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Testosterone and atherosclerosis

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Abstract

Hypoandrogenemia in men and hyperandrogenemia in women are associated with increased risk of coronary artery disease but also with visceral obesity, insulin resistance, low high-density lipoprotein (HDL) cholesterol, elevated triglycerides, low-density lipoprotein (LDL) cholesterol and plasminogen activator inhibitor (PAI-1). These gender differences and confounders render the precise role of *endogenous* androgens in atherosclerosis unclear.

Exogenous androgens, on the other hand, 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 pro-atherogenic, since it may reflect accelerated reverse cholesterol transport instead.

Short-term application of supraphysiological doses of exogenous T can reduce the severity and frequency of angina pectoris and improve the electrocardiographic signs of myocardial ischaemia; long-term effects have not been investigated. Nonetheless, interpretations of the effects of pharmacological doses of androgens on arterial compliance and flow-mediated dilatation in particular must be treated with circumspection also because at physiological concentrations, beneficial, neutral, and detrimental effects on vascular reactivity can be observed.

Testosterone exerts 'pro-atherogenic' effects on macrophage function by facilitating the uptake of modified lipoproteins and an 'anti-atherogenic' effect by stimulating efflux of cellular cholesterol to HDL.

In the majority of animal experiments, exogenous testosterone exerted neutral or beneficial effects on the development of atherosclerosis.

In conclusion, the overall effect of administration of testosterone on cardiovascular-disease risk is difficult to assess because androgens have such an extraordinary array of effects in vivo. When dealing with a complex multifactorial condition such as CAD, it is premature to assume that clinical benefits can be derived from manipulation of the sex steroid milieu – even when these assumptions are based on biologically plausible mechanisms or, indeed, on cross-sectional risk-factor observational data. Neither needs the therapeutic use of testosterone in men be restricted by concerns regarding cardiovascular side effects.

Keywords: Coronary artery disease; Androgens; Testosterone; Atherosclerosis; High-density lipoproteins; Polycystic ovary syndrome; Vasoreactivity; Hormone replacement

1. Introduction

Male gender is one of the classic risk factors for coronary artery disease (CAD), and the average life expectancy for men with CAD is about 8 years less than that of women. The presence of androgens and the lack of estrogens are often regarded as proximate causes for the disproportionately shorter duration of survival among men. With the prospect of much wider therapeutic application of androgens (e.g., for contraception; treatment of aplastic anaemia or sarcopenic, osteopenic and dysphoric states, and chronic systemic conditions; and treatment of physiologic ageing), one important question is whether androgen treatment might increase the risk for, or severity, of CAD.

This review addresses five questions related to androgens and coronary artery disease: (1) Do observational studies provide any evidence for associations between serum levels of endogenous androgens and

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CAD endpoints? (2) Which effects on CAD endpoints or symptoms are exerted by application of exogenous testosterone (T) or suppression of endogenous T? (3) What can be learned from animal studies of the effects of androgens on atherosclerosis? (4) How do androgens affect cardiovascular-disease risk factors? (5) How do androgens affect the function of vascular cells involved in the pathogenesis of atherosclerosis [1]?

2. Lessons learned from observational studies

It is important to emphasize the limitations of observational studies in demonstrating the associations between serum levels of endogenous androgens and CAD. These studies have been characterized by extremely variable CAD endpoints (e.g., mortality; morbid conditions such as myocardial infarction [MI] and angina; and angiography-, ultrasound- or post-mortem-based diagnosis or unspecified cardiac events), heterogeneous study groups, and diverse selection criteria. For example, most CAD patients are taking medications and have modified their lifestyle, factors which could distinguish them from controls. In some studies, selection of poorly matched controls may have introduced biases. Among patients, the time interval from MI to sampling varied from 3 months to several years and was not always standardized for diurnal variation of hormone levels. In addition, in the majority of the studies, no adjustment was made for confounding factors. For example, the diagnoses of hypoandrogenemia in men and hyperandrogenemia in women were confounded by various concurrent metabolic disorders, including obesity, insulin resistance, dyslipidaemia, and impaired fibrinolysis, which are associated with chronic illnesses, including CAD and lower serum levels of T.

2.1. Endogenous T and CAD in men

In 16 of 32 cross-sectional studies [1,2] serum levels of T were lower in patients with CAD compared with healthy controls. The other 16 studies showed no difference in T levels when comparing cases and controls. In none of the studies were high levels of T associated with CAD. In all of the studies, an inverse association was found between levels of free T and CAD [1,2]. None of six longitudinal studies in men revealed any significant association between serum levels of T and future risk of CAD events [1,2].

2.2. Endogenous androgens and CAD in women

In contrast with the neutral or even beneficial associations between levels of endogenous T and cardiovascular-disease risk in men, the few retrospective or cross-sectional case-control studies in women revealed pro-atherogenic associations between androgens and CAD [1]. However, there is limited prospective data on the importance of T as a cardiovascular-disease risk factor in women. In a report by Barrett-Connor and Goodman-Gruen [3] documenting a 19-year follow-up of 651 postmenopausal women, serum levels of T, bioavailable T, and androstendione did not differ among the women with a history of CAD and those without such a diagnosis at baseline. Cardiovascular-disease mortality during follow-up was not associated with any particular androgen serum level [3].

