CLINICAL STUDY

Association of endogenous testosterone with blood pressure and left ventricular mass in men. The Tromsø Study

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Abstract

Objective: To test the hypothesis that lower endogenous testosterone levels are associated with higher blood pressure, left ventricular mass, and left ventricular hypertrophy.

Design: Population-based cross-sectional study.

Methods: Sex hormone levels, measured by immunoassay, anthropometric measurements and resting blood pressure were studied in 1548 men aged 25–84 years; echocardiography was completed in 1264 of these men. Partial correlations and multiple regressions were used to estimate the associations between sex hormones, blood pressure and left ventricular mass by height. Analyses of variance and covariance were used to compare men with categorical hypertension and left ventricular hypertrophy.

Results: In age-adjusted partial correlations, total testosterone and sex hormone-binding globulin (SHBG) were each inversely associated with systolic blood pressure (SBP) ($P < 0.001$). Men with categorical hypertension (SBP ≥ 140 or diastolic blood pressure (DBP) ≥ 90 mmHg) had lower levels of total and free testosterone and SHBG before ($P < 0.001$, $P = 0.011$ and $P < 0.001$, respectively) and after ($P < 0.001$, $P = 0.035$ and $P = 0.002$, respectively) adjusting for body mass index (BMI). Total testosterone and SHBG were each inversely associated with left ventricular mass ($P < 0.001$), and men with left ventricular hypertrophy had significantly lower levels of total testosterone ($P = 0.042$) and SHBG ($P = 0.006$); these associations were no longer significant after adjusting for BMI.

Conclusion: The results of the present study are consistent with the hypothesis that lower levels of testosterone in men are associated with higher blood pressure, left ventricular mass, and left ventricular hypertrophy. The reduced associations after adjusting for BMI suggest that the association of low testosterone levels with blood pressure and left ventricular mass is mediated by obesity.

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Introduction

Both systolic and diastolic hypertension are established risk factors for coronary heart disease (CHD). In men, blood pressure increases with age while testosterone, especially free testosterone decreases. Lower endogenous testosterone levels were reported with increasing systolic (SBP) and diastolic blood pressure (DBP) in one case-control study (1), and low levels of testosterone have also been described in hypertensive men (2–4). In cross-sectional studies an inverse relationship between endogenous testosterone and blood pressure has been reported by some (5) but not all (6–8) investigators. Left ventricular hypertrophy is associated with both hypertension and increased cardiovascular morbidity

and mortality (9), and it has been suggested that testosterone could be influential in modulating left ventricular mass (10). Little is known, however, about the relationship between endogenous testosterone, left ventricular mass and left ventricular hypertrophy in men.

In the fourth survey of the Tromsø Study, a population-based multipurpose study, which took place in 1994 to 1995, serum testosterone, blood pressure and body size were measured in 1605 men aged 25 years and older, and echocardiography was performed in 1316 of these men. We used this large database to investigate the possible relationships between endogenous testosterone levels and blood pressure, left ventricular mass and left ventricular hypertrophy.

Materials and methods

Study population

All inhabitants aged ≥ 25 years living in the municipality of Tromsø were invited to participate in the fourth survey of the Tromsø Study. This fourth survey consisted of two screening visits 4 to 12 weeks apart, and 27 159 individuals attended the first visit. All subjects aged 55 to 74 years, random 5% samples of subjects in other age groups up to age 84 years, as well as 308 men aged 40 to 54 years (selected on the basis of high total cholesterol and/or low high density lipoprotein cholesterol) were invited to a second visit for more extensive examinations. A total of 6891 subjects (3393 men) attended the second visit, representing 79% of the eligible population (11). Serum samples were drawn for future analysis of sex hormones in a random sample of 1605 men, and echocardiography was performed in 1316 of them. Of the 1605 men who had samples drawn for sex hormone analyses, 1565 men had sufficient serum for all hormone assays. Eight of these men whose total testosterone levels were below the assay detection level, seven men whose sex hormone-binding globulin (SHBG) levels were above the assay reference range and two men using androgen therapy were excluded, leaving 1548 men for the blood pressure analyses and 1264 men for the left ventricular mass and left ventricular hypertrophy analyses.

Questionnaires

All participants completed two self-administered questionnaires, checked by trained nurses, including information about smoking habits, physical activity, alcohol consumption and medical history. From this questionnaire a physical activity score was computed adding together the hours of easy and vigorous physical activity, with the hours of vigorous activity receiving double weight. The alcohol intake of beer, wine and hard liquor consumed during an average 2-week period was also scored, assuming an equal amount of alcohol in one glass of each type.

