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Endogenous Sex Steroid Hormones, Lipid Subfractions, and Ectopic Adiposity in Asian Indians

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Abstract

Background: Estradiol, testosterone (T), and sex hormone binding globulin (SHBG) levels are associated with lipid subfractions in men and women. Our objective was to determine if associations are independent from adipose tissue area among Asian Indians.

Methods: We used data from 42 women and 57 Asian Indian men who did not use exogenous steroids or lipid-lowering medications. Lipoprotein subfractions including low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), and intermediate density lipoprotein (IDL) were assessed by ion mobility spectrometry. Intra-abdominal adiposity was assessed by computed tomography. Multivariable regression models estimated the association between sex hormones with lipoprotein subfractions before and after adjustment for adiposity.

Results: Among women, lower $_{log}$SHBG levels were associated with smaller $_{log}$LDL particle size and higher $_{log}$triglycerides, $_{log}$VLDL, and $_{log}$IDL, although these associations were attenuated with adjustment for visceral adiposity in particular. Among women, lower $_{log}$SHBG levels was significantly associated with lower $_{log}$medium LDL and $_{log}$small LDL concentrations even after consideration of visceral and hepatic adiposity and insulin resistance as represented by the homeostasis model assessment of insulin resistance (HOMA-IR). Among men, lower $_{log}$SHBG was also associated with smaller $_{log}$LDL peak diameter size and higher $_{log}$triglycerides and $_{log}$VLDL, even after adjustment for HOMA-IR and adiposity. Relationships between sex steroids and lipid subfractions were not significant among women. Among men, higher total testosterone was associated with higher $_{log}$HDL and $_{log}$LDL particle size, and lower $_{log}$triglycerides and $_{log}$VLDL, but these associations were partially attenuated with adjustment for adiposity and HOMA-IR.

Conclusions: Among Asian Indians, SHBG is associated with more favorable lipid subfraction concentrations, independent of hepatic and visceral fat.

Introduction

Asian Indians represent a quarter of the world's population and are the second fastest growing ethnic group in the United States, with over 3 million U.S. residents.[1] Both native and migrant Asian Indians have a high prevalence of diabetes, hypertension, and cardiovascular disease (CVD).[2] However, Asian Indians develop these conditions at relatively low body mass indices compared with other racial/ethnic groups.[2] Thus, the pilot Metabolic Syndrome and Atherosclerosis in South Asians Living in America (MASALA) study was designed to characterize the relationship between metabolic syndrome and CVD risk in Asian Indians.[1]

The high risk of the population and unique adiposity composition also makes it an ideal one in which to study the relationship between sex steroids and lipids, both of which have been associated with visceral and hepatic adiposity and CVD risk.[3] Smaller, denser low-density lipoprotein (LDL) particles are associated with greater risk CVD risk, an effect that has been found in some studies to be independent of standard lipid levels, and higher total LDL particle concentrations generally reflect denser LDL.[4] Similarly, lipoprotein (a) or Lp(a), an LDL-like particle, increases risk of CVD independent of conventionally measured lipid levels.[5] Finally, recent studies suggest that the high-density lipoprotein (HDL) inflammatory properties are better predictors

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of the anti-atherogenic properties of HDL than HDL cholesterol levels.[6] No studies have examined the relationship between endogenous sex hormone profiles and measures of lipoprotein heterogeneity in Asian Indians[7] or whether such associations persist after visceral and hepatic adiposity are considered. However, in examinations of conventional lipid profiles, greater androgenicity (as represented by higher testosterone [T], lower estradiol [E2], and lower sex hormone binding globulin [SHBG]) has been associated with more atherogenic profiles in women and less atherogenic profiles in men, and such associations are partially mediated and/or attenuated after adjustment for visceral and hepatic adiposity.[8]

Therefore, we examined the relationships between endogenous sex hormone levels and lipoprotein subclasses using data from preliminary studies of the MASALA study, a cohort of Asian Indians in the United States.[9] The aim of this secondary analysis was to determine whether more androgenic profiles were associated with more atherogenic lipoprotein subfraction profiles in women and less atherogenic lipoprotein profiles in men and whether such associations persisted after consideration of adipose tissue mass.

Methods

From August 2006 to October 2007, the pilot MASALA study enrolled 150 community-dwelling individuals (75 men and 75 women) living in the San Francisco Bay area who self-identified as Asian Indian.[9] Participants were aged 45–84 years and had no known CVD. Detailed study methods have been described elsewhere.[9] Briefly, this pilot was population-based, with random sampling of households in the San Francisco Bay Area with Asian Indian surnames from the California Health Interview Survey. Individuals were eligible for the study if they were free from physician-diagnosed CVD (myocardial infarction, stroke, transient ischemic attack, congestive heart failure, angina, coronary artery bypass graft surgery, percutaneous cardiovascular interventions). Persons were excluded if they could not speak or understand Hindi or English. For the purposes of this study, individuals from other South Asian countries were excluded. Sixty-one percent of eligible participants who were able to be contacted by telephone were enrolled. The Institutional Review Board at the University of California, San Francisco approved the study protocol, and all study participants provided written informed consent. For this analysis, we excluded participants who were premenopausal, as blood draws were not coordinated to the menstrual cycle, as well as participants who were using lipid-lowering medication or who were currently using exogenous sex steroid therapy, leaving a total of 42 women and 57 men.

