Endogenous Sex Hormones and Progression of Carotid Atherosclerosis in Elderly Men

Majon Muller, MD, PhD; Annewieke W. van den Beld, MD, PhD; Michiel L. Bots, MD, PhD; Diederick E. Grobbee, MD, PhD; Steven W.J. Lamberts, MD, PhD; Yvonne T. van der Schouw, PhD

Background—The burden of atherosclerosis especially afflicts the increasing older segment of the population. Recent evidence has emphasized a protective role of endogenous sex hormones in the development of atherosclerosis in aging men.

Methods and Results—We studied the association between endogenous sex hormones and progression of atherosclerosis in 195 independently living elderly men. Participants underwent measurements of carotid intima-media thickness (IMT) at baseline in 1996 and again in 2000. At baseline, serum concentrations of testosterone (total and free) and estradiol (total and free E_2) were measured. Serum free testosterone concentrations were inversely related to the mean progression of IMT of the common carotid artery after adjustment for age ($\beta=-3.57$; 95% CI, −6.34 to −0.80). Higher serum total and free E_2 levels were related to progression of IMT of the common carotid artery after adjustment for age ($\beta=0.38$; 95% CI, −0.11 to 0.86; and $\beta=0.018$; 95% CI, −0.002 to 0.038, respectively). These associations were independent of body mass index, waist-to-hip ratio, presence of hypertension and diabetes, smoking, and serum cholesterol levels

Conclusions—Low free testosterone levels were related to IMT of the common carotid artery in elderly men independently of cardiovascular risk factors. **(*Circulation*. 2004;109:2074-2079.)**

Key Words: atherosclerosis ■ carotid arteries ■ hormones ■ men

Cardiovascular disease (CVD) is the prime cause of death among the elderly in Western countries and is a major determinant of chronic disability.[1] The burden of atherosclerosis especially afflicts the increasing older segment of the population. Several factors such as increasing age, hypercholesterolemia, hypertension, cigarette smoking, diabetes mellitus, obesity, and male sex accelerate the rate of progression of atherosclerosis.[2] It has been suggested that, with aging, other factors such as inflammation and endogenous sex hormone levels have an increasing independent effect on the risk of CVD.[3] Although men do not experience an abrupt reduction in endogenous sex hormone production, an age-associated decrease in the levels of endogenous sex hormones does occur.[4] Whether changes in endogenous sex hormone levels in men have an impact on CVD has remained largely unknown, but in the past decade, more attention has been given to the importance of testosterone and estrogens in the cause, prevention, and treatment of male CVD.[5,6]

The putative (anti)atherogenic mechanism of sex hormones is not well understood, but several hypotheses have been proposed. Some data suggest that testosterone may affect the development of CVD by modulating risk factors such as diabetes,[7] obesity,[8] hypertension,[9] and hypercholesterolemia.[10–12] Furthermore, androgens have been shown to dilate the coronary, aortic, and brachial vasculature by both endothelial-dependent and -independent mechanisms.[13] In addition, androgen receptors have been demonstrated within the cardiovascular system found within human and animal systemic arteries, including aortic, coronary, pulmonary, and carotid arteries, and may mediate the effects of androgens on the arterial wall.[14]

Recent population-based studies have emphasized a protective role of endogenous sex hormones in the development of atherosclerosis in aging men.[15,16] However, to the best of our knowledge, no other study investigated the relationship between endogenous sex hormone levels and progression of atherosclerosis in the oldest old. Therefore, we studied whether serum testosterone and estradiol levels predict progression atherosclerosis as measured by intima-media thickness (IMT) of the carotid artery in a population of independently living men 77 to 96 years of age.

Methods

Subjects

In 1996, names and addresses of all male inhabitants ≥70 years of age were drawn from the municipal register of Zoetermeer, a medium-sized town in the midwestern part of the Netherlands, and 1567 men were invited as described previously.[16] A total of 886 men

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From the Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht (M.M., M.L.B., D.E.G., Y.T.v.d.S.), and Department of Internal Medicine, Erasmus University Medical Center, Rotterdam (A.W.v.d.B., S.W.J.L.), the Netherlands.