Measurements

Height and weight were measured in subjects wearing light clothing without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Blood pressure was measured with an automatic device (Dinamap Vital Signs Monitor 1846, Critikon Inc., USA), after subjects were seated for 2 min. Three recordings were made at 2-min intervals, and the mean of the last two were used in this report. Echocardiography was performed by medical doctors, using a VingMed CFM 750 (VingMed Sound A/S, Horten, Norway). Subjects

were examined in a supine, left lateral position with a combined 3.25 MHz mechanical and a 2.5 MHz Doppler probe. The echocardiographic examinations were performed using the standard apical and parasternal long- and short-axis views. Left ventricular diastolic dimensions from one heart cycle were measured online from standard two-dimensional guided M-mode registrations according to the recommendations of the American Society of Echocardiography (12), using EchoPAC software (VingMed Sound). Registrations were regarded as adequate for measurement if both margins of the septum and the posterior wall were visible throughout one heart cycle. Left ventricular mass was calculated using the correction formula of Devereux *et al.* (13). Left ventricular mass was indexed for height to control for the increase in left ventricular mass with increasing height, without masking the increase in left ventricular mass with increasing BMI (14). Left ventricular hypertrophy was defined by sex-specific 97.5 percentiles of left ventricular mass by height in a healthy reference group, aged 25–76 years, from the total echo sample (15). The cut-off normal value for men was $<145.5 \text{ g}/\text{m}$.

The presence of valvular heart disease was evaluated by two dimensional colour Doppler for mitral insufficiency, colour M-mode for aortic insufficiency and pulsed wave Doppler for mitral and aortic stenosis.

Blood was drawn by venipuncture from non-fasting men between 0800 and 1600 h. Serum samples were stored at -70°C , until they were first thawed for analyses of sex hormones in 2001. Determination of total testosterone and SHBG was performed on Immulite 2000 (Diagnostic Product Corp., Los Angeles, CA, USA). Free testosterone values were calculated from total testosterone and SHBG using a fixed albumin of $43.0 \text{ g}/\text{l}$ according to the method of Vermeulen *et al.* (16). Coefficients of variation were based on assays of pooled human sera at two hormone levels run in parallel each day. The intra- and interassay coefficients of variation for total testosterone were 3.5% and 5% respectively at a concentration above $1 \text{ nmol}/\text{l}$ and 12% and 20% respectively in the range 0.1 (limit of detection) – $1.0 \text{ nmol}/\text{l}$. For SHBG the intra- and interassay coefficients of variation were 3.5% and 6% respectively and the limit of detection was $1.0 \text{ nmol}/\text{l}$.

Statistical analyses

Partial correlations were used to determine the cross-sectional associations between blood pressure, left ventricular mass indexed by height, and individual hormone levels adjusted for age, BMI or waist circumference and life-style factors. Multiple regression analyses, with blood pressure or left ventricular mass indexed by height as the dependent variable were performed to evaluate the independent contribution of testosterone, age, BMI or waist circumference, and life-style factors. Baseline distribution of testosterone

and SHBG was examined by categorically defined hypertension (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg including individuals on medication for hypertension) or left ventricular hypertrophy (≥ 145.5 g/m) in age-specific categories. Analysis of covariance was used to calculate adjusted means of testosterone and SHBG by blood pressure and left ventricular hypertrophy status. The covariates age and BMI were included as continuous variables in the model. All statistical tests were two-tailed with statistical significance defined as $P < 0.05$. The data were analyzed using the SPSS statistical package for Windows version 10.1 (SPSS Inc., Chicago, IL, USA).

Ethics

The Tromsø Regional Ethics Committee approved the study, and all participants gave written informed consent.

Results

The characteristics of the 1548 men are summarized in Table 1. Approximately 50% had hypertension of whom 13.4% were using medication for hypertension. A history of CHD was reported by 15.2%, stroke by 2.6% and diabetes by 3.2%.

In age-adjusted partial correlations total testosterone and SHBG were inversely and significantly (all $P < 0.001$) associated with DBP ($r = -0.10$ and $r = -0.13$ respectively) and SBP ($r = -0.12$ and

$r = -0.13$ respectively). There was a weak inverse association between free testosterone and SBP ($r = -0.05$; $P = 0.038$). Both DBP and SBP were positively associated with BMI ($r = 0.24$; $P < 0.001$ and $r = 0.18$; $P < 0.001$ respectively) and with alcohol consumption ($r = 0.07$; $P = 0.010$ and $r = 0.08$; $P = 0.003$ respectively). SBP was also inversely associated with the number of cigarettes smoked ($r = -0.06$; $P = 0.019$).

