Original Article

Low Testosterone Level Is an Independent Determinant of Endothelial Dysfunction in Men

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We investigated whether a low plasma testosterone level is related to endothelial dysfunction in men with coronary risk factors. One hundred and eighty-seven consecutive male outpatients (mean age±SD: 47±15 years) who underwent measurement of flow-mediated vasodilation (FMD) of the brachial artery using ultrasonography were enrolled. The relationship between plasma hormones and FMD was analyzed. Total and free testosterone and dehydroepiandrosterone-sulfate (DHEA-S) were significantly correlated with %FMD (r=0.261, 0.354 and 0.295, respectively; p<0.001), while estradiol and cortisol were not. %FMD in the highest quartile of free testosterone was 1.7-fold higher than that in the lowest quartile. Multiple regression analysis revealed that total and free testosterone were related to %FMD independent of age, body mass index, hypertension, hyperlipidemia, diabetes mellitus and smoking (β=0.198 and 0.247, respectively; p<0.01), and were independent of age, body mass index, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, fasting plasma glucose, smoking and nitroglycerin-induced dilation (β=0.196 and 0.227, respectively; p<0.01). DHEA-S was not significantly related to %FMD in multivariate analysis. In conclusion, a low plasma testosterone level was associated with endothelial dysfunction in men independent of other risk factors, suggesting a protective effect of endogenous testosterone on the endothelium. (*Hypertens Res* 2007; 30: 1029–1034)

***Key Words*: androgen, sex hormone, vasodilation, endothelium, risk factor**

Introduction

Androgen levels decline with advancing age in men (*1*, *2*). Decreases in hormonal activity have been considered physiologic, but are often associated with the pathological process of aging, which includes such effects as erectile dysfunction, osteopenia, sarcopenia, depressed mood and cognitive impairment (*1*, *3*). Also, not all but many recent observational studies have shown that a low plasma testosterone level is associated with advanced atherosclerosis (*4*, *5*), and a higher incidence of cardiovascular disease (*6*), suggesting that endogenous testosterone may protect against the development of cardiovascular disease in men. The inverse correlations between testosterone and coronary risk factors such as obesity (*4*, *7*) and high blood pressure (*8*, *9*), plasma lipids (*4*, *7*, *8*), and plasma glucose (*7*, *10*) may provide insight into the mechanism of the effect of testosterone on cardiovascular disease. Furthermore, anti-ischemic (*11*, *12*) and endothelium-dependent vasodilating (*13*, *14*) effects of testosterone supplementation have been reported. These findings led us to hypothesize that men with a low plasma testosterone level would have impaired vasomotor function.

To test this hypothesis, we conducted a cross-sectional sur-

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Table 1. Characteristics of Study Subjects (*N*=187)

Age (years)	47±15	[20–79]
Body mass index (kg/m^2)	25.7±4.6	[19.0–47.2]
Risk factors		
Hypertension (*n* (%))	69 (37)	
Hyperlipidemia (*n* (%))	82 (44)	
Diabetes mellitus (*n* (%))	37 (20)	
Current smoker (*n* (%))	87 (46)	
Hemodynamic and vascular measurements		
Systolic blood pressure (mmHg)	126±16	[98–185]
Diastolic blood pressure (mmHg)	76±13	[53–128]
%FMD	5.4±3.7	[0.0–20.2]
%NTG	13.6±5.0	[1.6–27.2]
Carotid IMT (mm)	0.96±0.36	[0.3–1.4]
Blood chemistry and hormones		
Total cholesterol (mmol/L)	5.23±1.00	[3.06–8.70]
HDL cholesterol (mmol/L)	1.28±0.42	[0.67–3.42]
Triglycerides (mmol/L)	1.73±1.40	[0.36–9.94]
Fasting plasma glucose (mmol/L)	5.78±1.10	[4.21–12.54]
Hemoglobin A1c (%)	5.5±1.3	[3.9–10.4]
Total testosterone (nmol/L)	17.4±5.7	[4.6–33.6]
Free testosterone (pmo/L)	61.0±22.5	[18.7–166.8]
DHEA-S (μmol/L)	4.78±2.51	[0.56–11.96]
Estradiol (pmol/L)	120±31	[50–216]
Cortisol (nmol/L)	375±133	[83–742]