Indirect evidence for the atherogenicity of androgens in women was derived from the findings of clinical studies showing that women with CAD were affected more frequently than controls by clinical symptoms of androgen excess, such as hirsutism and polycystic ovaries [4–7]. Cross-sectional data consistently showed a strong obesity-independent association of androgen excess in women with a cluster of cardiovascular-disease risk factors, including insulin resistance, dyslipidaemia, and impaired fibrinolysis. It was therefore suggested that the chronically abnormal hormonal and metabolic milieu found in women with polycystic ovary syndrome (PCOS), starting from adolescence, may predispose these women to premature atherosclerosis. Based on calculated risk profiles, women with PCOS were predicted to have a 7-fold increased relative risk for MI. In agreement with this notion, results of a study combining angiography and pelvic ultrasound in 143 women aged $\leqslant 60$ years indicated there were significant associations between the presence of polycystic ovaries and the presence and severity of CAD and a family history of MI, as well as with elevated levels of insulin and triglycerides and lower levels of high-density lipoprotein cholesterol (HDL-C) [8]. In two case-control studies, significantly increased carotid artery intima-media thickness was found in women with PCOS compared with age-matched controls independent of body mass index (BMI), fat distribution, and other risk factors [9,10]. In contrast, in the single long-term longitudinal study, no association was found between the incidence of PCOS and that of CAD [11]. The authors compared the mortality and morbidity rates of 786 of 1028 women diagnosed with PCOS between 1930 and 1979 with 1060 age-matched controls over a mean period of 30 years. Despite significantly increased prevalences of diabetes, hypertension, and hypercholesterolaemia among women with PCOS, the standardized odds ratios of CAD mortality and morbidity were only slightly and insignificantly increased in women with PCOS [11]. Hence, CAD risk in women with PCOS may have previously been overestimated. However, estrogen treatment in women with PCOS may have counteracted the effects of these risk factors.

3. Lessons learned from interventions

3.1. Androgen deprivation

Historic studies comparing the life spans of castrated inmates and castrated singers with that of their intact counterparts did not reveal any differences in the incidence of total or cardiovascular-disease-related mortality [12,13]. Cross-gender sex-hormone treatment of 816 male-to-female transsexuals aged 18–86 years with administration of ethinyl E2 100 μg/day and cyproterone acetate 100 mg/day for 7734 patient-years was not associated with any significant difference in cardiovascular-disease mortality or morbidity compared with the general population of men, despite a 20-fold increase in venous thromboembolic complications [14]. These data were interpreted as indicating that the abolition of testicular androgens by pre- or postpubertal castration does not change the rate of cardiovascular-disease mortality in men.

3.2. Androgen excess from anabolic steroid abuse

No formal case-control study has been conducted of anabolic-androgenic steroid (AAS) abuse in young men presenting with acute MI. Nevertheless, a review of the literature covering a 12-year period (1987–1998) identified 17 case reports of cardiovascular events (11 acute MI, 4 cardiomyopathy, and 2 stroke) in young male body builders who used extremely high supraphysiological doses of AAS [15]. Although the number of current and former AAS users has increased since the 1960s (there are presently over 1 million AAS users in the USA alone), there does not seem to have been any increase in the frequency of reported vascular events amongst likely users and ex-users of AAS.

3.3. Exogenous T in men with CAD

The long-term effects of exogenous T on rates of coronary events have not been investigated. However, in several small studies, therapeutic doses of T reduced the severity and frequency of angina pectoris and improved electrocardiographic signs of myocardial ischaemia. Webb and colleagues [16] showed that a single intravenous (iv) bolus of 2.3 mg T increased time to 1-mm ST segment depression by 66 seconds in 14 men with CAD and low plasma T levels. The plasma level of T increased from 5.2 to 117 nmol/l, 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. Infusion of T over a 3-min period into the coronary arteries of 13 men with an established diagnosis of CAD during coronary angiography at supraphysiological doses of 8 μmol/l, but not at the physiological dose of 8 nmol/l, led to significant increases in coronary vessel diameter and blood flow at all four doses of T tested. These results have been confirmed by a similar study in 14 men with established CAD [17].

3.4. Exogenous androgen treatment in women

There is increasing interest in the use of T as part of postmenopausal hormone replacement therapy (HRT), particularly to improve reported impairment of sexual function [18]. Whether the concurrent use of T will impact the perceived benefits of estrogen HRT on the cardiovascular system is currently unknown. In a 20-year (1975–1994) retrospective survey of the Amsterdam Gender Dysphoria Clinic, 293 female-to-male transsexuals aged 17–70 years (mean age, 34 years) were treated with oral T undecanoate 160 mg/day or T (Sustanon) 250 mg intramuscularly (im) every 2 weeks (range: 2 months to 41 years; total exposure: 2418 patient-years) [14]. There was no excess of cardiovascular-disease mortality or morbidity (or excess mortality or morbidity from all causes) compared with the general population of Dutch women.

4. Animal studies

The influence of androgens on the development and progression of experimentally induced atherosclerosis has been investigated in five animal models with diet-induced atherosclerosis and in three mouse models genetically susceptible to atherosclerosis.