In multiple regression analyses, total testosterone, age, BMI or waist circumference, and alcohol consumption were significantly and independently associated with systolic blood pressure; age, BMI or waist circumference and alcohol consumption were associated with diastolic blood pressure (Table 2). Excluding men on treatment for hypertension did not materially change the results.

In the 1264 men with valid data on left ventricular mass by height, there was an inverse age-adjusted partial correlation between left ventricular mass by height and total testosterone ($r = -0.10$; $P < 0.001$) and SHBG ($r = -0.14$; $P < 0.001$). Left ventricular mass by height was positively associated to both systolic ($r = 0.26$; $P < 0.001$) and diastolic ($r = 0.24$; $P < 0.001$) blood pressure, and with BMI ($r = 0.39$; $P < 0.001$). Left ventricular mass by height was not associated with lifestyle factors. In multiple regression analyses, age, systolic blood pressure, valvular heart disease and BMI or waist circumference were each independently associated with left ventricular mass by height. Total testosterone and SHBG were independently associated with left ventricular mass by height before but not after BMI or waist circumference was added to the model (Table 3). Excluding men with CHD did not materially change these results.

Table 4 shows mean hormone levels by categorical hypertension including men with hypertensive medication. In age-adjusted analyses, men with hypertension had lower levels of total testosterone (12.4 vs 14.0 nmol/l; $P < 0.001$), free testosterone (201 vs 210 pmol/l; $P < 0.011$), and SHBG (48.4 vs 54.7 nmol/l; $P < 0.001$). Adjusting for BMI weakened but did not change these associations. In addition, these differences were consistent within age-specific groups. In age-adjusted analysis, men with left ventricular hypertrophy had lower levels of total testosterone (12.6 vs 13.5 nmol/l; $P < 0.042$) and SHBG (47.1 vs 51.6 nmol/l; $P < 0.006$). However, these associations were no longer present after adjusting for BMI.

Additional analyses adjusting for time of day and season of venipuncture did not materially change these associations.

Discussion

Views on the relationship between testosterone and blood pressure are conflicting. In cross-sectional studies

Table 1 Characteristics of the 1548 Tromsø men (mean $\pm$ S.D. or percent).

Age (years)	60.3 $\pm$ 10.1
(Range)	(25–84)
Anthropometric characteristics	
Height (cm)	175.2 $\pm$ 6.9
Weight (kg)	80.3 $\pm$ 12.1
Body mass index (kg/m ²)	26.1 $\pm$ 3.4
Waist circumference (cm)	95.4 $\pm$ 9.2
Hormone levels	
Total testosterone (nmol/l)	13.2 $\pm$ 5.1
Free testosterone (pmol/l)	205 $\pm$ 75
Sex hormone-binding globulin (nmol/l)	51.6 $\pm$ 22.3
Systolic blood pressure (mmHg)	140.8 $\pm$ 19.8
Diastolic blood pressure (mmHg)	81.7 $\pm$ 11.5
Echocardiography*	
Left ventricular mass (g)	201.4 $\pm$ 60.9
Left ventricular mass by height (g/m)	115.0 $\pm$ 34.8
Smoking – cigarettes/day: none/1–10/11 or more (%)	67/26/7
Alcohol – units/two weeks: none/1–14/15 or more (%)	35/58/7
Physical activity score: 0–3/4–6/7–9 (%)	58/23/19

*Data on echocardiography is available in 1264 men.

Alcohol units: one glass of beer, wine or hard liquor (assuming an equal amount of alcohol in one glass of each type); physical activity score: 0–3 is equivalent to occasional or regular easy activity, whereas 7–9 would involve vigorous exercise on a weekly basis.

Table 2 Multiple regression analyses with systolic and diastolic blood pressure as dependent variables.

Independent variable	Dependent variable: SBP		Independent variable	Dependent variable: SBP	
	β	P		β	P
Model 1			Model 1		
Total testosterone	-0.12	<0.001	SHBG	-0.13	<0.001
Age	0.28	<0.001	Age	0.33	<0.001
Model 2*			Model 2*		
Total testosterone	-0.07	0.005	SHBG	-0.05	0.057
Age	0.30	<0.001	Age	0.33	<0.001
Alcohol	0.07	0.004	Alcohol	0.07	0.005
BMI**	0.15	<0.001	BMI**	0.15	<0.001
Independent variable	Dependent variable: DBP		Independent variable	Dependent variable: DBP	
	β	P		β	P
Model 1			Model 1		
Total testosterone	-0.10	<0.001	SHBG	-0.13	<0.001
Age	0.16	<0.001	Age	0.21	<0.001
Model 2*			Model 2*		
Total testosterone	-0.03	0.300	SHBG	-0.03	0.370
Age	0.19	<0.001	Age	0.20	<0.001
Alcohol	0.06	0.014	Alcohol	0.06	0.016
BMI**	0.23	<0.001	BMI**	0.23	<0.001