Correspondence to Yvonne T. van der Schouw, PhD, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, PO Box 85500, Room D 01.335, 3508 GA Utrecht, The Netherlands. E-mail y.t.vanderschouw@umcutrecht.nl

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did not respond to the mailed invitation, which mentioned that only subjects who lived independently and had no severe mobility problems could participate. After exclusion of subjects who did not live independently and those who were not physically or mentally able to visit the study center independently, 403 men 73 to 94 years of age (25.7%) participated. All participants provided written informed consent, and the Medical Ethics Committee of the University Hospital Rotterdam approved the study. No additional health-related eligibility criteria were used. The baseline examination in 1996 comprised a structured interview, physical examination, blood sampling, and measurements of IMT of the carotid arteries at the research center.

We reinvited the 327 participants who were still living for a second examination in 2000, of whom 245 (61%) participated. Because of technical problems and inability to visit the study center, 50 of the 245 participants were ineligible to undergo a second measurement of the carotid IMT.

Procedure

At baseline, presence of CVD was defined as symptoms of or treatment for angina pectoris, congestive heart failure, or intermittent claudication or as a medical history of myocardial infarction or cerebrovascular accident. Participants were asked about current use of medications and smoking history. Height and weight were measured at baseline with participants in the standing position but not wearing shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured at the level of the umbilicus, and hip circumference was measured at the level of the greater trochanter. The average of 2 readings was used in the analyses. Waist-to-hip ratio (WHR), which is a measure of upper body adiposity, was calculated from these 2 measurements. Serum total, LDL, and HDL cholesterol and triglyceride concentrations were measured by use of commercially available radioimmunoassay kits.

Measures of Progression of Atherosclerosis

Carotid IMT was taken as a quantitative measure of generalized atherosclerosis[17] and measured by ultrasonography of both the left and right common carotid arteries with a 7.5-MHz linear-array transducer. At baseline, an ATL Ultramark IV (Advanced Technology Laboratories, Inc) was used, and follow-up measurements were obtained with an Acuson Aspen ultrasound device. A careful search was conducted for all interfaces of the near and far walls of the distal common carotid artery.[18] One trained sonographer performed both the baseline and follow-up measurements. The actual IMT measurements were performed offline. At baseline, an automatic edge-detection program (Artery Measurement System) was used as described previously[19]; follow-up measurements were obtained with Image-Pro (Image-Pro Plus, Media Cybernetics).[20] The baseline and follow-up echographic images were read by separate readers. To assess possible systematic differences between these readers, IMT measurements were assessed by scanning 10 subjects on a second occasion from which the IMT images were independently read by both readers. The absolute mean difference between the readers was 0.09 mm. Progression of IMT of the common carotid artery was defined as the difference between the IMT of the follow-up measurement and the baseline measurement, taking the previously mentioned between-reader difference in IMT of 0.09 mm into account.

Hormone Measurements

At baseline, blood samples were collected in the morning after an overnight fast. Serum concentrations of total testosterone (nmol/L), free testosterone (nmol/L), and sex hormone–binding globulin (nmol/L) were measured by radioimmunoassay with commercial kits (Diagnostic Systems Laboratories, Inc). The free testosterone radioimmunoassay uses a [^{125}I]-labeled testosterone analog, which has a low affinity for SHBG and albumin. This analog competes with the unbound testosterone in the test sample for binding to specific anti-testosterone polyclonal antibodies that have been immobilized on the assay tube. This competitive binding format allows direct estimation of unlabeled free testosterone levels in unextracted samples. Serum concentrations of estradiol (E_2) (nmol/L) were also measured by radioimmunoassay with commercial kits (Diagnostic Systems Laboratories, Inc). As a measure of biologically active E_2, free E_2 (pmol/L) was calculated according to the method described by Södergård et al,[21] taking the concentration of testosterone into account. Albumin (g/L) was measured by photometry with a commercial kit (ALB, Boehringer).

