Cardiovascular Risk Factors in Men: The Role of Gonadal Steroids and Sex Hormone-Binding Globulin

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Males have higher risk of cardiovascular disease (CVD) than premenopausal females. Gonadal steroids are probably involved in the gender difference in CVD, but previous results have been conflicting. We investigated the associations between CVD risk factors and sex hormones in a cross-sectional designed study of 508 healthy males, aged 41 to 72 years. We determined total testosterone (T), sex hormone-binding globulin (SHBG), free androgen index (FAI), and estradiol (E2) and studied their relationship to body fat mass (BF), blood pressure (BP), aortic compliance, left ventricular mass (LVM), and plasma lipids (total cholesterol, high-density lipoprotein [HDL], low-density lipoprotein [LDL], very–low-density lipoprotein [VLDL], and triglycerides). In quartile analyses after adjustment for confounders (age, body mass index [BMI], alcohol consumption, and smoking), SHBG and E2 were positively associated with HDL, while FAI was negatively associated with HDL. T and SHBG were negatively associated with VLDL and triglycerides, while FAI was positively associated with VLDL and triglycerides. T and SHBG were negatively associated with BMI and BF, while FAI and E2 were positively associated with BMI and BF. E2 was negatively associated with LVM. No hormone varied with total cholesterol, LDL, BP, and aortic compliance in the adjusted analyses. In multiple regression analyses, SHBG was the main predictive variable of HDL, VLDL, and triglycerides explaining 12%, 17%, and 17% of the variation, respectively. No other hormones were selected as predictive variables for VLDL and triglycerides, but E2, T, and FAI were selected in the HDL regression, explaining 3%, 2%, and less than 1%, respectively. Our regression analyses illustrate the diverging results when investigating associations between gonadal steroids and lipids with and without SHBG adjustment. Atherogenic lipid profile in males is associated with low SHBG, low T levels, and a high FAI. Males with high E2 levels may have a less atherogenic lipid profile and lower LVM. SHBG is a key hormone in the association between sex hormones and plasma lipids. We suggest that conflicting results of cross-sectional and intervention studies of sex hormones and lipids, in part, may be explained by interindividual differences or changes in SHBG. Thus, further studies on the potential role of SHBG in the development of ischemic heart disease (IHD) should be performed.

MALES HAVE A considerably higher risk of cardiovascular disease (CVD) in comparison to premenopausal females. After menopause, the incidence of CVD in women increases, diminishing the gender difference in morbidity and mortality from CVD. These observations suggest that sex hormones may be involved in the pathogenesis of atherosclerosis, either as independent risk factors or mediated via other risk factors, such as plasma lipids or insulin levels.

During the past decades, several studies on the association between androgens and known CVD risk factors, such as plasma lipids and blood pressure (BP) in males, have been conducted. However, the results have been conflicting. Most cross-sectional studies find a less atherogenic lipid profile with increasing endogenous total testosterone (T), mainly a positive correlation between T and high-density lipoprotein (HDL)-cholesterol (for review, see Barrett-Connor[1] and Tchernof et al[2]).[3] Additionally, an inverse relationship between BP levels and endogenous T have been reported.[4] In accordance with these findings, a recent nested case-control study found a higher prevalence of CVD risk factors in the low total T group compared with the normal T group.[5] Prospective follow-up studies have failed to show any predictive value of basal sex hormone level on the development of cardiovascular disease or death.[6-9] In contrast to the cross-sectional studies, interventional studies find that administration of exogenous T to healthy males leads to decreasing HDL-cholesterol,[10-12] while androgen treatment therapy in hypogonadal males leads to decreasing total cholesterol with no concomitant change in HDL-cholesterol.[13,14] Thus, the association between T and plasma lipids in males is still unclear, and the role for endogenous gonadal steroids in the development of CVD deserves further attention.

Therefore, we determined sex hormone-binding globulin (SHBG), total T, free androgen index (FAI), and estradiol (E2) in a random sample of healthy men (aged 41 to 72 years), participating in the large population-based study Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA). The aim was to investigate significant relationships between sex hormones and classical CVD risk factors, such as age, BP, plasma lipids (total cholesterol, lipoproteins, triglycerides), obesity, and smoking and to investigate the relative importance of the hormones studied in predicting CVD risk factor levels. Additionally, aortic compliance and left ventricular mass (LVM), possible independent risk factors of CVD,[15,16] were analyzed for associations with plasma levels of gonadal steroids.