Values except risk factors are expressed as the mean±SD [range]. %FMD, percent flow-mediated dilation of brachial artery; %NTG, percent nitroglycerin-induced dilation of brachial artery; IMT, intima-media thickness of common carotid artery; HDL, high-density lipoprotein; DHEA-S, dehydroepiandrosterone-sulfate.

vey of 187 men by examining flow-mediated dilation of the brachial artery (%FMD) and plasma sex hormones, and showed that a low testosterone level was associated with endothelial dysfunction.

Methods

Subjects

One hundred and eighty-seven consecutive male outpatients of our department, who underwent examination of vasomotor function of the brachial artery and intima-media thickness (IMT) of the carotid artery in our department, were enrolled. The subjects were referred to our department to check for cardiovascular disease or risks. All of them were in chronic stable condition. A history was taken, and physical examination and laboratory tests were performed in all subjects. Subjects with a history of cardiovascular disease, including stroke, coronary heart disease, congestive heart failure or peripheral arterial disease, malignancy, overt endocrine disease or use of steroid hormones were excluded, because these conditions may have a serious influence on both plasma sex hormones and endothelial function. Subjects who showed a carotid IMT >1.5 mm were also excluded, because such subjects might have significant subclinical atherosclerosis. The characteristics of the study subjects are shown in Table 1.

Table 2. Pearson's Correlation Coefficients between Age, Vascular Measurements and Plasma Hormones

	Age	%FMD	Carotid IMT
Total testosterone	0.057	0.261†	0.003
Free testosterone	−0.288†	0.354†	−0.259†
DHEA-S	−0.604†	0.295†	−0.356†
Estradiol	0.155*	−0.062	0.234*
Cortisol	−0.047	0.081	−0.082

%FMD, percent flow-mediated dilation of brachial artery; IMT, intima-media thickness of common carotid artery; DHEA, dehydroepiandrosterone-sulfate. $^\dagger p<0.001$, $*p<0.05$

Seventy-six percent of the subjects had one or more of the classical coronary risk factors, such as hypertension, hyperlipidemia, diabetes mellitus or current smoking. Hypertension, hyperlipidemia and diabetes mellitus were defined according to the diagnostic criteria (*15–17*) or if the subjects were taking any medications for these diseases. Ninety-five percent of the hypertensive subjects were treated: 77% with calcium antagonists, 18% with angiotensin-converting enzyme inhibitors, 12% with diuretics and 7% with β-blockers. Seventy-seven percent of the hyperlipidemic subjects were treated with statins, and 81% of the diabetic subjects were treated with oral hypoglycemic agents. None of the study subjects were taking nitrates. Each subject gave written informed consent before enrollment in this study. The study protocol was approved by the ethics committee of the Graduate School of Medicine, The University of Tokyo.

Vascular Measurement

Vasomotor function of the brachial artery was evaluated using an ultrasound machine according to the method described previously (*18*). Briefly, endothelium-dependent %FMD was measured as the maximal percent change of the vessel diameter after reactive hyperemia. Subsequently, endothelium-independent nitroglycerin-induced vasodilation (%NTG) was measured as the maximal percent change of the vessel diameter after sublingual administration of nitroglycerin spray (0.3 mg; Toa Eiyo Co., Tokyo, Japan). Carotid IMT was evaluated using an ultrasound machine as described previously (*18*). The same examiner performed the measurements of FMD throughout this study. The subjects were examined in the morning after a 14-h overnight fast, and reclined on the bed for 15 min in a quiet, temperature-controlled (22–24°C) room before measurements.