Sociodemographic characteristics and medical history were assessed via questionnaire, and use of statins, niacin, and fibrates were assessed by medication inventory. Participant weight was determined using a digital scale, height was measured with a stadiometer, and waist circumference was taken using a measuring tape at the site of greatest waist circumference. Visceral and subcutaneous adiposity and hepatic liver-to-spleen attenuation ratio (values <1 represented higher amounts of hepatic fat) were measured using computed tomography (CT) (Philips Medical Systems). Visceral and subcutaneous abdominal fat were measured at the L4–L5 level after participants were positioned supine. Non-enhanced CT images of liver and spleen density were used to quantify hepatic fat content. CT scans were digitally recorded for batched readings by a trained research assistant; intra-abdominal adipose tissue area was quantified by delineating the intra-abdominal cavity at the innermost aspect of the abdominal and oblique muscle walls surrounding the cavity.[10]

Participants were asked to provide blood samples after a 12-hour fast. Fasting serum insulin was measured by radioimmunoassay (Millipore), and the homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as a proxy measure of insulin resistance using the formula $[I_0\ (\mu IU/mL) \times G_0\ (mmol/L)/22.5]$.[11] Total cholesterol, triglycerides, and HDL were measured by enzymatic methods (Quest), and LDL was calculated.[12] Lipoprotein particle subfractions were assessed at the Children's Hospital Oakland Research Institute using ion mobility techniques that have been previous described.[13] Briefly, ion mobility uses an electrospray procedure to obtain direct lipoprotein particle counts as a function of particle size; direct comparison of ion mobility techniques with other methods have demonstrated similar relationships with degree of atherosclerosis.[13] Particle concentrations (nmol/L) were determined for subfractions defined by the following size intervals: very low-density lipoprotein (VLDL): large (42.40–54.70 nm), medium (33.50–42.39 nm), small (29.60–33.49 nm); intermediate density lipoprotein (IDL): large (25.00–29.59 nm), small (23.33–24.99 nm); LDL: large LDL1 (22.46–23.32 nm), medium LDL2a (22.20–22.45 nm) and LDL2b (21.41–22.19 nm), small LDL3a (20.82–21.40 nm) and LDL3b (20.49–20.81 nm), and very small LDL4a (19.90–20.48 nm) and LDL4b (19.00–19.89 nm). Based upon correlations previously observed between lipoprotein subfraction measurements,[13] LDL fractions 1 and 2a were classified as large LDL, fraction 2b as medium LDL, fraction 3a as small LDL, and fractions 3b, 4a, and 4b as very small LDL. LDL particle concentration was defined as the sum of LDL subfraction measurements. Intermediate density lipoprotein (IDL) fraction 1 was classified as large IDL and IDL2 as small IDL. For the purposes of this analysis, LDL particle concentrations were calculated as the sum of LDL particle concentrations, and VLDL fractions consisting of small, medium, and large particles were combined.[13] Interassay variation was less than 15% for all subfractions.

Lp(a) was measured with the BNII nephelometer [N Latex Lp(a) Reagent; Dade Behring Inc.) utilizing a particle enhanced immunonepholometric assay at the Laboratory for Clinical Biochemistry Research at the University of Vermont. The assay range was 10.0–160 mg/dL and the minimum detectable level of Lp(a) was 0.20 mg/dL. Intraassay coefficients of variation (CVs) range from 1.8% to 4.1% and interassay CVs range from 2.0% to 5.3%. [14] The HDL-inflammatory index was assessed at the University of California Los Angeles, and methods have been previously described.[15] HDL was isolated from blood samples using dextran sulfate precipitation. The LDL, necessary in the cell-free assay for testing the ability of HDL to protect against LDL oxidation, was aliquotted and cryopreserved in sucrose. Dichlorofluorescein-diacetate was dissolved in fresh methanol at 2.0 mg/mL, incubated at room temperature, and protected from light for 30 min, which resulted in the release of dichlorofluorescein that produced an intense fluorescence upon interaction with oxidized lipid. Fluorescence was determined using a plate reader (Spectra Max, Gemini XS; Molecular Devices) at an excitation wavelength of

485 nm, an emission wavelength of 530 nm, and a cutoff of 515 nm with the photomultiplier sensitivity set at medium. For this study, the coefficient of variation for this assay was 9.6%. HDL-inflammatory index was calculated by normalizing the assay values obtained for LDL alone as 1.0. If the addition of a test HDL resulted in a value of 1.0 or greater, the test HDL was classified as pro-inflammatory or dysfunctional. Conversely, if the addition of the standard normal LDL together with a test HDL resulted in a value less than 1.0, the test HDL was classified as anti-inflammatory.