Larsen et al. [19] compared the effects of im injections of T enanthate or placebo on atherosclerosis in castrated male rabbits. Serum cholesterol levels were titrated by a cholesterol-rich diet. After 17 weeks of treatment, there was no difference between the 19 rabbits in the treated group and the 17 rabbits in the placebo group in terms of cholesterol levels in the aorta. Likewise, treatment with the artificial androgen stanozolol did not lead to significant changes in the extent of atherosclerotic lesions [20].

Bruck et al. [21] found sex-specific effects of T on the development of atherosclerotic lesions in rabbits. Thirty-two castrated male rabbits and 32 ovariectomized female rabbits were divided into four groups of eight animals that were fed a cholesterol-rich diet and received either placebo, E2-valerate alone, T-enanthate alone, or a combination of T-enanthate and E2-valerate. After 12 weeks of treatment, the degree of intimal thickening was reduced in rabbits of both sexes receiving the combination therapy, in female rabbits receiving E2 alone, and in male rabbits receiving T alone. The sex-specific antiatherogenic effects of T and E2 were independent of changes in lipoprotein levels.

Alexandersen et al. [22] showed that castration per se in male rabbits resulted in a doubling of aortic atherosclerosis compared with sham-operated controls. This

effect was reversed by administration of oral T undecanoate 80 mg/day by way of a lipid-dependent mechanism. In addition, 25 mg T enanthate im delivered twice weekly raised circulating T levels 10-fold and decreased the degree of aortic atherosclerosis by lipid-independent mechanisms. This finding suggests that androgens administered at pharmacological doses may have effects on the vasculature that protect against atherosclerosis.

In contrast to the beneficial or neutral effects of T on atherosclerosis in male rabbits, treatment of male chicks with T resulted in a dose-dependent increase of aortic atherosclerosis [23]. Treatment of female ovariectomized cynomolgus monkeys with T for 24 months also increased the size of atherosclerotic plaques in the coronary artery by a factor of 2 compared with both untreated animals either with or without ovariectomy. The increase in atherosclerotic plaque size was independent of changes in lipid levels and, surprisingly, occurred despite improved endothelial reactivity [24].

Two studies investigating the effect of castration and administration of exogenous T on atherosclerosis in apoE-deficient mice demonstrated conflicting results. Elhage et al. [25] found that castration at the age of 4 weeks had no effect on the degree of atherosclerosis in either male or female mice. In both sexes, application of subcutaneous T pellets for 8 weeks significantly decreased serum levels of cholesterol and inhibited the development of fatty streak lesions in the sinus aortae by about 30%. However, von Dehn et al. [26] found that suppression of T by 100 μg of the GnRH antagonist cetrorelix every 48 h led to a decrease in the degree of atherosclerosis in both the sinus aortae and in the ascending aorta, despite increases in cholesterol levels in male mice and decreases of HDL-C levels in female mice. Insertion of a silastic implant with 35 mg T increased serum levels to 6.1 ng/ml in male mice and led to small but significant increases in cholesterol levels and in the presence of atherosclerotic lesions in male mice. Despite an increase of T levels to 10.1 ng/ml, female mice showed no change in lipid levels and fewer atherosclerotic lesions. The discrepant results between the two studies may have resulted from the higher dosages of T used in the second study. Another study performed in LDL-receptor knockout mice also found an anti-atherogenic effect of T; however, that effect was blunted by the parallel use of an aromatase inhibitor [27]. Therefore, the anti-atherogenic effect was ascribed to estradiol rather than T [27].

5. Effects of T on cardiovascular-disease risk factors

The net effect of T on cardiovascular-disease risk is difficult to assess for at least six main reasons. First, the effects of T on cardiovascular-disease risk factors are contradictory, depending on whether associations with endogenous T or effects of exogenous T have been investigated. Second, the associations between serum concentrations of endogenous T and cardiovascular-disease risk factors are confounded by mutual interactions between endogenous androgens, body fat distribution, and insulin sensitivity. Third, exogenous T has profound effects on several risk factors, some of which at first glance appear beneficial, namely the lowering of lipoprotein(a) (Lp(a)), insulin, fibrinogen, and plasminogen activator inhibitor type 1 (PAI-1); whereas, other effects are considered adverse, namely the lowering of HDL-C. Fourth, the causal relationship between some of the aforementioned risk factors and atherosclerosis has not been proven, though it is suspected. Of special importance are results of experimental and clinical studies indicating that therapeutically induced changes in HDL-C concentrations may not necessarily be accompanied by changes in the risk of cardiovascular-disease [28]. Fifth, T can exert its metabolic effects either directly or by means of its metabolites E2 and dihydro-T. The effects of T and E2, in particular, can be either additive (for example on Lp(a)) or counter-regulatory (for example on HDL-C). Sixth, polymorphisms in the genes of the androgen receptor, sex-hormone binding globulin (SHBG) and 5α-reductase regulate genomic effects and the bioavailability of T and dihydro-T, respectively. Thus, at a given serum concentration, the metabolic effects of T can be diverse.

5.1. Associations of endogenous T with cardiovascular-disease risk factors

The results of several cross-sectional population studies have shown statistically significant correlations between plasma levels of T and various risk factors, although the associations were opposite in men and women.