METHODS AND MATERIALS

Participants

A total of 518 consecutive male participants in the MONI 10 survey attended the present study. Details of the MONI 10 (MONItoring of trends and determinants in cardiovascular diseases) population are

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described elsewhere.[17] In brief, a random sample consisting of 2,656 Danish men and women from the referral area of Glostrup County Hospital participated in the MONI 10 survey. Participants were recruited from the national population register without prior knowledge of health status. Participants represented 4 age cohorts, 41 to 42 years, 51 to 52 years, 61 to 62 years, and 71 to 72 years. Sex hormones were determined in the MONI 10 subpopulation consisting of 518 male subjects. Of these, 10 men were excluded due to hypogonadism with extremely low total T levels (< 2 nmol/L), leaving 508 men, aged 41 to 42 years (n = 137), 51 to 52 years (n = 160), 61 to 62 years (n = 130), and 71 to 72 years (n = 81) for the present analyses.

All subjects completed a questionnaire on current and prior diseases, intake of medication, and an extensive questionnaire on behavioral risk factors. Previously diagnosed ischemic heart disease (IHD) or cerebral stroke were reported by 35 men (6.9%), and statistical analyses were conducted with and without these subjects.

Blood samples were obtained in the fasting state between 7:30 AM and 12:30 PM. Samples were drawn from an antecubital vein, clotted, centrifuged, and serum was stored at –20°C until hormone analyses, which were performed in the subsequent 12-month period.

Methods

Blood pressure (BP) was measured in the sitting position after 5 minutes of rest using a standard mercury sphygmomanometer, and the mean of 2 values for systolic and diastolic pressure was used in the subsequent analyses. All measurements were performed by the same nurse, who prior to the study was tested for normal hearing.

Body fat (BF) mass was determined by bioelectrical impedance using a BIA-103 (RJL-system, Detroit, MI) analyzer with a 50 KHz, 800 A device. In brief, BF was estimated using the following multiple regression equation for BF mass: BF (kg) = 0.755BW - 0.279 Ht^2/R - 0.231Ht + 0.077 Age + 14.941 (Ht = heigth (cm), R = impedance (ohm), BW = body weight [kg]). This equation was previously derived from a representative sample of participants in the MONICA study and validated by comparison to BF assessment by reference methods.[18]

LVM was determined from a standard M-mode left parasternal echocardiographic recording using a Toshiba (Tokyo, Japan) 60A echocardiograph equipped with a 3.5 MHz transducer. Left ventricular diameters were obtained according to the Penn-cube method as the mean of 6 determinations (3 in the longitudinal axis and 3 in the short axis).[19] LVM was calculated according to the equation: LVM (g) $=1.04\ (LVIDd + PWTd + VSTd)^3 - (LVIDd)^3 - 13.6$ (LVIDd = left ventricular internal diameter diastolic [cm], PWTd = posterior wall thickness diastolic [cm], VSTd = ventricular septum thickness diastolic [cm]). All echocardiographic recordings were performed by the same investigator (S.L.R.).

Left ventricular mass index (LVMI) is defined as LVM in relationship to body surface area, LVMI = LVM(g)/body surface(m^2).

Aortic compliance was estimated as pulse wave velocity (PWV) using transducers placed over the carotid and femoral arteries.[20] PWV is inversely related to aortic compliance (C) according to the formula $PWV = \sqrt{(\pi \times r^2/C)}$, in which r^2 can be considered constant on a group basis.

Serum lipids were determined in venous blood samples obtained in the fasting state using a commercial assay (CHOD-PAP; Boehringer, Manheim, Germany).

Hormones

SHBG was determined by a time-resolved immunoflurosence assay (Delfia; Wallac Oy, Turku, Finland) with a sensitivity of 0.23 nmol/L. Intra- and interassay coefficients of variation were 5.8% and 6.4%, respectively. Testosterone was determined by a radioimmunoassay (Diagnostic Products, Los Angeles, CA) with a sensitivity of of 0.23 nmol/L. Intra- and interassay coefficients of variation were 3.8% and 8.6%, respectively. FAI was calculated as (T × 100/SHBG). E2 was determined by radioimmunoassay (Immunodiagnostic Systems, Bolton, UK) with a sensitivity of 18 pmol/L. Intra- and interassay coefficients of variation were 7.5% and 12.9%, respectively.