E2, T, and SHBG were measured at the Reproductive Endocrine Research Laboratory at the University of Southern California. SHBG was measured by a solid-phase, two-site chemiluminescent assay on the Immulite Analyzer (Siemens Healthcare Diagnostics). The SHBG assay sensitivity is 1 nmol/L and the interassay CV is <10%. E2 and T were quantified in serum (0.5 mL) by a previously described radioimmunoassay (RIA) method.[10] Prior to RIA, steroids were extracted with hexane:ethyl acetate (3:2), and then E2 and T were separated from each other and their metabolites by Celite column partition chromatography. The assay sensitivities for the E2 and T RIAs are 2 pg/mL and 1.5 ng/dL, respectively. The interassay coefficients of variation (CVs) for these assays are 11%, 13%, and 12% at 15, 36, and 101 pg/mL and 8%, 12%, and 12% at 13, 30, and 96 ng/dL, respectively. Free E2 and T were calculated using a validated algorithm based on derived equations,[16,17] taking the concentrations of total T, total E2, and SHBG into account and assuming a fixed albumin concentration of 3.5 g/dL.

We compared baseline characteristics of participants by sex using chi-squared tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. We used a series of sex-stratified multivariable regression models to examine the association between endogenous sex steroid hormones and lipoprotein subfractions, before and after adjustment for visceral adiposity and hepatic density. To meet model assumptions, lipoprotein subfractions and endogenous sex hormone levels were log-transformed. Finally, we examined the associations between sex hormones and LDL and IDL subfractions; models containing both men and women were constructed and an interaction term for sex and SHBG included. The prevalence of metabolic syndrome

Table 1. Descriptive Statistics for Respondents, Shown as Median (Interquartile Ratio) Unless Indicated as *N* (%)

	Women (n = *42*)	*Men* (n = *57*)	P-*value*
Age (years)	53 (7.6)	56 (14)	0.13
Current smoking, *n* (%)	0 (0)	0 (0)	0.99
Number of alcoholic beverages per week	0 (1)	2 (7)	<0.01
Systolic blood pressure >130 mm Hg or diastolic blood pressure >85 mm Hg, *n* (%)	5 (12)	32 (56)	<0.01
Waist circumference >102 cm, *n* (%)		18 (32)	
Waist circumference >88 cm, *n* (%)	22 (52)		
HDL <40 mg/dL, *n* (%)		20 (35)	
HDL <50 mg/dL, *n* (%)	15 (35)		
Triglycerides >150 mg/dL, *n* (%)	5 (12)	23 (40)	<0.01
Fasting glucose >100 mg/dL, *n* (%)	7 (17)	26 (46)	<0.01
Presence of metabolic syndrome, *n* (%)	6 (14)	13 (23)	0.29
BMI (kg/m^2)	24.3 (5.4)	25.9 (5.8)	0.21
Waist circumference (cm)	89 (18)	97 (13)	<0.01
Visceral fat area (per SD cm^2)	98.2 (53.7)	145.0 (66.5)	<0.01
Liver-to-spleen attenuation ratio (HU)	1.3 (0.2)	1.2 (0.3)	0.17
HOMA-IR (mg/dL • mIU/L)	2.0 (1.4)	3.1 (3.3)	<0.01
Total E2 (*p*g/mL)	16.5 (59.5)	41.0 (14.5)	<0.01
Total T (ng/dL)	27.1 (13.7)	415.9 (340.2)	<0.01
SHBG (nmol/L)	46.2 (29.1)	27.9 (15.5)	<0.01
Free E2 (*p*g/mL)	0.4 (0.9)	1.2 (0.5)	<0.01
Free T (ng/dL)	4.8 (3.3)	128.5 (75.1)	<0.01
LDL cholesterol (mg/dL)	109 (40)	113 (43)	0.31
HDL cholesterol (mg/dL)	53 (20)	44 (14)	<0.01
Triglycerides (mg/dL)	109 (54)	117 (104)	0.13
VLDL combined (mg/dL)	125.0 (56.1)	23.0 (21.0)	0.01
LDL peak diameter (Å)	222.7 (5.0)	218.5 (10.0)	<0.01
LDL particle concentration (nmol/L)	1789.2 (430.8)	1986.4 (533.1)	<0.01
HDL inflammatory index	0.37 (0.36)	0.39 (0.51)	0.66
Lipoprotein a (g/L)	0.18 (0.23)	0.12 (0.17)	0.12
Large IDL (nmol/L)	145.6 (67.2)	164.5 (75.3)	0.09
Small IDL (nmol/L)	227.2 (89.9)	185.0 (58.6)	<0.01
Large LDL (nmol/L)	268.8 (110.5)	250.5 (113.3)	0.41
Medium LDL (nmol/L)	234.9 (113.7)	302.1 (137.7)	<0.01
Small LDL (nmol/L)	138.8 (72.0)	232.2 (250.2)	<0.01
Very small LDL (nmol/L)	172.3 (54.6)	199.8 (179.8)	<0.01