Table 3. Age-Adjusted Regression Coefficients between Vascular Measurements and Plasma Hormones

	%FMD	Carotid IMT
Total testosterone	0.282†	−0.050
Free testosterone	0.324†	−0.090
DHEA-S	0.262†	0.036
Estradiol	−0.005	0.139
Cortisol	0.071	−0.053

Standardized regression coefficients by multiple regression analyses with %FMD or carotid IMT as a dependent variable and age and each of the hormones as independent variables are shown. †$p<0.001$. %FMD, percent flow-mediated dilation of brachial artery; IMT, intima-media thickness of common carotid artery; DHEA, dehydroepiandrosterone-sulfate.

Table 4. Regression Coefficients between %FMD and Plasma Hormones Adjusted for Coronary Risk Factors

	Model 1	Model 2	Model 3	Model 4
Total testosterone	0.198§	0.210§	0.216§	0.196§
Free testosterone	0.247§	0.266§	0.255§	0.227§
DHEA-S	0.091	0.150	0.175	0.170
Estradiol	0.033	0.024	0.061	−0.001
Cortisol	0.012	−0.001	−0.004	−0.073

Standardized regression coefficients by multiple regression analyses with %FMD as a dependent variable and coronary risk factors (covariates used in each analysis are listed below) and each of the hormones as independent variables are shown. §$p<0.01$. Model 1: age, body mass index, hypertension, hyperlipidemia, diabetes mellitus, and current smoking. Model 2: age, body mass index, systolic blood pressure, total cholesterol, HDL cholesterol, fasting plasma glucose and current smoking. Model 3: Model 2 plus carotid intima-media thickness. Model 4: Model 2 plus percent nitroglycerin-induced dilation of brachial artery. %FMD, percent flow-mediated dilation of brachial artery; DHEA-S, dehydroepiandrosterone-sulfate; HDL, high-density lipoprotein.

Plasma Hormones

Blood sampling was performed in the morning of the vascular measurement after a 14-h overnight fast, to measure plasma hormones and other chemical parameters. Plasma total and free testosterone, dehydroepiandrosterone-sulfate (DHEA-S), estradiol and cortisol concentrations were determined using sensitive radioimmunoassays by a commercial laboratory (SRL Inc., Tokyo, Japan). The intra-assay coefficients of variation for these measurements were less than 5%.

Data Analysis

The values are expressed as the means±SD in the text. Pearson's simple correlation coefficients between age, vascular measurements and plasma hormones were determined. Standardized regression coefficients from multiple regression analysis of vascular measurements in relation to age, coronary risk factors and plasma hormones were determined. Differences between the groups were analyzed using one-factor ANOVA, followed by Newman-Keuls' test. A value of $p<0.05$ was considered statistically significant.

Results

Changes in Plasma Hormones and Vascular Measurements According to Age and Coronary Risk Factors

Plasma levels of free testosterone and DHEA-S declined with age, while those of total testosterone and cortisol did not significantly change (Table 2). Conversely, estradiol showed a weak but significant positive correlation with age. %FMD decreased ($r=-0.365$, $p<0.001$) and carotid IMT increased ($r=0.546$, $p<0.001$) with advancing age.

The subjects with hypertension, hyperlipidemia or diabetes mellitus showed impaired %FMD compared to those without these diseases (hypertension, 3.8±2.4 *vs*. 6.3±4.0; hyperlipidemia, 4.4±3.4 *vs*. 6.1±3.7; diabetes mellitus, 3.1±2.4 *vs*. 5.9±3.7; $p<0.01$ for each). %FMD in the patients taking antihypertensive agents, statins or hypoglycemic agents was comparable to or smaller than that in the patients without medical agents (hypertension, 3.8±2.5 *vs*. 4.6±1.3, n.s.; hyperlipidemia, 3.9±2.7 *vs*. 6.3±4.7, $p<0.05$; diabetes mellitus, 2.6±2.0 *vs*. 4.9±3.0, $p<0.05$), suggesting that the favorable effects of medical treatment on endothelial function, if present, might have been lost in patients with a long history of coronary risk factors. In contrast, no significant associations were found between any of the plasma hormones and either coronary risk factors or medications.

