animal studies, in contrast to the conflicting data on the effects of exogenous T on diet-induced atherosclerosis, DHEA administration to rabbits seems to consistently decrease atherosclerosis. Thus, all five studies (Table 2 and Refs. 117 and 405–408) in intact or castrated male and female rabbits treated by DHEA for between 5 and 30 wk showed a significant reduction in the extent of spontaneous or balloon injury-induced aortic or cardiac transplant atherosclerotic lesions independently of changes in lipids. DHEA may therefore be considered favorable under these rather artificial experimental conditions. Although estrogens were not measured in these studies, it is probable that DHEA administered in pharmacological doses to animals (rabbits) with little endogenous adrenal androgen production would be converted to estrogenic metabolites with potent actions on the vascular endothelium. This is supported by the findings of Hayashi *et al.* (408), who demonstrated that the antiatherogenic effects of DHEA in ovariectomized female rabbits can be partially (50%) blocked by the aromatase inhibitor fadrozole. Together with the fact that no specific receptors for DHEA have yet been identified, it is plausible that this steroid primarily acts as a prohormone for more potent metabolites. In the context of atherosclerosis, pharmacological doses of DHEA may well be feeding the estrogenic rather than the androgenic bioconversion pathways. One should therefore be circumspect in extrapolating to man the apparent beneficial actions of DHEA suggested by animal studies because exposure to similarly high pharmacological doses has not been investigated and may not be acceptable clinically.

In experimental studies, DHEA facilitates fibrinolysis (409) and inhibits platelet aggregation (410), lipid accumulation in mouse macrophage foam cell cultures (411), and proliferation and migration of vascular SMC lines (412). DHEA has also been shown to reduce IL-6, IL-1$\beta$, and TNF$\alpha$ in mouse macrophages (413) and IL-6 in human mononuclear cells *in vitro* (414) and to reduce TNF$\alpha$ in mice and rats *in vivo* (415, 416). The use of animals with negligible physiological adrenal androgen production and pharmacological doses of DHEA again render these *in vivo* and *in vitro* experimental data of doubtful relevance to man.

In summary, the epidemiological and experimental data on the relationship between DHEA and CAD are inconsistent and unconvincing. No definitive conclusions or clinical recommendations can be drawn from the existing evidence base.

## IX. Estrogens and Cardiovascular Disease in Men

There is compelling evidence indicating that an increasing number of physiological actions of T in men are mediated by the ERs after conversion to estradiol by site-specific aromatases in target tissues (368). The extraglandular production of estrogens (with circulating androgens as the immediate precursor substrate) may therefore play a role in male cardiovascular physiology and pathophysiology. Estrogens are generally regarded as antiatherogenic and therefore protective against CAD. Estrogens increase HDL-C, decrease Lp(a), and prevent lipid peroxidation. There is also evidence from animal models and *in vitro* experiments that estrogens exert direct effects on the vascular wall. Estradiol mediates vasodilatation and inhibits SMC proliferation/migration, modulates the vascular inflammatory response by inhibiting cytokine activation and expression of cell adhesion molecules, and inhibits platelet aggregation and adhesion (for reviews, see Refs. 11, 274, 295, and 327–331). Thus, vasoprotection by estrogens can be mediated via either classical ER-mediated genomic (*e.g.*, cell proliferation, structural remodeling, or lipid distribution) or the rapid nongenomic pathways (*e.g.*, changes in vasomotor tone). The latter may involve direct action via L type calcium channels in vascular SMCs or membrane ER-mediated activation of NO synthase and cGMP-dependent calcium-activated potassium channels (314). ER$\alpha$, ER$\beta$, aromatase, and 17$\beta$-hydroxysteroid dehydrogenase are expressed in many cell types in the vasculature (see *Section VII.A*).

The importance of locally produced estrogens from aromatization of T in males for cardiovascular health has been tantalizingly highlighted by recent human and transgenic mouse models of aromatase deficiency and estrogen resistance. In two men with undetectable circulating estradiol and estrone and high T due to P450 aromatase deficiency (417, 418), dyslipidemia with elevated total and LDL-C and triglyceride and decreased HDL-C was associated with insulin resistance (in the first patient only). These metabolic abnormalities were correctable by low-dose oral or transdermal estrogen replacement. In a 28-yr-old male with a null mutation in ER$\alpha$ gene causing estrogen resistance (419), insulin resistance, acanthosis nigricans, and impaired glucose tolerance were apparent. HDL-C and LDL-C were low. Intact hepatic ER$\beta$ may have prevented full expression of dyslipidemia. Ultrafast electron beam computed tomography imaging showed calcium deposition in the proximal left anterior descending coronary artery, indicating the presence of premature atherosclerosis (420). Flow-mediated, endothelial-dependent, and NO-activated brachial artery vasodilation (membrane ER-mediated) in response to hyperemia was absent, showing marked endothelial dysfunction (421). Preservation of response to nitroglycerin indicates that NO action in vascular SMCs is intact. The nongenomic rapid vasodilation in response to a sublingual dose of 2 mg of estradiol also remained intact.

These recent findings suggest that estrogens are important in maintaining normal carbohydrate and lipid metabolism as well as normal endothelial-dependent, NO-mediated vasodilatation in men. They are compatible with data from transgenic knockout models confirming that ER$\alpha$ is important in preventing adipocyte hypertrophy, obesity, insulin resistance, and hypercholesterolemia (422–424), and maintaining basal NO release from vascular endothelium (425) in male animals and ER$\beta$ in vascular smooth muscle may also regulate vascular sensitivity to estradiol (316, 426). The favorable effects of estrogens on HDL-C that have been demon-

strated are also in accord with clinical studies using aromatase inhibitors in normal men (see *Section VI.C.1*) and the prevention of early atherosclerosis in LDL-receptor-deficient mice (122a).