BMI, body mass index; E2, estradiol; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HU, Hounsfield units; IDL, intermediate density lipoprotein; LDL, low-density lipoprotein; SHBG, sex hormone binding globulin; T, testosterone; VLDL, very low-density lipoprotein.

was determined using the modified Adult Treatment Panel III definition.[18] The analysis was completed using SAS version 9.3, SAS Institute.

Results

Participant characteristics are shown in Table 1. Few participants were smokers and most women had less than one alcoholic beverage per week. Despite the lack of known CVD, elevated waist circumference, and HDL abnormalities were common among women and elevations in glucose, hypertension, and elevated triglycerides were common among men. Fourteen percent of women had metabolic syndrome, compared with 23% of men. Men had higher levels of visceral fat but similar levels of hepatic density compared with women. Despite the fact that the majority of adults were not obese, the median HOMA-IR for both men and women were elevated.[19] Consistent with the lack of baseline CVD, the

Table 2. Associations Between Sex Hormones and Lipoprotein Fractions in Women, Beta-coefficient (*P*-value)

	SHBG	*E2*	*T*
log LDL (mg/dL)			
Unadjusted	–0.03 ($P=0.09$)	–0.05 ($P=0.06$)	0.01 ($P=0.92$)
Model 1*	–0.08 ($P=0.20$)	–0.03 ($P=0.31$)	0.03 ($P=0.65$)
Model 1, and visceral adiposity (SD^3)	–0.09 ($P=0.27$)	–0.03 ($P=0.29$)	0.05 ($P=0.52$)
Model 1, and hepatic density (HU)	–0.09 ($P=0.23$)	–0.03 ($P=0.32$)	0.01 ($P=0.90$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	–0.09 ($P=0.33$)	–0.03 ($P=0.38$)	0.0 ($P=0.97$)
log HDL (nmol/L)			
Unadjusted	0.15 (0.06)	0.0 ($P=0.81$)	0.04 ($P=0.68$)
Model 1*	0.13 ($P=0.12$)	0.01 (0.78)	0.06 ($P=0.58$)
Model 1, and visceral adiposity (SD^3)	0.0 ($P=0.93$)	0.02 ($P=0.57$)	–0.01 ($P=0.91$)
Model 1, and hepatic density (HU)	**0.21 ($P=0.05$)**	0.04 ($P=0.38$)	0.09 ($P=0.45$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	0.09 ($P=0.45$)	0.03 ($P=0.48$)	0.02 ($P=0.88$)
log Triglycerides (mg/dL)			
Unadjusted	**−0.27 ($P=0.008$)**	0.0 ($P=0.93$)	–0.25 ($P=0.054$)
Model 1*	**−0.33 ($P=0.002$)**	0.03 ($P=0.55$)	–0.25 ($P=0.08$)
Model 1, and visceral adiposity (SD^3)	–0.16 ($P=0.20$)	0.02 ($P=0.74$)	–0.14 ($P=0.27$)
Model 1, and hepatic density (HU)	**−0.24 ($P=0.05$)**	–0.03 ($P=0.53$)	–0.20 ($P=0.11$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	–0.17 ($P=0.24$)	–0.03 ($P=0.62$)	–0.14 ($P=0.28$)
log VLDL (nmol/L)			
Unadjusted	–0.14 ($P=0.18$)	–0.07 ($P=0.15$)	–0.17 ($P=0.21$)
Model 1*	**−0.23 ($P=0.02$)**	–0.02 ($P=0.72$)	–0.14 ($P=0.27$)
Model 1, and visceral adiposity (SD^3)	–0.20 ($P=0.13$)	–0.03 ($P=0.56$)	–0.09 ($P=0.49$)
Model 1, and hepatic density (HU)	**−0.52 ($P=0.0005$)**	–0.06 ($P=0.25$)	–0.08 ($P=0.47$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	–0.15 ($P=0.33$)	–0.06 ($P=0.31$)	–0.09 ($P=0.51$)
log LDL peak diameter (Å)			
Unadjusted	**0.01 ($P=0.02$)**	0.0 ($P=0.19$)	0.01 ($P=0.33$)
Model 1*	**0.02 ($P=0.02$)**	0.0 ($P=0.16$)	0.01 ($P=0.37$)
Model 1, and visceral adiposity (SD^3)	**0.01 ($P=0.03$)**	0.0 ($P=0.22$)	0.01 ($P=0.76$)
Model 1, and hepatic density (HU)	**0.02 ($P=0.01$)**	0.0 ($P=0.63$)	0.01 ($P=0.38$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	0.02 ($P=0.07$)	0.0 ($P=0.70$)	0.0 ($P=0.55$)
LDL particle concentration (nmol/L)			
Unadjusted	**−0.13 ($P=0.01$)**	–0.02 ($P=0.39$)	–0.06 ($P=0.36$)
Model 1*	**−0.16 ($P=0.002$)**	–0.02 ($P=0.52$)	–0.04 ($P=0.51$)
Model 1, and visceral adiposity (SD^3)	–0.11 ($P=0.08$)	–0.02 ($P=0.51$)	0.0 ($P=0.98$)
Model 1, and hepatic density (HU)	**−0.16 ($P=0.01$)**	–0.02 ($P=0.41$)	–0.03 ($P=0.56$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	–0.11 ($P=0.12$)	–0.02 ($P=0.54$)	–0.03 ($P=0.64$)
log Lp(a) (g/L)			
Unadjusted	0.12 ($P=0.60$)	–0.11 ($P=0.29$)	0.24 ($P=0.43$)
Model 1*	0.05 ($P=0.83$)	–0.07 ($P=0.56$)	0.28 ($P=0.37$)
Model 1, and visceral adiposity (SD^3)	0.04 ($P=0.91$)	–0.07 ($P=0.57$)	0.27 ($P=0.41$)
Model 1, and hepatic density (HU)	0.05 ($P=0.88$)	–0.11 ($P=0.44$)	0.18 ($P=0.59$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	0.0 ($P=0.99$)	–0.11 ($P=0.45$)	0.10 ($P=0.78$)
log HDL inflammatory index			
Unadjusted	–0.13 ($P=0.44$)	0.03 ($P=0.70$)	0.15 ($P=0.49$)
Model 1*	–0.15 ($P=0.34$)	0.07 ($P=0.39$)	0.18 ($P=0.37$)
Model 1, and visceral adiposity (SD^3)	0.05 ($P=0.88$)	0.06 ($P=0.48$)	0.30 ($P=0.14$)
Model 1, and hepatic density (HU)	–0.13 ($P=0.54$)	0.08 ($P=0.36$)	0.17 ($P=0.44$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	–0.04 ($P=0.87$)	0.10 ($P=0.29$)	0.31 ($P=0.17$)