Statistical Methods

All statistical analyses were performed using the SPSS computer package, 1989 to 1997, release 8.0.0 (SPSS, Chicago, IL).

Relationships between hormones and CVD risk factors were investigated using analysis of variance (ANOVA) used on risk factors split by hormone quartile categories. This statistical approach was chosen because it is more robust and does not assume linearity between the independent and dependent variable. Due to positively skewed distributions, systolic BP, PWV, LVM, LVMI, very–low-density lipoprotein (VLDL) cholesterol, triglycerides, E2, and SHBG required log-transformation before statistical analyses. Adjustment for confounders was performed using General Linear Model (GLM), in which age, body mass index (BMI), and systolic BP were included as continuous covariates, while smoking (yes/no) and alcohol (less than 7, 7 to 21, ≥ 21 drinks weekly) were included as explanatory factors together with hormone quartile groups. Age was included as a continuous variable due to the linear relationship between age and CVD risk factors, which was assured for every risk factor/age correlation by studying residual plots.

In multiple regression analyses with CVD risk factors as dependent variables, a forward stepwise method was used with a probability of F to enter ≤ 0.05 and probability of F to remove ≥ 0.10. Alcohol and smoking were entered as dummy variables, 2 dummy variables for alcohol (1 for low and 1 for high consumption) and 1 dummy variable for current smoking.

Linear regression was used to test hormone levels versus age and χ^2 test was used to test significant variations between hormone quartile groups regarding current smoking, medication, and medical history. The effect of smoking on hormones was investigated by comparing hormone levels in the smoker versus nonsmoker group using t test.

Study subjects represented 4 age cohorts: 41 to 42 years, 51 to 52 years, 61 to 62 years, and 71 to 72 years. These age cohorts are referred to as age 40, 50, 60, and 70 years, respectively, in all results, tables, and figures. The significance level of all statistical tests was chosen as $\alpha = 0.05$.

Ethical Considerations

The study was approved by the ethics committee for Copenhagen County (project no. KA 93054) and conducted in accordance with the Second Helsinki Declaration. All participants gave their written informed consent.

RESULTS

Baseline clinical and metabolic characteristics are given in Table 1. Table 2 shows the observed medians of every risk factor split by hormone quartile groups. Significant differences between groups are shown for the adjusted GLM models.

A minor proportion of the men (1% to 2%) were currently using insulin, oral antidiabetics, nitroglycerine, or cholesterol-lowering medicine, while 10.6% reported current use of antihypertensive medicine (angiotensin-converting enzyme [ACE] inhibitors, diuretics, or β-blocking agents). However, there were no significant differences in medication between quartile groups of any hormone.

Previously diagnosed IHD was prevalent in 5.1% and cerebral stroke in 2.0% of the men. Median hormone levels in these patients were not significantly different from hormone levels in

Table 1. Clinical and Metabolic Characteristics of 508 Male Study Subjects

	No.				Percentiles		
	Valid	Missing	Mean	SD	5th	50th	95th
Age (yr)	508	0	53.1	10.4	40.0	50.0	70.0
Height (cm)	508	0	176.0	6.6	166.0	175.5	187.5
Weight (kg)	508	0	82.0	12.5	64.1	81.6	101.9
BMI (kg/m^2)	508	0	26.5	3.9	21.1	26.0	32.8
BF mass (kg)	508	0	21.4	7.6	10.8	20.4	34.3
Lean body mass (kg)	508	0	60.6	6.3	50.5	60.1	71.8
BF (%)	508	0	25.5	5.7	16.1	25.5	34.4
Systolic BP (mm Hg)	508	0	131.8	19.9	105.0	128.5	171.0
Diastolic BP (mm Hg)	508	0	84.3	10.5	69.0	83.0	103.0
PWV (m/sec)*	506	2	11.9	3.3	8.3	11.0	17.9
LVM (g)	470	38	212.4	63.6	131.2	201.4	332.7
LVMI (g/m^2)	470	38	107.3	31.0	68.6	101.6	168.3
Cholesterol, total (mmol/L)	507	1	6.08	1.02	4.63	5.99	7.84
Cholesterol, HDL (mmol/L)	507	1	1.30	.37	.83	1.25	2.02
Cholesterol, LDL (mmol/L)	496	12	4.08	.95	2.67	4.00	5.73
Cholesterol, VLDL (mmol/L)	496	12	.69	.35	.29	.59	1.40
Triglycerides (mmol/L)	507	1	1.54	.95	.62	1.27	3.29
SHBG (nmol/L)	508	0	43.7	20.3	19.0	40.0	82.6
Testosterone (nmol/L)	508	0	18.1	5.5	10.0	17.4	28.1
FAI	508	0	46.4	16.7	23.8	43.9	77.3
E2 (pmol/L)	508	0	122.6	39.8	70.0	117.0	195.6

* Aortic compliance is inversely correlated to PWV.

disease-free men. Multiple regression and ANOVA with (n = 508) and without (n = 473) these subjects gave essentially similar results. One percent had a medical history of diabetes.