Relationship between Plasma Hormones and Vascular Measurements

First, simple correlation coefficients between plasma hormones and vascular measurements were determined. As shown in Table 2, %FMD was positively correlated with total testosterone, free testosterone and DHEA-S. Carotid IMT was negatively correlated with free testosterone and DHEA-S, and was positively correlated with estradiol. There was no significant correlation between cortisol and vascular measurements.

Next, age-adjusted regression coefficients were determined, because age was correlated with both hormones and vascular measurements, as mentioned above. The results showed that none of the hormones was significantly related to carotid IMT, and estradiol was not related to either of the vascular measurements (Table 3). In contrast, total testosterone, free testosterone and DHEA-S were significantly related to %FMD, independent of age.

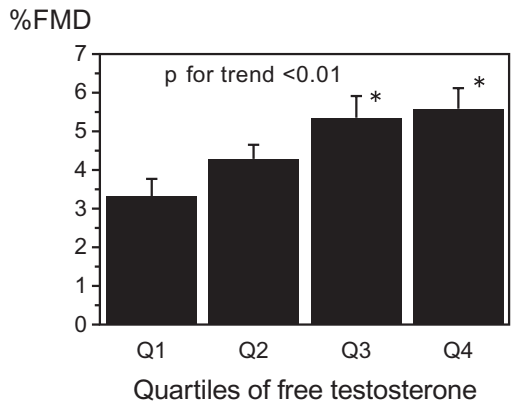

Fig. 1. *Percent flow-mediated dilation of the brachial artery (%FMD) according to quartiles of plasma free testosterone. Values are expressed as the means ± SEM. *p < 0.05 vs. Q1.*

Finally, multiple regression analyses were performed to exclude the influence of coronary risk factors on the relationship between hormones and %FMD. As shown in Table 4, total and free testosterone were related to %FMD, independent of age, body mass index, hypertension, hyperlipidemia, diabetes mellitus and current smoking (Model 1), and were independent of age, body mass index, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, fasting plasma glucose and current smoking (Model 2). Furthermore, the relationship between total and free testosterone and %FMD was significant after addition of carotid IMT to the model (Model 3), suggesting that the relationship was not attributable to the effect of testosterone on the development of subclinical atherosclerosis. Also, the statistical result was unchanged after %NTG of the brachial artery to the model (Model 4), indicating that testosterone is related to endothelial function independent of arterial compliance. DHEA-S, estradiol and cortisol were not significantly related to %FMD in similar multivariate analyses (Table 4). As shown in Fig. 1, %FMD showed a stepwise increment according to quartiles of free testosterone, and %FMD in the highest quartile of free testosterone was 1.7-fold higher than that in the lowest quartile.

Discussion

In this cross-sectional study, both total and free testosterone levels were positively correlated with %FMD, a surrogate marker of clinical atherosclerosis that reflects endothelial function (*19*, *20*). Adjustment for potential confounders such as age, coronary risk factors and %NTG had little influence on the results. These results suggest that testosterone level is an independent determinant of endothelial vasomotor function in men.

A number of studies have shown an association between low testosterone level and cardiovascular disease (*6*, *21*, *22*) or risk factors (*4*, *5*, *7–10*), but others have shown no association (*23*, *24*) and have reported that a low level of DHEA-S (*6*, *25*, *26*) or estradiol (*24*) is associated with cardiovascular disease. Also, a positive association between the cortisol:testosterone ratio and the incidence of coronary heart disease has been reported (*27*). Therefore, we added DHEA-S, estradiol and cortisol to the present analysis. However, our results showed that estradiol and cortisol were not related to %FMD. The ratio of cortisol to total testosterone ($r=-0.162$, $p<0.05$) and that of cortisol to free testosterone ($r=-0.194$, $p<0.05$) were significantly related to %FMD in simple regression analyses, but statistical significance was not found in multiple regression analyses (data not shown). DHEA-S was positively correlated with %FMD, but the statistical significance disappeared after adjustment for coronary risk factors. Taking these results together, testosterone was the only steroid hormone that was significantly related to %FMD in the multivariate analyses.