*Model 1 includes age (years), current smoking, and number of drinks per week.
Figures in *bold* are significant.

HDL inflammatory index was not elevated.[15] Men had greater levels of total E2 and total T and lower levels of SHBG compared with women. Men also had higher LDL article concentrations and higher concentrations of smaller lipid subfractions including medium LDL, small LDL, and very small LDL compared with women.

Table 2 shows the associations between SHBG and lipid subfractions and standard triglycerides, HDL, and LDL among women. Lower SHBG levels were associated a more atherogenic profile, particularly, higher levels of triglycerides, VLDL, smaller LDL peak diameter size, and greater particle concentrations. These relationships were attenuated by adjustment for visceral adiposity in particular. Levels of total E2 and T were not associated with lipid subfractions or activity before or after adjustment.

Table 3 shows the associations between sex hormones and lipoprotein subgroups and activity in men. Similar to women, lower SHBG was associated with a more atherogenic profile, particularly with higher levels of triglycerides, VLDL, and smaller peak diameter size. These associations

Table 3. Associations Between Sex Hormones and Lipoprotein Subfractions in Men

	SHBG	*E2*	*T*
log LDL (mg/dL)			
Unadjusted	–0.05 ($P=0.51$)	0.09 ($P=0.51$)	0.04 ($P=0.58$)
Model 1*	0.05 ($P=0.60$)	0.10 ($P=0.45$)	0.13 ($P=0.12$)
Model 1, and visceral adiposity (SD^3)	0.03 ($P=0.33$)	0.06 ($P=0.66$)	0.15 ($P=0.13$)
Model 1, and hepatic density (HU)	0.05 ($P=0.57$)	0.10 ($P=0.54$)	0.15 ($P=0.11$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	0.00 ($P=0.97$)	–0.02 ($P=0.91$)	0.11 ($P=0.32$)
log HDL (mg/dL)			
Unadjusted	**0.23 ($P=0.003$)**	**0.29 ($P=0.03$)**	**0.22 ($P=0.006$)**
Model 1*	0.16 ($P=0.07$)	**0.25 ($P=0.05$)**	**0.17 ($P=0.03$)**
Model 1, and visceral adiposity (SD^3)	0.13 ($P=0.19$)	0.24 ($P=0.08$)	0.16 ($P=0.11$)
Model 1, and hepatic density (HU)	0.16 ($P=0.09$)	**0.31 ($P=0.04$)**	**0.18 ($P=0.04$)**
Model 1, visceral adiposity, hepatic density, HOMA-IR	0.11 ($P=0.29$)	0.27 ($P=0.10$)	0.15 ($P=0.19$)
log Triglycerides (mg/dL)			
Unadjusted	**−0.61 ($P<0.0001$)**	0.02 ($P=0.94$)	**−0.54 ($P<0.001$)**
Model 1*	**−0.52 ($P=0.002$)**	0.14 ($P=0.59$)	**−0.44 ($P=0.006$)**
Model 1, and visceral adiposity (SD^3)	**−0.41 ($P=0.02$)**	0.20 ($P=0.47$)	–0.32 ($P=0.10$)