In men, plasma T levels were frequently found to have positive correlations with serum levels of HDL-C as well as inverse correlations with plasma levels of triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), fibrinogen, and PAI-1 [1,29–31]. However, serum levels of T have even stronger inverse correlations with BMI, waist circumference, waist-hip-ratio (WHR), amount of visceral fat, and serum levels of leptin, insulin and free fatty acids. After adjustment for these measures of obesity and insulin resistance, the correlations of the cardiovascular-disease risk factors with T, but not with visceral fat or insulin, lost their statistical significance [29–31]. These findings indicate that a low serum level of T in eugonadal men is a component of the metabolic syndrome, which is characterized by the presence of obesity, glucose intolerance or overt type 2 diabetes mellitus, arterial hypertension, hypertriglyceridaemia, low HDL-C, and a procoagulatory and anti-fibrinolytic state, and for which insulin resistance is thought to be an important etiological

factor. Therefore, the frequently observed association of high T levels with a more favourable cardiovascular-disease risk factor profile in men probably does not reflect direct regulatory effects of T on lipoprotein metabolism and the haemostatic system. Accordingly, in some populations, these associations disappeared when serum levels of free T instead of total T were correlated with lipids and other cardiovascular-disease risk factors. In accordance with these factors, a low number of CAG repeats in the androgen receptor, which increases its sensitivity to T, was associated with reduced levels of HDL-C and leptin, as well as low body fat mass and BMI [32,33]. One reason for the discrepancy between the biological effects and the associations of endogenous T with various cardiovascular-disease risk factors is the negative regulatory effect of insulin on the production of SHBG so that insulin resistance causes low levels of SHBG and thereby low levels of total T [34].

Women present with the opposite associations between endogenous androgens and obesity, insulin, and cardiovascular-disease risk factors. In cross-sectional studies, serum levels of T were found to have significant positive correlations with BMI and leptin levels [1,29]. 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 [29]. Moreover, in a prospective study, 20% of women with SHBG levels below the 5th percentile developed type 2 diabetes mellitus within the 12-year follow-up period [35]. Thus, in women, hyperandrogenism is a component of the insulin-resistance syndrome. In agreement with this, women with PCOS frequently present with hypercholesterolaemia, low HDL-C, hypertriglyceridaemia, elevated fibrinogen levels, PAI-1, and a family history of diabetes mellitus. Because many women with PCOS are overweight, and most if not all are insulin resistant, it is a matter of debate as to whether these symptoms are secondary to obesity and insulin resistance or whether hyperandrogenemia itself contributes to obesity, insulin resistance, and hyperinsulinaemia [4–7].

Longitudinal studies have been used to investigate the effect of puberty, and hence the effect of endogenous sex hormones, on cardiovascular-disease risk factors in children. Prepubertal boys and girls do not differ significantly in their serum lipid and lipoprotein levels. In contrast with girls, in whom levels of HDL-C and LDL-C change little at puberty, sexually maturing boys experience a decrease in HDL-C levels and increases in LDL-C and triglyceride levels [36]. However, these changes may not reflect effects of sex hormones only, since they are confounded by other endocrinological changes, for example in the growth hormone axis, which also regulates lipoprotein metabolism.

5.2. Effects of exogenous T on cardiovascular-disease risk factors

5.2.1. HDL

In the majority of studies, substitution of T in hypogonadal men decreased HDL-C levels [1,37]. Treatment with supraphysiological doses of T or androgen-like anabolic steroids in eugonadal men decreased HDL-C levels by about 20% or more. Conversely, castration as well as biochemical suppression of endogenous T by GnRH antagonists increased HDL-C [1]. These observations, and the finding of low HDL-C levels in men with aromatase deficiency or estrogen resistance, suggest that physiological tissue levels of E2 play a role in maintaining physiological levels of HDL-C in men.

Because low HDL-C concentration is an important risk factor for CAD, and because isolated HDL in vitro and in vivo exerts several potentially anti-atherogenic actions, lowering of HDL-C levels by administration of T is considered to increase cardiovascular-disease risk factors. However, epidemiologic studies have not proved a causal association between low HDL-C concentration and elevated risk of CAD. Instead, low levels of HDL-C frequently occur concurrent with other indicators of metabolic syndrome and markers of chronic inflammation, and therefore may be merely a surrogate marker for a separate but linked pro-atherogenic condition. Moreover, in a study using transgenic animal models, only increases of HDL-C induced by overproduction of apoA-I, but not by inhibition of HDL catabolism, were consistently found to prevent atherosclerosis [38]. Therefore, the mechanism of HDL modification – rather than changes in levels of HDL-C per se – appears to determine the degree of anti-atherogenicity of HDL modification.

Two genes involved in the catabolism of HDL are up-regulated by T, namely hepatic lipase (HL) and scavenger receptor B1 (SR-B1). 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. 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 [39]. HL hydrolyses phospholipids on the surface of HDL, thereby facilitating the selective uptake of HDL lipids by SR-B1. The activity of HL in postheparin plasma is increased after administration of exogenous T [40] and is slightly decreased by suppression of T after GnRH antagonist treatment [41]. The increases in both SR-B1 and HL activities are consistent with the HDL-lowering effect of T. Interestingly, in transgenic mice, over-expression of SR-B1 or HL caused a dramatic fall in HDL-C levels but inhibited rather than enhanced the degree of atherosclerosis [38]. This again demonstrates the difficulty

in extrapolating the HDL-lowering effect of T to increased cardiovascular-disease risk.