*Smoking and physical activity were not significant and were excluded. **Using waist circumference in the model instead of BMI gave the same result.

an inverse association with both systolic and diastolic blood pressure has been reported by some investigators (5), in agreement with our finding, but not by all (6–8). In a longitudinal study of 66 men over 13 years (former participants of the Multiple Risk Factor Intervention Trial, MRFIT) no association was found between testosterone and blood pressure (17).

We found lower levels of total testosterone in hypertensive men, consistent with four previous reports (2–5), while two other investigators reported no difference (18, 19). It is, however, important to notice that no study has reported a positive association between testosterone and blood pressure or found higher levels of testosterone in hypertensive men.

Table 3 Multiple regression analyses with left ventricular mass as dependent variable.

Independent variable	Dependent variable: left ventricular mass by height		Independent variable	Dependent variable: left ventricular mass by height	
	β	P		β	P
Model 1			Model 1		
Total testosterone	-0.10	<0.001	SHBG	-0.15	<0.001
Age	0.16	<0.001	Age	0.22	<0.001
Model 2			Model 2		
Total testosterone	-0.08	0.005	SHBG	-0.12	<0.001
Age	0.08	0.002	Age	0.13	<0.001
Systolic blood pressure	0.19	<0.001	Systolic blood pressure	0.19	<0.001
Diastolic blood pressure	0.09	0.019	Diastolic blood pressure	0.09	0.028
Model 3			Model 3		
Total testosterone	-0.08	0.002	SHBG	-0.12	<0.001
Age	0.06	0.027	Age	0.11	<0.001
Systolic blood pressure	0.18	<0.001	Systolic blood pressure	0.18	<0.001
Diastolic blood pressure	0.11	0.004	Diastolic blood pressure	0.11	0.006
Valvular disease	0.20	<0.001	Valvular disease	0.20	<0.001
Model 4			Model 4		
Total testosterone	0.02	0.431	SHBG	0.02	0.393
Age	0.10	<0.001	Age	0.09	0.001
Systolic blood pressure	0.18	<0.001	Systolic blood pressure	0.20	<0.001
Valvular disease	0.21	<0.001	Valvular disease	0.21	<0.001
BMI*	0.36	<0.001	BMI*	0.37	<0.001

*Using waist circumference in the model instead of BMI gave the same result.

Table 4 Mean and s.d. total and free testosterone and SHBG levels by categorical hypertension and by left ventricular hypertrophy (LVH).

	No hypertension (SBP < 140 and DBP < 90)			Hypertension (SBP ≥ 140 and/or DBP ≥ 90)				No LVH (< 145.5 g/m)			LVH (≥ 145.5 g/m)			
Age (years)	<i>n</i>	Mean	S.D.	<i>n</i>	Mean	S.D.	<i>P</i>	<i>n</i>	Mean	S.D.	<i>n</i>	Mean	S.D.	<i>P</i>
Total testosterone (nmol/l)														
25–39	59	15.3	6.0	15	13.3	4.4		72	15.0	5.8	2	12.0	1.5	
40–49	87	14.2	5.0	37	12.6	5.0		103	14.1	5.1	8	10.3	2.3	
50–59	271	14.0	5.3	182	12.1	4.6		353	13.4	5.3	55	12.8	4.1	
60–69	285	13.8	4.8	320	12.4	5.1		381	13.4	5.1	77	12.6	5.4	
70–84	81	13.4	5.4	211	12.3	5.1		167	12.8	5.4	46	12.5	4.7	
All 25–84 (age-adjusted)	783	14.0	5.2	765	12.4	4.9	<0.001	1076	13.5	5.3	188	12.6	4.7	0.042
All (age- and BMI-adjusted)		13.7			12.7		<0.001		13.3			13.6		0.453
Free testosterone (pmol/l)														
25–39		307	109		265	67			300	104		259	56	
40–49		254	77		252	84			255	79		232	71	
50–59		226	81		206	62			218	76		225	66	
60–69		196	58		188	62			195	62		190	66	
70–84		168	63		171	59			172	57		169	63	
All 25–84 (age-adjusted)		210	82		201	66	0.011		210	78		209	769	0.794
All (age- and BMI-adjusted)		209			202		0.035		209			212		0.625
SHBG (nmol/l)														
25–39		36.5	15.3		34.1	14.9			36.2	15.4		30.0	4.2	
40–49		42.0	16.5		33.9	15.4			40.7	16.8		27.6	12.4	
50–59		49.2	19.2		43.8	19.1			47.7	19.5		42.3	16.3	
60–69		57.3	20.4		52.1	23.1			55.5	22.5		51.1	21.1	
70–84		69.2	28.2		58.6	22.8			61.5	24.4		60.9	17.1	
All 25–84 (age-adjusted)		54.7	21.9		48.4	22.7	<0.001		51.6	22.0		47.1	20.2	0.006
All (age- and BMI-adjusted)		53.1			50.0		0.002		50.8			52.2		0.357