Hormones Versus Classical Risk Factors

Plasma lipids. Plasma triglycerides, HDL, and VLDL cholesterol varied significantly with all hormones in the unadjusted analyses. After adjustment for confounders (Table 2), HDL, VLDL, and triglycerides retained their high significant association with hormone levels, except for HDL versus total T, which became near significant ($P = .059$). An atherogenic lipid profile (low HDL, high VLDL, and high triglycerides) was associated with low SHBG, low total T, and high FAI after adjustment for age, BMI, and alcohol. The picture was more complex for E2. HDL remained fairly constant, while VLDL and triglycerides increased from the first to the third E2 quartile group, where after VLDL and triglycerides decreased and HDL increased markedly in the upper E2 quartile group.

Total cholesterol and LDL did not vary with hormone quartiles neither in the unadjusted (except for E2) nor in the adjusted models.

Body fatness. BF mass and BMI varied with quartile groups of all hormones studied, achieving significant levels in both ANOVA and adjusted GLM (Table 2). Low BMI and low

Table 2. Cardiovascular Risk Factors (medians) Split by Hormone Quartile Groups

	SHBG Quartiles				T Quartiles				FAI Quartiles				E2 Quartiles			
	≤25th	25-50th	50-75th	>75th	≤25th	25-50th	50-75th	>75th	≤25th	25-50th	50-75th	>75th	≤25th	25-50th	50-75th	>75th
Cholesterol, total (mmol/L)*	6.16	5.78	6.01	5.98	5.86	6.04	6.19	5.89	6.01	5.90	5.97	6.05	5.71	6.06	6.04	6.12
Cholesterol, HDL (mmol/L)*	1.10	1.22	1.25	1.42¶	1.13	1.23	1.30	1.31	1.40	1.27	1.18	1.16#	1.23	1.18	1.19	1.40¶
Cholesterol, LDL (mmol/L)*	4.09	3.88	4.01	4.00	3.94	3.99	4.03	3.96	3.96	3.99	4.01	4.04	3.92	4.17	4.11	3.84
Cholesterol, VLDL (mmol/L)*	0.85	0.59	0.56	0.47¶	0.74	0.61	0.59	0.50**	0.50	0.55	0.60	0.76¶	0.52	0.59	0.65	0.60#
Triglycerides (mmol/L)*	1.88	1.25	1.21	1.00¶	1.63	1.32	1.26	1.06**	1.06	1.18	1.30	1.69¶	1.10	1.25	1.50	1.31**
BMI (kg/m^2)†	27.0	26.4	25.7	24.4¶	27.4	26.8	25.6	24.6¶	25.0	26.2	26.4	26.3**	25.4	26.1	25.9	27.1#
BF mass (kg)†	22.8	21.4	19.3	17.8¶	23.4	22.6	19.5	18.2¶	18.6	20.1	21.4	21.8#	19.5	20.4	21.0	22.3#
Systolic BP (mm Hg)‡	130.0	129.0	125.5	130.0	133.0	131.0	126.0	123.0	133.0	132.0	127.5	123.0	124.0	125.0	131.5	135.0
Diastolic BP (mm Hg)‡	85.0	86.0	82.0	80.0	87.0	85.0	82.0	80.0	81.0	85.0	85.0	82.0	82.0	82.0	83.0	85.0
LVM (g)§	210.5	208.3	191.7	195.6	209.2	201.7	199.3	193.4	195.4	209.2	196.3	206.3	202.8	203.7	199.4	200.2#
LVMI (g/m^2)§	102.7	103.1	95.0	103.1	103.9	101.5	98.3	100.8	102.6	103.8	99.5	99.6	102.7	100.7	99.8	101.6
PWV (m/sec)‖	10.9	10.8	11.1	11.3	11.3	11.3	10.7	10.9	11.7	11.4	10.8	10.5	10.2	10.8	11.3	12.0

* Adjusted for alcohol/BMI/age in multivariate model.
† Adjusted for smoking/alcohol/age in multivariate model.
‡ Adjusted for smoking/BMI/age in multivariate model.
§ Adjusted for sysBP/BMI/age in multivariate model.
‖ Adjusted for sysBP/age in multivariate model.
¶ $P < .0005$ in adjusted model.
# $P < .05$ in adjusted model.
** $P < .005$ in adjusted model.