5.2.2. Lipoprotein(a)

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 they coincide with other cardiovascular-disease risk factors [42]. Although genetics have a tremendous influence on Lp(a) levels, administration of T to men significantly decreased serum levels of Lp(a) (25–59%). Conversely, Lp(a) levels increased by 40–60% in controls and patients in whom endogenous T levels were suppressed by treatment with the GnRH antagonist cetrorelix or the GnRH agonist buserelin [1,43,44]. The Lp(a)-lowering effect of T is independent of E2, which also reduces Lp(a) levels. It is not known how T regulates Lp(a). It is also not known whether changes in Lp(a) induced by T will affect cardiovascular-disease risk. Interestingly, in the Heart and Estrogen/progestin Replacement Study (HERS) study, postmenopausal HRT prevented coronary events only in those women who had elevated Lp(a) at baseline and who experienced a decrease of Lp(a) levels by treatment with conjugated equine E2 and medroxyprogesterone [45].

5.2.3. The haemostatic system

In agreement with an important role of thrombus formation in the pathogenesis of acute coronary events and stroke, prospective studies have identified various haemostatic variables as cardiovascular-disease risk factors [46]. Among these haemostatic variables are fibrinogen and the fibrinolysis inhibitor PAI-1 or tissue plasminogen activator antigen. 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 weeks of treatment [47]. In this study the doubling of T levels also initially led to significant decreases of PAI-1, protein S, and protein C, as well as to increases of antithrombin and β-thromboglobulin. Likewise, PAI-1 levels were decreased in men who received the anabolic androgen stanozolol. However, suppression of T in patients with prostate cancer or benign prostatic hypertrophy by treatment with the non-steroidal antiandrogen bicalutamide or the GnRH agonist leuprolide exerted no significant effects on plasma fibrinogen levels [48]. In agreement with the lowering effects of T on PAI-1 concentrations, T inhibited the secretion of PAI-1 from bovine aortic endothelial cells in vitro. Taken together, the current data indicate that T lowers fibrinogen and PAI-1 levels. However, these anti-coagulatory and pro-fibrinolytic effects may be opposed by pro-aggregatory effects on platelets, because high dosages of androgens were found to decrease cyclooxygenase activity and thereby increase platelet aggregability.

5.2.4. Obesity and insulin sensitivity

Numerous observations suggest inter-relationships between androgens, body fat distribution, and insulin sensitivity, of which the latter two are also involved in the regulation of HDL metabolism and triglyceride metabolism [49]. However, it is not clear whether androgens regulate adipose tissue and insulin sensitivity as opposed to adipocytes and insulin regulating T levels. It is probable that the relationship is bi-directional.

Morbidly obese and insulin-resistant men frequently have low serum levels of T that increase upon weight loss [50]. In contrast, E2 levels are elevated in obese men and decrease with weight loss. It has therefore been suggested that obesity causes hypotestosteronaemia by increased aromatisation of T to E2 in the adipose tissue. In support of the hypothesis that insulin plays a role in the determination of T levels in men, infusion of insulin during euglycaemic clamp increased T levels in obese men but not in lean men [51]. On the other hand, hypogonadal men are frequently obese and have increased levels of leptin and insulin [52]. Body weight, leptin levels, and insulin levels decreased upon substitution of T in hypogonadal men [53,54]. Even treatment of eugonadal obese men with T led to a decrease of visceral fat mass and, in parallel, improved insulin sensitivity and corrected dyslipidaemia. Results of a different experiment showed the opposite: suppression of T by the GnRH-antagonist cetrorelix increased serum levels of leptin and insulin [41]. Moreover, male carriers of the more T-sensitive androgen receptor gene alleles with a low number of CAG repeats have less body fat than carriers of less T-sensitive androgen receptor gene alleles, with a high number of CAG repeats [33]. These data indicate that, in men, the dominant action in the bidirectional relationship is that T reduces fat mass, especially in the abdomen, and improves insulin action. In agreement with this deduction, androgens activate the expression of β-adrenergic receptors, adenylate cyclase, protein kinase A, and hormone-sensitive lipase in adipocytes [49]. As a result, T stimulates lipolysis and thereby reduces fat storage in adipocytes.

In women, mutual interrelationships have also been observed between T, adipose tissue, and insulin sensitivity, though these relationships apparently are opposite to those seen in men. On the one hand, insulin sensitivity contributes to the pathogenesis of hyperandrogenemia in women with PCOS. Insulin stimulates androgen synthesis in the ovaries via its cognate receptor and the inositolglycan pathway [55]. The ovaries remain sensitive to insulin when other tissues, such as fat and muscle tissues, are resistant, and the subsequent hyperinsulinaemia can augment the luteinizing hormone- and adrenocorticotrophic hormone-dependent

hyperandrogenism in insulin-resistant women with PCOS [56]. As evidence of this, research has shown that treatment of insulin resistance in women with PCOS using metformin or the insulin-sensitizer troglitazone significantly decreased serum levels of insulin and T, independent of BMI or gonadotropin levels [57,58]. Furthermore, plasma levels of HDL-C increased while plasma PAI-1 levels decreased concurrent with treatment of insulin resistance. These data imply that hyperinsulinaemia contributes to the functional ovarian hyperandrogenism in women with PCOS. By contrast, lowering androgen levels by means of treatment with GnRH agonists and androgen receptor blockade in hyperandrogenic women also was found to improve insulin sensitivity and lipid profile [59,60]. The magnitude of these changes, however, is less than that usually encountered in PCOS. Therefore, since short-term lowering of ovarian androgens by laparoscopic ovarian cauterization did not alter insulin or lipid levels [61], androgens probably only aggravate rather than account for insulin resistance in women with PCOS.