TABLE 1. Interobserver and Intraobserver Precision Results of the Hormone Measurements

	Interassay Coefficient, %	Intra-Assay Coefficient, %
Total testosterone	10.5	8.1
Free testosterone	9.7	6.2
Total estradiol	10.2	5.6
Sex hormone–binding globulin	4.4	3.0

Data Analyses

Distributions of anthropometric and lifestyle characteristics, sex hormone concentrations, and CVD risk factors were expressed as mean and SD.

In multivariate linear regression models, baseline testosterone and estradiol levels were investigated in relation to progression of IMT. Results were described as the linear regression coefficient (β), its SE, and the 95% CI. To compare the impact of the several sex hormones on the progression of IMT, we also presented standardized linear regression coefficients. Because an association was present between several hormone concentrations and age, multiple regression analysis was used to adjust for age. Estimates were further adjusted for BMI (kg/m^2), WHR, hypertension (yes/no), diabetes (yes/no), smoking status (current/former/never), and serum cholesterol (total, HDL, LDL, triglyceride) levels (nmol/L). To assess whether a threshold was present, multivariate models using ANCOVA analyses were used to estimate mean (SE) progression in IMT across tertiles of sex hormones. Trend analyses were performed through linear regression analysis. Data analyses were performed with SPSS statistical software (version 11.5). Table 1 gives the interobserver and intraobserver precision results.

Results

Mean age of the population at baseline was 77.2 years (range, 73 to 91 years). Descriptive values of the sex hormone levels, general characteristics, and data on IMT of the common carotid artery at baseline are presented in Table 2. A number of participants were taking medications for hypertension (n=42) and diabetes (n=12). At baseline, 37 of the 195 men smoked at the time of the investigation, whereas 96 subjects did not but had smoked previously. Sixty-two subjects had never smoked. Mean IMT at follow-up measurement was 1.09 mm (range, 0.69 to 2.51 mm). Taking the systematic error (0.09 mm) of the IMT measurement into account, the progression rate of IMT was 0.01 mm/y. In our population, serum total testosterone, total E_2, and free E_2 were not significantly associated with age (data not shown). Serum free testosterone was significantly inversely related with age [$\beta=-0.008\pm0.002$ (nmol/L)/10 y; $P<0.01$]. Serum total testosterone concentrations were inversely related to BMI [$\beta=-0.15\pm0.07$ (nmol/L)/(kg/m2); $P=0.03$]. Serum free testosterone, total E_2, and free E_2 were not significantly associated with BMI. Endogenous total testosterone was not related to progression of IMT of the common carotid artery,

TABLE 2. Baseline Characteristics of 195 Ambulatory Elderly Dutch Men Studied in Terms of Serum Sex Hormone Levels and Carotid Atherosclerosis

	Mean	SD
General characteristics		
Age, y	77.2	3.1
BMI, kg/m^2	25.5	3.0
WHR	0.98	0.05
Cholesterol risk factors, nmol/L		
Total	5.86	1.11
LDL	3.89	0.98
HDL	1.34	0.33
Triglycerides	1.37	0.75
Serum sex hormone concentrations		
Total testosterone, nmol/L	9.00	2.78
Free testosterone, nmol/L	0.032	0.011
Total estradiol, nmol/L	0.101	0.063
Free estradiol, pmol/L	2.56	1.54
Sex hormone–binding globulin, nmol/L	30.9	14.0
Albumin, g/L	45.8	2.5
Common carotid artery IMT, mm	0.96	0.15
Medical history (presence), %		
Cardiovascular disease	29.2	
Diabetes	6.2	
Hypertension	21.5	
Smoking (current), %	19.0	