Several studies (*4*, *8*) have assayed bioavailable testosterone, non–globulin-bound or free plus albumin-bound testosterone (*28*), whereas others have measured total (*5*, *7*, *21–24*) or free (*6*, *9*, *10*, *21*, *22*) testosterone in the plasma. These differences in assays might influence the results. In this study, we did not analyze bioavailable testosterone, because a direct assay is not available in Japan, and we did not measure the levels of sex hormone binding globulin and albumin, which are needed to estimate the value of bioavailable testosterone. However, both total and free testosterone levels were positively associated with %FMD, although free testosterone showed a stronger impact throughout the statistical analyses. Accordingly, we believe that the assays do not affect our conclusion that testosterone level is an independent determinant of endothelial vasomotor function in men.

The mechanisms by which testosterone regulates vasomotor function should be discussed. Short-term intracoronary administration of testosterone has been reported to elicit vasodilation and increased blood flow in men (*29*) and in animals (*30*). A supra-physiologic dose of testosterone induced relaxation of isolated blood vessels *in vitro* (*31*). These direct vasodilator actions of testosterone observed at higher concentrations seem to be endothelium- and androgen receptor-independent, and to be mediated *via* membrane ion channels of smooth muscle cells (*31*). On the other hand, both acute (*13*) and chronic (*14*) supplementation of testosterone in men enhanced %FMD without affecting the basal diameter of the brachial artery, suggesting an endothelium-dependent vasodilator action of testosterone. We also showed that the relation between %FMD and testosterone was not altered after adjustment for %NTG, further supporting the action of testosterone on endothelial function. Although the existence of androgen receptors in endothelial cells is recognized (*32*), the cellular and molecular mechanism linking testosterone to endothelial release of vasoactive agents such as nitric oxide is uncertain. We recently found that ginsenoside Rb1 stimulated nitric oxide production and endothelial nitric oxide synthase activ-

ity *via* androgen receptors in human aortic endothelial cells (*33*). To date, however, there has been no experimental evidence showing a direct effect of testosterone on endothelial nitric oxide synthesis. Another less likely hypothesis is that estradiol converted from testosterone by aromatase might exhibit vasoreactivity. Although the plasma level of estradiol was not correlated with %FMD in the present study, tissue conversion of testosterone into estradiol might play a role. Further *in vitro* and animal studies will be needed to clarify these issues.

The results of this study do not imply that testosterone has favorable effects in women. In fact, in a preliminary study, we observed that the plasma testosterone level was not related to FMD in postmenopausal women (unpublished observation). It has been reported that testosterone may impair endothelial function in women, and especially in young women with polycystic ovary syndrome (*34*) and women taking high-dose androgens (*35*). Aortic rings obtained from female rats treated with testosterone showed a significant decrease in prostacyclin synthesis (*36*), supporting the idea that testosterone influences vasoconstriction in women. Taken together, these results indicate that the vascular responses to testosterone are clearly different between men and women. Gender differences in the steroid hormone receptor expression in arteries (*37*, *38*) might play a mechanistic role.

This study has some limitations. First, since this was a cross-sectional study, the causal relationship between testosterone and vasomotor function could not be determined. Endothelial dysfunction might be associated with a reduction in blood flow of endocrine organs, leading to decreased hormone production. Longitudinal studies following the subjects might add some information. Secondly, a population bias was possible. The study subjects ranged from young to elderly men with or without coronary risk factors. Consequently, the results might have been different if homogeneous subjects in terms of age and health status had been studied. In our subgroup analyses according to age and coronary risk factors and in multiple regression analyses including drug classes, comparable regression coefficients were obtained between testosterone and %FMD, although the statistical power was weakened (data not shown).

In summary, a low plasma testosterone level was associated with endothelial dysfunction in men independent of other risk factors, suggesting a protective effect of testosterone on the endothelium. This finding provides mechanistic insight into the role of endogenous testosterone in the development of cardiovascular disease in men.

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