The complex interplay between the endocrine actions of androgens and the paracrine or autocrine actions of locally produced estrogens in target tissues is critical for isosexual physiological regulation of metabolic and vascular functions by sex hormones in both men and women. Better understanding of these mechanisms is likely to yield new opportunities for selective abrogation or stimulation of specific effects with the goal of cardiovascular disease prevention and amelioration in the future.

## X. Summary and Conclusion

The gender difference in CAD cannot be explained on the basis of endogenous sex hormone exposure. None of the epidemiological studies in the literature showed a positive association between T and CAD in men to suggest that high levels of this androgen may be a risk factor, with all the longitudinal studies consistently showing a lack of relationship. Data on women also do not suggest that endogenous T plays a causal or protective role for CAD, but PCOS patients undoubtedly have an adverse risk profile. Whether this leads to increased premature heart disease is currently unclear. Observational studies on DHEAS do not support the hypothesis that DHEAS deficiency is a risk factor for CAD in men or women.

In men, endogenous T is correlated positively with HDL-C and negatively with LDL-C, triglycerides, fibrinogen, and PAI-1. In women, these relationships are reversed. However, hypoandrogenemia in men and hyperandrogenemia in women are confounded by central obesity and insulin resistance. These associations are therefore uninformative with respect to a direct pro- or antiatherogenic role of androgens.

Interventional studies generally do not show a causal relationship between T exposure and the development of CAD. Short-term studies suggest T treatment may improve exercise ECG in men with established CAD. The majority of animal experiments found exogenous T and DHEA(S) to exert neutral or beneficial effects on atherosclerosis in male and detrimental effects in female animals.

Exogenous androgens induce both apparently beneficial and deleterious effects on cardiovascular risk factors by decreasing serum levels of HDL-C, PAI-1 (apparently deleterious) Lp(a), fibrinogen, insulin, leptin, and visceral fat mass (apparently beneficial) in men as well as women. However, androgen-induced declines in circulating HDL-C should not automatically be assumed to be proatherogenic, because these declines may reflect accelerated reverse cholesterol transport instead.

Supraphysiological concentrations of T stimulate vasorelaxation including coronary arteries; but at physiological concentrations, beneficial, neutral, and detrimental effects on vascular reactivity can be observed. They may involve direct and indirect, endothelium-dependent and endothelium-independent, genomic and nongenomic modes of action. The importance of locally converted estradiol on vascular reactivity in men has recently been demonstrated in estrogen-resistant and aromatase-deficient models. T exerts proatherogenic effects *in vitro* on macrophage functions by facilitating the uptake of modified lipoproteins and an antiatherogenic effect by stimulating SR-B1-mediated cholesterol efflux of cellular cholesterol to HDL.

In conclusion, endogenous androgens do not show any consistent association with CAD. Androgens can exert both apparently beneficial and deleterious actions on a multitude of factors implicated in the pathogenesis of atherosclerosis and CAD. Current evidence does not permit any meaningful assessment of the net effects of T or DHEAS on cardiovascular disease risks in men or women.

## XI. Clinical Implications

On the basis of current evidence, efforts to exploit the wider therapeutic benefits of T in men should not be deterred or hampered by concerns regarding increased CAD risks. In the presence of evidence of androgen deficiency, the initiation and continuation of T replacement therapy is not contraindicated in male patients with known CAD.

In elderly men, it has been suggested (on the basis of short-term ECG changes in a few small studies only) that androgen replacement, in addition to possible benefits on muscle, bone, sexual, and mental functions, may also ameliorate CAD. Given the current lack of long-term morbidity and mortality data, it will be difficult to justify large-scale primary or secondary prevention trials specifically to investigate the possible benefits of androgens in CAD, especially when there are other established medical treatment modalities (*e.g.*, weight reduction, smoking cessation, exercise, aspirin, statins, antihypertensives, beta-blockers, and vasodilators) that are of proven benefit. One way out of this conundrum may be to target high-prevalence patient groups (*e.g.*, type 2 diabetics, hyperlipidemics) for smaller-scale interventional studies and also to incorporate subclinical noninvasive disease endpoints (*e.g.*, circulatory markers of endothelial dysfunction, ultrasonic carotid intimal-media thickness, and waveform and computer tomography scanning of coronary artery calcification) in addition to morbidity and mortality.

In women, the possibility that spontaneous or induced hyperandrogenemia may be associated with increased risks for CAD should be seriously considered. Thus, clinical evidence of hyperandrogenism in PCOS is a biomarker for the metabolic diathesis associated with increased risks for type 2 (and gestational) diabetes, hypertension, stroke, and possibly CAD in later life. Because PCOS can affect 4–11% of premenopausal women (427, 428), this represents both an early and a valuable primary prevention opportunity in a substantial population of at-risk women. Insulin-sensitizing drugs (biguanides and thiazolidinediones) are increasingly being used in the management of current problems such as anovulation, oligomenorrhea, and hirsutism in women with PCOS (429). Whether these drugs, by improving insulin sensitivity and ameliorating hyperandrogenism and dyslipidemia, will also reduce the future risk of cardiovascular disease in PCOS patients should be a goal for future prospective investigations.

## Acknowledgments

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A.v.E. was supported by a grant from "Interdisziplinäres Zentrum für Klinische Forschung der Medizinischen Fakultät Münster," Germany (Project A3 on "Androgens as Modulators of Atherosclerosis").

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