BF were associated with high levels of SHBG and total T, whereas the opposite associations applied to E2 and FAI.

Blood pressure. Systolic BP was negatively associated with total T and FAI and positively associated with E2 in the unadjusted ANOVA. In the GLM adjusted for current smoking, BMI, and age, all associations between systolic BP and hormones became nonsignificant. However, after exclusion of men with previously diagnosed IHD or stroke, systolic BP was negatively associated with SHBG and total T in adjusted models.

Diastolic BP was negatively associated with SHBG and T in the unadjusted analyses, losing significance in the adjusted GLM models. Diastolic BP remained negatively associated with SHBG in adjusted models after exclusion of men with previously diagnosed IHD or stroke.

Age. Figure 1 shows gonadal hormones in relationship to age. SHBG levels were lowest in the younger age cohorts with a mean (95% confidence interval [CI]) of 34.9 (32.1 to 36.9) nmol/L in the 40-year-old males and 36.7 (34.4 to 39.2) nmol/L, 42.5 (39.4 to 46.0) nmol/L and 52.8 (48.6 to 57.4) nmol/L in the 50, 60, and 70-year-old males, respectively (Pearson's $r = .32$; $P < .0005$ when analyzed by linear regression). total T did not change significantly with age. FAI decreased from 55.4 (52.2 to 58.5) in the 40-year-old to 48.0 (45.6 to 50.3), 42.0 (39.5 to 44.5), and 35.0 (32.7 to 37.3) at age 50, 60, and 70 years, respectively ($r = -.42$; $P < .0005$). E2 increased slightly with age ($r = .11$; $P = .01$). Compared with the youngest age cohort, mean SHBG was 51% higher, mean FAI 37% lower, and mean E2 8% higher in the oldest age cohort. A partial correlation with BMI as covariate resulted in correlation coefficients of 0.36 (SHBG *v* age), -.42 (FAI *v* age), and 0.10 (E2 *v* age); *P* values remained unchanged.

Smoking. Forty-eight percent of the men were current smokers, defined as daily or occasionally smoking of any kind of tobacco. total T and SHBG varied significantly with current smoking, whereas FAI did not. E2 varied significantly with smoking only when adjusted for BMI. In general, current smokers had higher hormone levels. In the upper T quartile group, 62.5% of the men were current smokers compared with 32.5% in the lowest quartile group (χ^2, 26.3; $P < .0005$). In the upper SHBG quartile group, 55.5% were current smokers compared with 29.5% in the lowest quartile group (χ^2, 25.5; $P < .0005$). In the smoker group, BMI adjusted means of SHBG, T, and E2 were 42.3 nmol/L, 19.3 nmol/L, and 121.3 pmol/L, respectively, whereas the corresponding means in the nonsmoker group were 37.5 nmol/L ($P < .005$), 17.0 nmol/L ($P < .0005$), and 113.8 pmol/L ($P < .05$). No hormone was associated with cigarette pack-years.

Hormones Versus LVM and Compliance

LVM

In the unadjusted analyses, LVM and LVMI did not vary significantly with any hormone. When adjusted for systolic BP, BMI, and age (the main predictive variables of LVM), LVM was significantly associated with E2. The estimated means (95% CI) of LVM were 215.8 (206.1 to 225.9), 202.8 (194.1 to 211.8), 196.3 (187.5 to 205.6), and 200.9 (191.4 to 210.9) g in the first, second, third, and fourth E2 quartile categories, respectively ($P = .03$). The association between LVMI and E2 was near significant after adjustment for confounders ($P = .06$).

Aortic Compliance

High PWV (ie, low compliance) was associated with low FAI and high E2 levels, these associations being highly significant in the unadjusted ANOVA. When adjusted for systolic BP and age, which are the main predictive variables of compliance, PWV versus FAI became nonsignificant ($P = .91$) and PWV versus E2 near significant ($P = .061$), with higher PWV in the upper E2 quartile categories. Compliance was not associated with SHBG or total T.