Model 1, and hepatic density (HU)	**−0.49 ($P=0.003$)**	–0.11 ($P=0.71$)	**−0.39 ($P=0.02$)**
Model 1, visceral adiposity, hepatic density, HOMA-IR	**−0.46 ($P=0.02$)**	–0.02 ($P=0.95$)	–0.37 ($P=0.08$)
log VLDL (nmol/L)			
Unadjusted	**−0.60 ($P<0.0001$)**	–0.11 ($P=0.65$)	**−0.49 ($P=0.0007$)**
Model 1*	**−0.53 ($P=0.0003$)**	0.03 ($P=0.90$)	**−0.42 ($P=0.003$)**
Model 1, and visceral adiposity (SD^3)	**−0.42 ($P=0.009$)**	0.11 ($P=0.67$)	–0.27 ($P=0.12$)
Model 1, and hepatic density (HU)	**−0.29 ($P=0.03$)**	–0.16 ($P=0.56$)	**−0.40 ($P=0.009$)**
Model 1, visceral adiposity, hepatic density, HOMA-IR	**−0.47 ($P=0.005$)**	–0.08 ($P=0.78$)	–0.33 ($P=0.09$)
log LDL peak diameter (Å)			
Unadjusted	**0.02 ($P=0.002$)**	0.01 ($P=0.39$)	**0.02 ($P=0.009$)**
Model 1*	**0.02 ($P=0.01$)**	0.01 ($P=0.63$)	**0.02 ($P=0.02$)**
Model 1, and visceral adiposity (SD^3)	**0.02 ($P=0.04$)**	0.01 ($P=0.41$)	0.02 ($P=0.10$)
Model 1, and hepatic density (HU)	**0.02 ($P=0.02$)**	0.02 ($P=0.15$)	**0.02 ($P=0.04$)**
Model 1, visceral adiposity, hepatic density, HOMA-IR	**0.02 ($P=0.04$)**	0.02 ($P=0.18$)	0.02 ($P=0.08$)
log LDL particle concentration (nmol/L)			
Unadjusted	**−0.19 ($P=0.002$)**	0.06 ($P=0.60$)	**−0.14 ($P=0.04$)**
Model 1*	–0.14 ($P=0.052$)	0.09 ($P=0.39$)	–0.08 ($P=0.21$)
Model 1, and visceral adiposity (SD^3)	–0.08 ($P=0.29$)	0.16 ($P=0.16$)	0.0 ($P=0.99$)
Model 1, and hepatic density (HU)	–0.12 ($P=0.11$)	0.03 ($P=0.79$)	–0.06 ($P=0.45$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	–0.11 ($P=0.16$)	0.05 ($P=0.69$)	–0.03 ($P=0.69$)
log Lp(a) (g/L)			
Unadjusted	–0.15 ($P=0.56$)	–0.27 ($P=0.55$)	–0.16 ($P=0.55$)
Model 1*	**−0.58 ($P=0.04$)**	–0.46 ($P=0.31$)	–0.34 ($P=0.21$)
Model 1, and visceral adiposity (SD^3)	–0.61 ($P=0.06$)	–0.80 ($P=0.09$)	–0.37 ($P=0.25$)
Model 1, and hepatic density (HU)	–0.67 ($P=0.06$)	–0.75 ($P=0.13$)	–0.33 ($P=0.23$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	–0.44 ($P=0.20$)	–0.52 ($P=0.33$)	–0.11 ($P=0.76$)
log HDL inflammatory index			
Unadjusted	–0.40 ($P=0.14$)	0.13 ($P=0.80$)	–0.32 ($P=0.27$)
Model 1*	–0.19 ($P=0.54$)	0.18 ($P=0.72$)	–0.14 ($P=0.63$)
Model 1, and visceral adiposity (SD^3)	–0.37 ($P=0.32$)	0.20 ($P=0.72$)	–0.42 ($P=0.30$)
Model 1, and hepatic density (HU)	–0.27 ($P=0.41$)	0.52 ($P=0.39$)	–0.27 ($P=0.41$)
Model 1, visceral adiposity, hepatic density, HOMA-IR	–0.41 ($P=0.30$)	0.62 ($P=0.35$)	–0.57 ($P=0.21$)