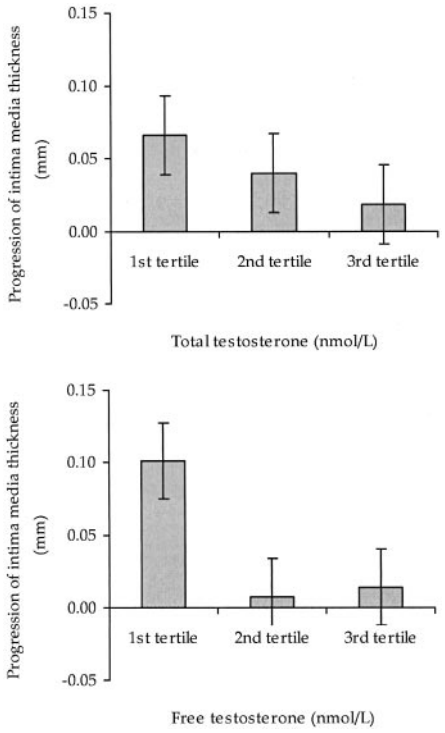

Figure 1. Progression of mean IMT of common carotid artery in tertiles of serum total and free testosterone concentrations (nmol/L). Adjustments were made for age. Probability values for trend tests over tertiles of total and free testosterone were 0.21 and 0.02. Limits in total testosterone level for different tertiles were as follows: first tertile, 0.17 to 7.92 nmol/L; second tertile, 7.92 to 9.76 nmol/L; and third tertile, 9.76 to 17.22 nmol/L. Limits for free testosterone level for different tertiles were as follows: first tertile, 0.13 to 2.75×10^{-2} nmol/L; second tertile, 2.75 to 3.57×10^{-2} nmol/L; third tertile, 3.57 to 7.27×10^{-2} nmol/L.

with or without adjustment for age and cardiovascular risk factors (Table 3). Serum free testosterone concentrations, however, were inversely related to the mean progression of IMT of the common carotid artery after adjustment for age (Table 3). The association between serum free testosterone levels and progression of IMT was independent of BMI, WHR, presence of hypertension and diabetes, smoking, and serum cholesterol levels (total, HDL, and LDL cholesterol and triglycerides) ($\beta=-3.40\pm1.42$; $P=0.02$). Higher serum total and free E_2 levels might be related to progression of IMT of the common carotid artery after adjustment for age (Table 3); however, probability values were borderline significant ($P=0.12$, and 0.08, respectively). Standardized linear regression coefficients show that baseline free testosterone levels are more strongly related to atherosclerosis than the other sex hormone levels. None of the results changed after adjustment for SHBG levels (data not shown).

Figures 1 and 2 suggest that there is a threshold for the relation of free testosterone and free E_2 levels with progression of IMT. Subjects with baseline free testosterone levels in the lowest tertile had a significantly more marked progression

TABLE 3. Regression Coefficients (Unstandardized and Standardized) Resulting From Use of Progression of IMT of the Common Carotid Artery as Dependent Variable and Endogenous Testosterone and Estradiol Levels as Independent Variables in a Study of 195 Elderly Men

	Progression of IMT*				
	Unstandardized		Standardized (per SD)		
	β†	95% CI	SD	β†	95% CI
Total testosterone, nmol/L	−0.007	−0.018–0.004	2.98	−0.022	−0.054–0.011
Free testosterone, nmol/L	−3.57	−6.34−0.80	0.013	−0.047	−0.084−0.011
Total estradiol, nmol/L	0.38	−0.11–0.86	0.058	0.022	−0.006–0.050
Free estradiol, pmol/L	0.018	−0.002–0.038	1.43	0.024	−0.003–0.054

*Estimates are adjusted for age.

†β=coefficient of linear regression; an increase of the independent variable by 1 unit is associated with a β increase of IMT.