Multiple Regression

To account for the close interrelationship among several of the hormones studied, stepwise multiple regression was used with selected CVD risk factors as dependent variables and hormones plus various confounders as explanatory variables. Table 3 summarizes the multiple regression results.

Predictors for PWV (ie, aortic compliance) were age, systolic BP, and E2, the latter accounting for less than 1% of the

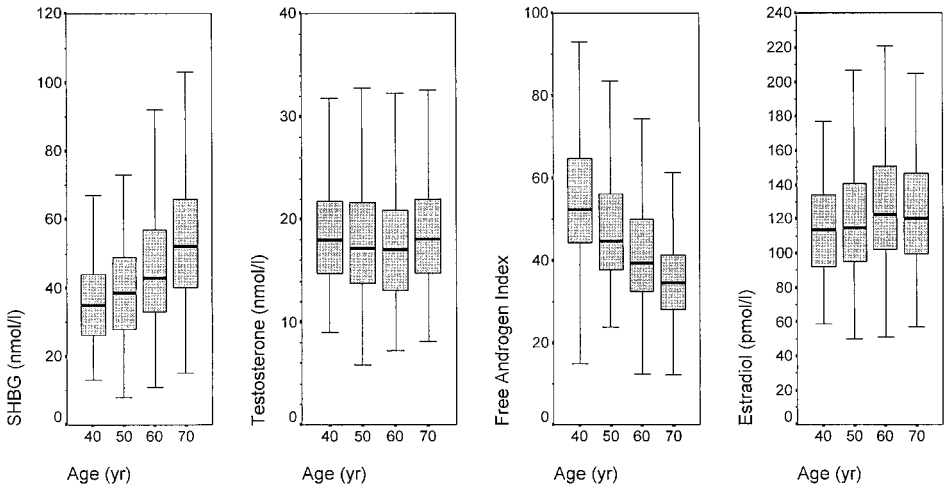

Fig 1. Boxplot of hormones (median, 25th, 75th percentiles, and whiskers) split by age.

Table 3. Multiple Linear Regression Models With Selected CVD Risk Factors as Dependent Variables

Dependent Variable	Predictive Variables in Final Model	B (SE)	Standardized Coefficient β	*P* Value	R^2 Change	ΣR^2	Excluded Variables
PWV*	Age	0.00449 (0.000)	0.444	<.0005	.365	.365	SHBG, T, FAI, Diastolic BP
	Systolic BP*	0.638 (0.059)	0.389	<.0005	.132	.497	BMI, Body fat mass, Smoking, Alcohol (low), Alcohol (high), LVM
	E2*	0.0613 (0.027)	0.075	.023	.006	.503	
LVM*	BF mass	0.00575 (0.001)	0.325	<.0005	.140	.140	SHBG, E2, T, FAI, BMI, Age, Smoking, PWV, Diastolic BP
	Systolic BP*	0.495 (0.082)	0.256	<.0005	.057	.197	
	Alcohol (high)†	−0.0484 (0.013)	−0.165	<.0005	.016	.213	
	Alcohol (low)†	−0.0240 (0.012)	−0.093	.039	.007	.221	
HDL	SHBG*	1.247 (0.291)	0.649	<.0005	.120	.120	BMI, Age, Alcohol (high)
	Alcohol (low)†	−0.135 (0.030)	−0.174	<.0005	.044	.164	
	BF mass	−0.0133 (0.002)	−0.275	<.0005	.038	.202	
	E2*	0.623 (0.116)	0.225	<.0005	.031	.233	
	T	−0.0232 (0.007)	−0.349	.001	.018	.251	
	Current smoking†	−0.0658 (0.029)	−0.089	.025	.008	.259	
	FAI	0.00546 (0.003)	0.247	.046	.006	.264	
VLDL*	SHBG*	−0.414 (0.049)	−0.371	<.0005	.166	.166	E2, T, FAI, BMI, Alcohol (low), Alcohol (high)
	BF mass	0.00825 (0.001)	0.296	<.0005	.087	.254	
	Current smoking‡	0.0493 (0.017)	0.117	.003	.012	.266	
	Age	0.00192 (0.001)	0.095	.024	.008	.273	
Triglycerides*	SHBG*	−0.442 (0.052)	−0.373	<.0005	.171	.171	E2, T, FAI, BMI, Alcohol (low), Alcohol (high)
	BF mass	0.00858 (0.001)	0.287	<.0005	.080	.251	
	Current smoking‡	0.0549 (0.018)	0.121	.002	.013	.264	
	Age	0.00182 (0.001)	0.083	.047	.006	.270	

NOTE. Stepwise procedure criteria: Probability of F to enter ≤0.050 and probability of F to remove ≥0.100.