*Model 1 includes age (years), current smoking, and number of drinks per week.
Figures in *bold* are significant.

remained significant even after adjustment for visceral adiposity, hepatic adiposity, and insulin resistance. Among men, higher levels of E2 were associated with greater concentrations of HDL, but this relationship was not significant after attenuation for visceral adiposity. In contrast to women, higher levels of total testosterone were associated with more favorable lipid profiles, particularly, higher levels of HDL, lower levels of triglycerides and VLDL, and larger LDL peak diameter size. These relationships were attenuated by visceral adiposity.

Table 4 shows the associations of SHBG with IDL and LDL particle sizes in men and women. Among women, higher SHBG levels were associated with lower concentrations of medium LDL and small LDL. These associations were not observed in men, although the *P*-value for the interaction term between sex and SHBG levels was not significant in any models, and the lack of significance may have been determined by sample size. In sensitivity analyses, free E2 and free T were not associated with lipid subfractions in women, similar to analyses of total E2 and total T. In men, free E2 and free T were not associated with lipid subfractions in men in fully adjusted models, and the associations between free T and lipid subfractions in men was attenuated, suggesting that SHBG was a significant contributor to the associations observed between total sex steroids and lipid subfractions (results not shown).

Discussion

Using a population-based sample of Asian Indians in the United States, we found that higher SHBG levels were associated with a slightly less atherogenic lipid profile among both men and women. This association persisted even after adjustment for CT measures of visceral adiposity and hepatic density and insulin resistance. Among men and women, sex steroids E2 and T did not have strong or consistent associations with lipid subclasses.

Few studies have examined the relationship between endogenous sex hormones and lipid subclasses.[7,20–22] Only one report has examined both men and women.[7] Similar to our findings, the Multi-Ethnic Study of Atherosclerosis (MESA) also reported that higher SHBG levels were associated with a less atherogenic profile in both men and women.[7] SHBG was directly associated with greater LDL particle size as well as

Table 4. Associations Between SHBG and Subfractions in Women and Men

	Women	*Men*
log Large IDL (nmol/L)		
Unadjusted model	–0.13 ($P=0.11$)	–0.19 ($P=0.054$)
Model 1	**−0.20 ($P=0.01$)**	–0.10 ($P=0.39$)
Model 1, and visceral adiposity (SD^3)	**−0.21 ($P=0.04$)**	–0.06 ($P=0.64$)
Model 1, and hepatic density (HU)	**−0.21 ($P=0.04$)**	–0.08 ($P=0.51$)
Model 1, visceral adiposity, hepatic density, and HOMA-IR	–0.22 ($P=0.07$)	–0.08 ($P=0.57$)
log Small IDL (nmol/L)		
Unadjusted model	0.15 ($P=0.08$)	0.10 ($P=0.18$)
Model 1	0.11 ($P=0.20$)	0.08 ($P=0.38$)
Model 1, and visceral adiposity (SD^3)	0.0 ($P=0.98$)	0.06 ($P=0.53$)
Model 1, and hepatic density (HU)	0.17 ($P=0.12$)	0.09 ($P=0.33$)
Model 1, visceral adiposity, hepatic density, and HOMA-IR	0.09 ($P=0.47$)	0.06 ($P=0.56$)
log Large LDL (nmol/L)		
Unadjusted model	–0.08 ($P=0.30$)	0.02 ($P=0.83$)
Model 1	–0.12 ($P=0.15$)	0.15 ($P=0.23$)
Model 1, and visceral adiposity (SD^3)	–0.05 ($P=0.65$)	0.19 ($P=0.16$)
Model 1, and hepatic density (HU)	–0.17 ($P=0.06$)	0.15 ($P=0.23$)
Model 1, visceral adiposity, hepatic density, and HOMA-IR	–0.05 ($P=0.61$)	0.18 ($P=0.22$)
log Medium LDL (nmol/L)		
Unadjusted model	**−0.28 ($P=0.003$)**	**−0.20 ($P=0.03$)**
Model 1	**−0.32 ($P<0.001$)**	–0.07 ($P=0.49$)
Model 1, and visceral adiposity (SD^3)	**−0.27 ($P=0.02$)**	–0.06 ($P=0.60$)
Model 1, and hepatic density (HU)	**−0.37 ($P=0.002$)**	–0.07 ($P=0.55$)
Model 1, visceral adiposity, hepatic density, and HOMA-IR	**−0.31 ($P=0.03$)**	–0.10 ($P=0.44$)
log Small LDL (nmol/L)		
Unadjusted model	**−0.38 ($P=0.002$)**	**−0.43 ($P=0.006$)**
Model 1	**−0.42 ($P=0.001$)**	–0.35 ($P=0.057$)
Model 1, and visceral adiposity (SD^3)	**−0.35 ($P=0.03$)**	–0.32 ($P=0.13$)
Model 1, and hepatic density (HU)	**−0.41 ($P=0.009$)**	–0.33 ($P=0.08$)
Model 1, visceral adiposity, hepatic density, and HOMA-IR	**−0.41 ($P=0.03$)**	–0.40 ($P=0.07$)
log Very small LDL (nmol/L)		
Unadjusted model	0.08 ($P=0.12$)	**−0.28 ($P=0.03$)**
Model 1	–0.16 ($P=0.06$)	**−0.31 ($P=0.05$)**
Model 1, and visceral adiposity (SD^3)	–0.10 ($P=0.35$)	–0.26 ($P=0.15$)
Model 1, and hepatic density (HU)	–0.15 ($P=0.12$)	–0.28 ($P=0.10$)
Model 1, visceral adiposity, hepatic density, and HOMA-IR	–0.15 ($P=0.22$)	–0.33 ($P=0.07$)