To our knowledge only one study has examined the relationship between testosterone levels and left ventricular mass and found no association in unadjusted or adjusted analyses (20). The prevalence of left ventricular hypertrophy was not reported. In contrast, we found an inverse association between total testosterone and left ventricular mass by height and lower testosterone levels in men with left ventricular hypertrophy. These associations were independent of age but were no longer present after adjusting for BMI or waist circumference, suggesting that the link between total testosterone and left ventricular mass by height is mediated by body fat or body fat distribution. Others have reported that low levels of total testosterone are associated with increasing obesity (1, 21, 22) and predict central obesity (23). Further, administration of low-dose testosterone to hypogonadal men increased fat-free mass and reduced total and central fat mass (24). If low levels of total testosterone increase overall obesity, especially central obesity, adjusting for BMI, waist circumference or waist-hip ratio to assess cardiovascular risk factors could be considered overadjusting. BMI may emerge as the strongest covariate because it is more precisely measured than testosterone.

In this study, we found lower levels of free testosterone in men with hypertension, but not in men with left ventricular hypertrophy. Free testosterone represents a small part (1–2%; in our study 1.6%) of total testosterone and the level is influenced both by

total testosterone and by SHBG levels (18). If total testosterone and SHBG decrease in parallel, free testosterone might be unchanged. We believe this could be a plausible explanation as to why free testosterone was not lower in men with left ventricular hypertrophy.

There could be several possible explanations for the association between testosterone, blood pressure and left ventricular mass by height. A genetic link between hypertension and serum testosterone levels has been suggested by a study showing that men with a family history of hypertension have lower levels of testosterone (25). Mice lacking the gene encoding the natriuretic peptide receptor A (Npr1) are characterized by both high blood pressure and low circulating levels of testosterone (26). Testosterone may exert antihypertensive effects on the vasculature as testosterone induces endothelium-independent relaxation of isolated rabbit and rat aorta (27, 28), and testosterone has also been shown to have a beneficial effect on myocardial ischemia (29) and coronary blood flow in men with CHD (30). These observations suggest a direct role for testosterone in modulating vessel resistance and arterial blood flow. In a recent study of patients with prostate cancer, treatment-induced hypogonadism was associated with increased central arterial pressure suggesting arterial stiffening (31). Arterial stiffening leads to changes in arterial pressure waveform, and ultimately increases central systolic blood pressure (32). Central pressure is the major determinant of left ventricular

afterload and the subsequent development of left ventricular hypertrophy (32, 33), and is an independent cardiovascular risk factor (9). However, the precise mechanism underlying the relationship between low testosterone levels, hypertension, and left ventricular mass by height is still elusive.

Limitations of this study should be considered. Hormone levels were based on a single serum sample, drawn between 0800 and 1600 h. Preferably, samples should have been drawn in the morning because of the diurnal variation of total and free testosterone; we observed the expected higher levels of both total and free testosterone in the morning, but adjusting for time and season of venipuncture did not change these associations or lack of associations. Blood samples were kept frozen for approximately 6.5 years at -70°C and hormone levels were measured when samples were thawed for the first time. Levels of steroid hormones have been shown to be relatively stable in frozen serum for up to 10 years (34, 35). SHBG has documented stability only in short-term frozen storage (35); reduced levels have been reported after longer storage (36). However, this would not be expected to alter the ordinal associations for the observed levels. We did not measure free testosterone but used calculated values. However, two independent investigators have recently reported that this calculation using fixed albumin is accurate and reliable (16, 37).

In summary, total testosterone was inversely associated with systolic blood pressure, and men with hypertension had lower levels of total and free testosterone and SHBG. Inverse associations were also seen between total testosterone and SHBG with left ventricular mass by height, and men with left ventricular hypertrophy had lower levels of total testosterone and SHBG. Results of the present study are consistent with the hypothesis that lower levels of testosterone in men are associated with CHD risk factors. We suggest that low testosterone-mediated obesity is a plausible mechanism for this association.

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