* Log-transformed values.

† Dummy variable. Low alcohol (less than 7 drinks/week), high alcohol (≥21 drinks/week).

‡ Dummy variable. Current smoking (yes/occasionally).

total variation in PWV. No hormone was included as predictive variable for LVM, and the final model consisted of BF mass, systolic BP, and alcohol consumption.

In the regression model for HDL (dependent), SHBG, alcohol (low consumption), BF mass, E2, T, smoking, and FAI were included as predictors. SHBG was the best predictive variable, accounting for 12% of the variance in HDL, whereas E2, T, and FAI explained 3%, 2%, and less than 1%, respectively. Remarkably, the estimated regression coefficient for T was negative, and the estimated regression coefficient for FAI positive, contrary to the GLM results, which suggested a positive association between HDL and T and a negative association between HDL and FAI. Partial correlation showed that the correlation coefficient for T versus HDL decreased slightly when controlling for E2 alone, but changed substantially from positive ($r = .17$; $P < .0005$) to negative, nonsignificant ($r = -.042$; $P = .35$) when controlling for SHBG alone.

In the regression models for VLDL and triglycerides as dependent variables, the predictors in both models were SHBG, BF mass, smoking, and age. SHBG was the best explanatory variable, accounting for 17% of the total variance in VLDL and triglycerides, which in both models, was considerably more than BF mass. No other hormone contributed significantly to either model. Thus, when controlling for SHBG, the lipids VLDL and triglycerides do not vary significantly with total T, FAI, and E2, in contrast to the results of GLM not controlling for SHBG.

DISCUSSION

In the present cross-sectional study of 508 healthy males aged 41 to 72 years, we found significant associations between SHBG, several gonadal steroids, and cardiovascular risk factors, primarily the plasma lipids, HDL, VLDL, and triglycerides, but no association with total cholesterol and LDL. Atherogenic lipid profile were correlated with low SHBG, low total T, and high FAI, while men in the upper E2 quartile group seemed to have a more favorable lipid profile. Additionally, E2 was negatively associated with LVM. No other hormone varied significantly with LVM or aortic compliance after adjustment for confounders.

As indexes of obesity, we used BF measurement by the impedance method and BMI. Central body fat distribution (CBF) was not assessed, although previous studies have suggested that CBF was a strong correlate of both plasma lipids and androgen levels in men. Inclusion of CBF in our models could possibly weaken the association between androgens, SHBG, and plasma lipids. However, several studies have shown that the association between SHBG and HDL-cholesterol remains significant after adjustment for waist-to-hip-ratio.[21,22]

Age, BF mass, and smoking were associated with some, but not all, sex hormones. We found higher serum levels of total T and SHBG, but not of FAI, among current smokers. Several prior studies have reported a similar positive association be-

tween smoking and SHBG or T.[3,23] Additionally, E2 levels were elevated in current smokers, after adjustment for BMI, in accordance with previous reports.[24,25]

When analyzed by GLM, the relationship between total T and plasma lipids was opposite to the relationship between FAI and plasma lipids, an observation explained by the interrelationship among SHBG, T, and FAI. SHBG correlated positively with total T ($r = .58$) and negatively with FAI ($r = -.70$), while total T and FAI correlated positively ($r = .11$), indicating that SHBG is a better predictor of FAI than T. When all hormones were included in stepwise multiple regression as explanatory variables, SHBG was the best predictor of HDL, VLDL, and triglycerides, and SHBG substantially reduced the variance in HDL otherwise explained by T and FAI. The regression analyses illustrate the diverging results when investigating associations between sex hormones and lipids with and without SHBG adjustment.