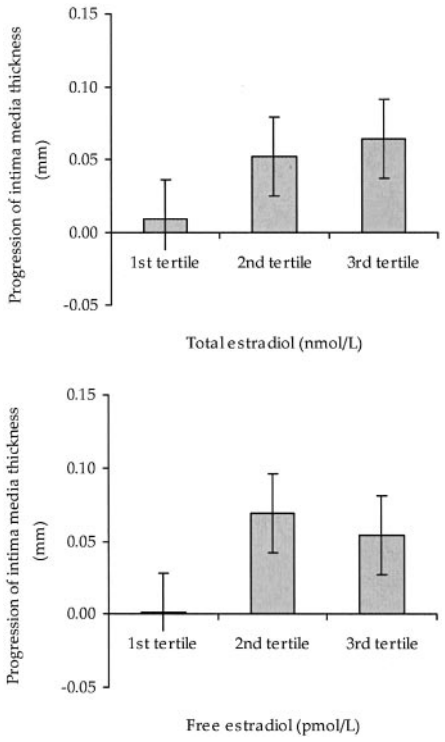

Figure 2. Progression of mean IMT of the CCA in tertiles of serum total and free estradiol concentrations (pmol/L). Adjustments were made for age. Probability values for trend tests over tertiles of total and free estradiol were 0.14 and 0.17. Limits in total estradiol level for different tertiles were as follows: first tertile, 0.012 to 0.067 nmol/L; second tertile, 0.067 to 0.112 nmol/L; and third tertile, 0.112 to 0.621 nmol/L. Limits in free estradiol level for different tertiles were as follows: first tertile, 0.29 to 1.75 pmol/L; second tertile, 1.75 to 2.78 pmol/L; and third tertile, 2.78 to 7.70 pmol/L.

of IMT (mean±SE progression, 0.101±0.026 mm) compared with subjects in the second and third tertiles (mean±SE progression, 0.007±0.027 and 0.014±0.026 mm, respectively); the probability value for the difference between extreme tertiles was 0.022. Subjects with baseline free E_2 levels in the second and third tertiles had a larger progression of IMT (mean±SE progression, 0.054±0.027 and 0.069±0.027 mm, respectively) compared with subjects in the first tertile (mean±SE, 0.001±0.027 mm). However, differences between tertiles did not reach statistical significance ($P=0.17$).

Discussion

In this prospective study of 195 independently living elderly men, we examined the association of endogenous sex hormone levels with progression of atherosclerosis. Results suggest that low free testosterone levels and high estradiol levels were related to thickening of the IMT of the common carotid artery. These associations were independent of cardiovascular risk factors.

To appreciate these findings, some issues need to be addressed. To the best of our knowledge, this study is the first to examine the effect of endogenous sex hormone levels on the progression of atherosclerosis in elderly men. Thickening of the IMT of the carotid artery is generally considered an early marker of generalized atherosclerosis[22] and has been associated with an unfavorable cardiovascular risk profile,[23] other localizations of atherosclerosis, and an increased risk of myocardial infarction and stroke.[17,24]

Our results are in agreement with results of other observational studies describing lower levels of total and free testosterone in association with atherosclerosis[15,16,25] and coronary artery disease.[26] Furthermore, recent cross-sectional studies have shown that low levels of free testosterone are associated with increased IMT in men with type 2 diabetes.[27,28] These results challenge the preconception that physiologically high levels of androgens in men account for their increased relative risk for coronary disease. However, a large-scale longitudinal study in men 40 to 80 years of age showed that no sex hormone measured (testosterone, androstenedione, estrone, or estradiol) was significantly associated with known CVD at baseline or with subsequent cardiovascular mortality or ischemic heart disease morbidity or mortality.[6]

Our estimate of IMT progression of 0.01 mm is comparable to the finding in another observational study in which average annual changes in mean common carotid IMT were between 0.007 and 0.01 mm.[29] It is difficult to indicate the clinical importance of the magnitude of change associated with the low free testosterone levels. However, men who have free testosterone levels in the lowest tertile have an IMT progression of 0.10 mm, which is 10 times the IMT progression in men with free testosterone level in the highest tertile (0.01 mm).