These findings do not preclude the possibility that androgens play an etiological role in PCOS. For example, experiments in rats and marmoset monkeys recently showed evidence for androgen imprinting. Transient intrauterine or perinatal exposure to T predisposed female animals to central adiposity and insulin resistance in adult life [62]. Administration of supraphysiological doses of exogenous T or other androgens to women or female cynomolgus monkeys increased BMI and the mass of both visceral fat and muscle and decreased insulin sensitivity [24]. Hence, there appears to be a vicious circle in which early androgen excess contributes to insulin resistance in adult women. The resulting hyperinsulinism contributes to the pathogenesis of PCOS and aggravates the hyperandrogenism and the associated clinical phenotype.

6. Effects of androgens on the function of vascular cells

6.1. Effects of androgens on vascular reactivity

An early hallmark of atherosclerosis is decreased vascular responsiveness to various hormonal stimuli, which is either due to endothelial dysfunction or to endothelium-independent disturbances in vascular smooth muscle cell physiology. As a result, decreased vasodilatation and enhanced vasoconstriction can lead to vasospasm and angina pectoris. Moreover, endothelial dysfunction also contributes to coronary events by promoting plaque rupture and thrombosis [63]. T can induce vasodilatation or vasoconstriction via endothelium-dependent or endothelium-independent mechanisms and by genomic or non-genomic modes of action. The diversity of these findings appears to be due to differences in species, gender, concomitant disease and, most importantly, the dosage of T administered.

Indicative of possible adverse effects associated with administration of T, the degree of nitrate-induced (and hence endothelium-independent) dilatation of the brachial arteries was shown to be significantly reduced in female-to-male transsexuals taking high-dose androgens [64]. In another case-control study, castrated patients with prostate cancer had a greater flow-induced (i.e., endothelium-dependent) dilatation of brachial arteries than did controls; whereas, the degree of endothelium-independent vasodilatation caused by administration of nitroglycerin did not differ between the groups [65]. In a group of 110 healthy men, we observed a positive association between the numbers of CAG repeats in exon 1 of the androgen receptor gene and endothelium-dependent as well as endothelial-independent vasodilatation. Thus, the greater the sensitivity to T, the less brachial arteries dilate in response to either increased blood flow or administration of nitrate [32].

In contrast to these observational studies, acute interventional studies with iv administration of T to male patients with CAD revealed apparently beneficial vasodilatory effects of T (see Section 3.3).

Likewise, 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 years in ovariectomized female cynomolgus monkeys, intracoronary injections of acetylcholine caused significant endothelium-dependent vasodilatation in treated, but not in untreated, animals. In contrast, endothelium-independent vasodilatation occurred normally in both groups [24]. In dogs, T induced vasodilatation of coronary arteries by both endothelium-dependent and -independent mechanisms [66,67]. The results of in vitro studies with isolated rings of coronary arteries and/or aortas from rats, rabbits, and pigs also showed that, in both sexes, T administration improved both endothelium-dependent and/or endothelium-independent vascular responsiveness [66–68]. However, it must be emphasized that all these studies employed supraphysiological doses of T in the micromolar range.

Teoh and colleagues [69] observed a direct vasodilatory effect of T on porcine coronary artery rings when administered at micromolar concentrations but no direct effect when administered at nanomolar dosages. In contrast, physiological doses of T inhibited the vasodilatory effects of bradykinin and calcium ionophores. Similarly, T inhibited the adenosine-mediated vasodilatation of rat coronary arteries and impaired endothelium-dependent relaxation of aortic rings from rabbits that were either made hypercholesterolaemic or that were exposed to tobacco smoke [70–72].

The cellular and molecular mechanisms by which T and E2 regulate vascular tone are little understood.

Evidence both for and against endothelium-dependent or endothelium-independent mechanisms has been found. Results of some studies suggest the involvement of endothelial nitric oxide [66,67,73]. In the coronary arteries of dogs, the aortas of rats, and the cerebral arteries of rats, the nitric oxide synthase inhibitor L-NMMA prevented T-induced vasodilatation. However, in another in vitro study, L-NMMA had no effect on T-induced vasodilatation of rabbit aortas and coronary arteries [68]. In agreement with the latter finding, Hishikawa and colleagues [74] found that in vitro expression of nitric oxide synthase in human aortic endothelial cells was stimulated by E2 but not by T. The involvement of prostaglandins is suggested by the observation that T increases the response of coronary arteries to prostaglandin F2α [71] and by the finding that dihydro-T increases the density of thromboxane receptors in rats and guinea pigs [75]. However, results of some in vivo and in vitro animal studies indicate that pretreatment with the prostaglandin synthesis inhibitor indomethacin had no effect on T-induced vasodilatation, so that the role of eicosanoids in mediating the actions of T on the arterial wall is still controversial.