Most clinical studies of exogenous T administration and plasma lipids focus on T and not SHBG. Two studies, reporting decreased HDL levels after peroral T administration, also determined SHBG, and both found significantly depressed SHBG levels,[10,26], while Zgliczynski et al[13] found no change in either HDL or SHBG after parenteral T administration. Indeed, exogenous T in supraphysiologic doses and peroral anabolic steroids suppress SHBG levels,[26] but whether the HDL decrease is mediated by SHBG or is related directly to T dose and route of administration, remains speculative. If we assume there is a causal relationship between SHBG and HDL cholesterol, this may explain the diverging results between cross-sectional and clinical intervention studies. Endogenous total T correlates positively with SHBG (in the present study, $r = .58$), and although SHBG is a better predictor of HDL than T, the close correlation between SHBG and total T explain the observed positive association between T and HDL in cross-sectional studies. Conversely, high doses of exogenous T suppress SHBG and thereby lower HDL-cholesterol levels. Parenterally administered T in physiologic doses does not reduce HDL, but the effect on SHBG in this case is less well documented.

As suggested by Pugeat et al,[27] a possible mechanism for SHBG-HDL interaction is the effect of sex steroids on hepatic lipoprotein lipase biosynthesis. This enzyme, which is central in the regulation of HDL, is stimulated by androgens and inhibited by estrogens, and by modulating the E2-T balance, SHBG might regulate the activity of hepatic lipoprotein lipase. Another possibility is a direct effect of SHBG on HDL metabolism via an unidentified SHBG receptor. However, it is well established that SHBG is associated with glucose metabolism, and SHBG levels correlate negatively with insulin and fasting glucose levels in vivo.[28] Furthermore, it is postulated that insulin downregulates SHBG synthesis in vitro.[29] In theory, the association between unfavorable lipid profile and low SHBG might reflect an effect of insulin on both.

Various studies, including the present, have found that low SHBG is associated with unfavorable levels of several strong CVD risk factors in both males[21,30] and females.[22] However, prospective studies have failed to detect any predictive value of baseline SHBG on subsequent development of CVD, except for postmenopausal females.[31] Theoretically, free T could be the sex hormone with causal effect on plasma lipids, and the inconsistent associations between free T and plasma lipids, reported in the literature, could rely on limitations in available methods of direct measurement of free T. In accordance with the free hormone hypothesis,[32] free T probably reflects the biologically active fraction of circulating T, and bioactive T must correlate negatively with SHBG in vivo.[33] Low SHBG (and consequently high free T) are associated with an atherogenic lipid profile, but with respect to other CVD risk factors, some evidence points to a beneficial effect of free T directly on coronary artery endothelium[34,35] and on the fibrinolytic system.[36,37] These opposing effects might explain the lack of prospective evidence for SHBG and T as independent CVD risk factors, at least in males.

Goodman-Gruen et al[6] found that women younger than 65 years had significantly higher SHBG levels than age-matched men, while there was no significant gender difference in SHBG over the age of 65. A protective role of endogenous E2 is another possible explanation for the gender difference in cardiovascular disease. We found a less atherogenic lipid profile in the upper E2 quartile group compared with the 3 lower E2 quartiles and a negative association between E2 and LVM, supporting a protective role for E2 on the cardiovascular system. In our study, the majority of men in the upper E2 quartile were either obese, heavy drinkers, or both (data not shown). Thus, a protective effect of E2 in these men is likely to be overruled by a deleterious impact on health from other risk factors associated with high endogenous E2 in men.

E2 and SHBG increased with older age, while FAI decreased. Total T was not significantly different between age cohorts. Several investigators have reported decreasing free T with age, mostly concomitant with decreasing total T, although the decrease in free T is often more pronounced.[38,39] However, these studies, including the present, share the common weakness of cross-sectional design; therefore, not allowing firm conclusions on cause-effect relationship. In theory, the observed high SHBG and low free T level in older men could be due to a higher probability of surviving into old age, perhaps caused by a favorable lipid profile, rather than a true age-related hormonal change.

In conclusion, our results from this cross-sectional study of 508 randomly selected, healthy males, suggest that: (1) low SHBG, low total T levels, and a high FAI are associated with increased cardiovascular risks, primarily mediated via an unfavourable plasma lipid profile. (2) Males in the upper E2 quartile group may have a more favorable lipid profile than others, and E2 seems to have a protective effect on left ventricular hypertrophy. (3) SHBG is a key hormone in the observed association between plasma lipids and sex hormones. We hypothesize a possible causal relationship between SHBG and HDL-cholesterol, which may explain previous inconsistent findings of studies on gonadal steroids and cardiovascular risk factors.

Focus on SHBG could prove valuable in future investigations of risk factors, accounting for the observed difference in cardiovascular morbidity and mortality between males and premenopausal females.

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