The mechanisms of the beneficial effect of testosterone on atherosclerosis in men are largely unknown. It has been suggested that testosterone may affect atherosclerosis through modulation of classic cardiovascular risk factors.[7–12] The fact that multivariate adjustment did not influence the association between testosterone and atherosclerosis in men in our study sample does not support this hypothesis and suggests that direct beneficial effects of testosterone on atherogenesis, probably mediated by the androgen receptor, may be involved.[14] To definitively demonstrate such actions of testosterone, randomized trials are needed. Several small-scale intervention studies have not shown consistent effects of testosterone replacement on cardiovascular function so far.[30–33] Another possible mechanism of testosterone could be the aromatization of testosterone into estradiol at the cellular level. Plasma levels of estradiol do not necessarily reflect tissue-level activity because peripherally formed estradiol is partially metabolized in situ; thus, not all enters the general circulation, with a fraction remaining only locally active.[34] Furthermore, it has been suggested that sex hormones have a sex-specific effect in the 2 sexes.[35] Animal studies evaluating the effect of androgens on the progression of atherosclerosis have shown beneficial effects when male animals were tested and detrimental effects when androgens were administered to female animals.[35]

Few studies have examined the relation between endogenous estradiol levels and atherosclerosis in men. Our findings that (free) E_2 levels are weakly associated with an increase in IMT, even after adjustment for cardiovascular risk factors, are in contrast with previous findings that showed no asso-

ciation of E_2 levels with atherosclerosis.[16,36] However, these cross-sectional studies did not investigate the relationship of free E_2 levels with atherosclerosis. In the present study, estradiol levels did not decline with age, which is in contrast to several other observational studies in older men.[37,38] These conflicting findings could be explained by the small age range of our study population. Furthermore, of the factors influencing plasma estradiol levels, plasma testosterone is a major determinant. The age-associated decrease in testosterone levels is scarcely reflected in plasma estradiol levels as a result of increasing aromatase activity with age and the age-associated increase in fat mass.[34]

We found a more consistent relation between free sex hormone levels and atherosclerosis compared with total sex hormone levels and atherosclerosis. This finding suggests that it is the unbound form of sex hormones that affects atherosclerosis or change in atherosclerosis. A possible explanation for this finding is that bioavailable sex hormones make up the fractions that are free or associated with albumin in the circulation,[4,21] and it is these fractions that have rapid access to target tissues.[39] As SHBG levels increase with age in men,[40] measurement of total testosterone or E_2 levels does not accurately reflect the actual levels of these steroids available to tissues.

The interpretability of the results may be limited by certain aspects of the study. Subjects who participated in the second examination (48% of the baseline population) might represent a healthier group because of selective mortality. If anything, however, this selection may have led to an underestimation of the true effect. Furthermore, some error could have occurred in the measurement of atherosclerosis resulting from 2 different readers of the sonographic images. Such an error would also have led to misclassification and limited precision and thus to underestimation of any true association between sex hormone levels and progression of IMT. Another issue is whether to adjust for baseline level of the outcome variable. It has been argued that baseline adjustments in studies on progression lead to an overestimation of results and at least to biased results.[29] Therefore, we took the unadjusted approach.

To overcome the potential effects of different ultrasound equipment and different readings on results, we also performed analyses in which we studied change over time in the ranking of a subject instead of studying progression of IMT in millimeters. These ranking analyses gave the same results in terms of direction and probability values. However, because a change in distribution is much more difficult to understand than progression of IMT, we chose to present the latter.

In summary, male low free testosterone levels are associated with progression of atherosclerosis in the oldest old independently of other cardiovascular risk factors. Furthermore, a tendency toward an association between high estradiol levels and IMT progression is observed. Relations between classic cardiovascular risk factors and CVD become increasingly complex with advancing age, and other factors such as sex hormone levels may be of increasing importance in relation to CVD risk in older individuals.[3]

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