It is unclear whether T regulates vasoreactivity by either genomic or non-genomic effects or both. Androgen receptor expression has been found in rat aortic smooth muscle and endothelial cells. Expression of the androgen receptor in human arterial cells has not been shown directly, although the association of endothelium-dependent and -independent vasoreactivity with the CAG repeat polymorphism in the androgen receptor provides some indirect evidence for the expression of the androgen receptor on vascular endothelial cells and smooth muscle cells, respectively [33]. Steroid hormones can also regulate vascular tone by non-genomic mechanisms that involve plasma membrane steroid receptors as well as modulation of cell membrane channels (e.g., ATP-sensitive, voltage-dependent, and calcium-activated potassium channels).

Several observations suggest that T, especially when administered at supraphysiological doses, modulates vascular tone via non-genomic modes of action and/or its secondary metabolites (e.g., E2). First, the androgen-receptor antagonists flutamide or cyproterone acetate did not inhibit the effects of T on rabbit or pig coronary arteries [68,69]. Second, the expression of aromatase in vascular endothelial cells [76] raises the possibility that T can be converted to E2 and that it exerts its vasoactive effects via activation of the estrogen receptors. However, neither the aromatase inhibitor aminogluthemide nor the estrogen-receptor antagonist ICI 182,780 prevented the T-induced vasodilatation [66]. Third, barium chloride attenuated the T-induced vasorelaxation of rabbit aortas and coronary arteries, indicating that T modulates the opening of potassium channels in vascular smooth muscle cells [68].

6.2. Effects of T on macrophage functions

Monocytes that migrate into the vascular wall differentiate into macrophages and bind lipoproteins that have permeated the endothelium and become modified within the arterial wall, for example, by oxidation. The uptake of modified lipoproteins by macrophages leads to the formation of large foam cells. These cells, together with T-lymphocytes, release inflammatory mediators, which stimulate the proliferation and migration of smooth muscle cells. Human monocyte-derived macrophages express the androgen receptor in a gender-specific manner. Macrophages of male donors exhibit a 4-fold higher expression of the androgen receptor than do macrophages of female donors [77]. There is also evidence that T regulates macrophage function by non-genomic effects via a G-protein-coupled, agonist-sequesterable plasma membrane receptor that initiates calcium- and 1,4,5-trisphosphate-signalling pathways [78].

Unregulated uptake of oxidatively modified lipoproteins via type A scavenger receptors leads to the intracellular accumulation of cholesteryl esters in macrophages and thereby to formation of foam cells [63,79]. E2 inhibits oxidation of LDL both in the presence and absence of cells, including macrophages. By contrast, T increases the oxidation of LDL by placental macrophages in vitro [80]. Moreover, dihydro-T dose-dependently stimulates the uptake of acetylated LDL by scavenger receptor type A and, hence, to intracellular cholesteryl ester accumulation in macrophages. In addition to the increased expression of the androgen receptor in male donors, this effect was seen in the macrophages of male, but not of female, donors. The stimulatory effect of dihydro-T was blocked by the androgen receptor antagonist hydroxyflutamide [77].

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 acylCoA–cholesterol:acyltransferase. The formed cholesteryl esters can be stored in the cytosol, giving the foamy appearance of lipid-laden macrophages [80]. 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 androstendione. The 17-hydroxy-steroids, which include T, are less effective. Cytosolic cholesteryl esters can be hydrolyzed by neutral cholesterol esterase (NCEH), which is activated by cyclic adenosine monophosphate (AMP). NCEH is more active in the adipose tissue of female rats than in the adipose tissue of male rats. Moreover, exogenous E2 increases NCEH activity in male rats and in female rats that have been ovariectomized. In vitro, E2, but not T, increases the activity of

NCEH in the murine macrophage cell line J774, probably by increasing the activity of a cyclic AMP-dependent protein kinase A [81].

Non-hepatic and non-steroidogenic cells cannot metabolize cholesterol and, therefore, can only dispose of excess cholesterol by secretion. Hence, cholesterol efflux from cells is central to the regulation of cellular cholesterol homeostasis. Non-specific, passive processes (i.e., aqueous diffusion) as well as specific, active processes (i.e., receptor-mediated) are involved. To date, two plasma membrane proteins are known to facilitate cholesterol efflux. Interaction of the scavenger receptor B1 with mature lipid-containing HDL is thought to facilitate cholesterol efflux by reorganizing the distribution of cholesterol within the 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 [38]. We have found that T up-regulates the expression of the scavenger receptor 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 [39].

Activated macrophages produce various cytokines, including chemotactic protein 1, interleukins (IL) 1 and 10 and tumour necrosis factor α (TNFα), 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 smooth muscle cell proliferation and migration) [63,80]. The effects of T on the production of cytokines and growth factors have not been studied in foam-cell macrophage models, only in unstimulated or lipopolysaccharide-stimulated macrophages. Whether these results are also valid for macrophages in the arterial wall is not known. For example, E2, but not T, inhibited the migration of monocytes in response to chemotactic protein 1. In J774 macrophages, T exerted potentially anti-inflammatory effects by stimulating IL-10 synthesis and by inhibiting the production of TNFα and nitric oxide [82].

6.3. Effects of T on arterial smooth muscle function

Rat arterial smooth muscle cells were found to express the androgen receptor. There is some evidence that T modulates endothelium-independent vasoreactivity, which supports the hypothesis that smooth muscle cells play a physiologic role in (see Section 6.1). Arterial smooth muscle cell proliferation, migration, and matrix production also play important roles in atherosclerosis [63]. However, the effect of T on these processes has not been investigated. Whereas E2 was shown to inhibit proliferation and migration of smooth muscle cells, T had no such effect [83,84]. Moreover, the protection of female rabbits from atherosclerosis by E2, but not the protection of male rabbits by T, was associated with decreased incorporation of 5′-bromo-2′-deoxyuridine into the DNA of neointimal cells, an in vivo marker of arterial smooth muscle cell proliferation [21].

7. Estrogens and cardiovascular disease in men

There is compelling evidence indicating that the physiological actions of T in men can be mediated by the estrogen receptors (ERs) following conversion to E2 by site-specific aromatases in target tissues [85]. The existence of two nuclear ER subtypes, α and β, as well as a membrane ER encoded by the same transcript as the α nuclear receptor, attest to the potential for many different biological estrogen effects. ERα, ERβ, and aromatase are detectable in the coronary arteries of the monkey and man [76,86,87]. The extra-glandular production of estrogens (with circulating androgens as the immediate precursor substrate) may therefore play a role in male cardiovascular physiology and pathophysiology.

In men, the importance to cardiovascular health of locally produced estrogens from aromatisation of T is highlighted by results of recent studies in human and transgenic mouse models of aromatase deficiency and estrogen resistance. In case reports of two men with undetectable circulating E2 and estrone levels concurrent with high T levels caused by P450 aromatase deficiency [88,89] both men had dyslipidaemia with elevated total cholesterol, LDL-C, and triglyceride levels, along with decreased HDL-C levels, and one man had insulin resistance. These metabolic abnormalities were correctable by administration of low-dose oral or transdermal estrogen replacement therapy. Insulin resistance, impaired glucose tolerance, and low HDL-C levels were also apparent in a 28-year-old man with a null mutation in the ERα gene causing estrogen resistance [90]. In addition, intact hepatic ERβ may have prevented full expression of dyslipidaemia. Ultrafast electron beam computed tomography imaging showed calcium deposition in the proximal left anterior descending coronary artery, indicating the presence of premature atherosclerosis. Flow-mediated brachial artery endothelial-dependent vasodilatation was absent, showing marked endothelial dysfunction [91].

These rare experiments of nature suggest that estrogens are important in maintaining normal carbohydrate and lipid metabolism as well as normal endothelial-dependent and, hence, nitric oxide-mediated vasodilatation in men. These results are compatible with data from transgenic knock-out models confirming that ERα

is important in preventing adipocyte hypertrophy, obesity, insulin resistance, and hypercholesterolaemia [92], and in maintaining basal nitric oxide release from the vascular endothelium in male animals. In addition, ERβ in vascular smooth muscle may also regulate vascular sensitivity to E2 [87,93,94]. The favourable effects of estrogens on HDL-C that were demonstrated are also in accord with results of clinical studies using aromatase inhibitors in normal men.

8. Conclusions and therapeutic implications

Current evidence indicates that the difference between genders in the incidence of CAD cannot be explained on the basis of ambient T exposure. Androgens can exert both beneficial and deleterious effects on a multitude of factors implicated in the pathogenesis of atherosclerosis and CAD. Thus, at present, it is not possible to determine the net effect of T on CAD.

What are the clinical implications of this ongoing uncertainty? In our view, the answer to this question must differentiate between concern for the possibility of cardiovascular side effects as a result of androgen treatment of endocrine and non-endocrine conditions on the one hand and whether T may be used for the prevention or even treatment of CAD on the other hand.

Efforts to fully develop the therapeutic benefits of T in the treatment of hypogonadism, osteoporosis, wasting and chronic consumptive disease, or for contraception in a wider male population should not be unduly deterred or hampered by concerns regarding increased risks of CAD. However, the possibility that spontaneous or induced hyperandrogenemia may increase the risks for CAD in women needs to be seriously considered.

Some clinicians argue that androgen replacement therapy for elderly men has the potential to prevent CAD, as well as having possible beneficial effects on muscles and bones and sexual and mental functions. However, androgens have such an extraordinary array of effects in vivo that it is hazardous to extrapolate isolated experimental findings to the wider clinical setting. It is premature to assume that clinical benefits can be derived from manipulation of the sex steroid milieu – even when these assumptions are based on biologically plausible mechanisms or, indeed, on cross-sectional risk-factor observational data – when dealing with a complex multifactorial condition such as CAD. Interpretations of the effects of pharmacological doses of androgens on arterial compliance and flow-mediated dilatation in particular must be treated with circumspection. For example, the lessons from estrogen HRT in postmenopausal women are especially salutary. However, despite the overwhelmingly positive but indirect evidence that HRT reduces the risk factors and disease incidence, recent controlled interventional studies have not confirmed that estrogens are an effective prevention of CAD in women [95–97]. Analogously, if HRT were to become an acceptable therapeutic entity for men, randomized interventional trials will be needed to assess clinical endpoints. In the absence of such information on T, priority must be given to treatment modalities that are proven to be effective in the prevention or treatment of CAD (e.g., weight reduction, smoking cessation, exercise, and therapy with aspirin, statins, anti-hypertensives, and vasodilators).

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