Figures in *bold* are significant.

inversely correlated with smaller LDL particle size. In contrast to our findings, SHBG was also directly related to HDL concentrations and inversely correlated with VLDL. Also in contrast to our findings, higher E2 levels were associated with a more atherogenic profile, consisting of smaller LDL particle size and higher HDL concentrations.

This difference in results may also be due to our smaller sample size, as the association between SHBG and HDL concentrations in the current study was borderline before adjustment for measures of adiposity. We may also have not found associations between SHBG and HDL and VLDL and between E2 and lipoprotein levels due to the adjustment for adiposity by CT; in our report, the association between SHBG and VLDL concentrations was attenuated after adjustment for visceral adiposity and hepatic density.

The difference in results may also be due to differences in endogenous sex steroid levels and/or lipid levels in the MASALA versus MESA populations. Participants in MESA were white, African American, Hispanic, or Chinese, whereas participants in MASALA were Asian Indian. Comparisons of Pakistani, non-Hispanic white European, and African Caribbean men living in England have reported lower total T and SHBG levels in Pakistanis than in other racial/ethnic groups.[23] (Racial/ethnic comparisons of sex steroids in South Asian women vs. other populations have not been reported.) Moreover, previous comparisons of racial/ethnic groups have noted that even after adjustment for visceral, subcutaneous, and hepatic density, Asian Indians have lower insulin sensitivity using hyperinsulinemic euglycemic clamp studies compared with other Asian groups.[24] In turn, insulin sensitivity is a known modifier of lipid particle size and activity, with greater insulin resistance contributing to lower HDL levels.[25] Thus, relationships between sex hormones and HDL may have been attenuated in the current analysis.

In one report examining middle-aged men, SHBG levels were inversely correlated with apolipoprotein A1, the major protein component of HDL, and higher T levels were directly correlated with apolipoprotein B, the major protein component of LDL.[26] LDL subfractions were not examined, but this report suggests that the relationships between SHBG and lipids might extend to other lipoprotein subfractions. Of note, associations were attenuated with adjustment for waist-to-hip ratio—an anthropometric proxy for body fat—underlining the importance of adjusting for this confounder.[26] Studies examining correlations between endogenous sex hormone profiles with lipid subfractions among women with and without polycystic ovarian syndrome have had conflicting results. In one report, total T was not associated with lipid subfractions, including LDL subclasses [21]; associations between SHBG or other sex steroids with and lipid fractions were not reported. Another study noted that SHBG was the strongest correlate of LDL particle size and number, as well as VLDL number and size.[22] Although total T was not correlated with lipid fractions, lower T after adjustment for SHBG did predict increased LDL particle size. Finally, greater total T levels correlated with lower HDL in another report, but associations between SHBG and lipid subfractions were not reported.[20]

Strengths of this report include the novel assessments of lipid subfractions and sex hormone profiles in Asian Indians, a population at high risk for atherogenesis. The cohort, while small, was also population-based rather than referral center based. Additional strengths include the CT assessment of intra-abdominal adipose tissue depots, an important measurement given the strong correlations between sex hormones and fat in midlife adults. The primary limitation of the study is its small sample size and conduction of multiple comparisons, and it is possible that we were underpowered to detect relatively weaker associations between sex steroids and lipoproteins, particularly in an ethnic group that may have low sex hormone levels. We note that premenopausal women have higher estradiol as well as testosterone levels, and it is possible that this population has a different pattern of associations with lipoproteins than reported here. The MASALA pilot study also did not have measurements of ApoA1 and ApoB or HDL subfractions. However, a larger MASALA cohort consisting of approximately 900 South Asians (including Asian Indians as well as other South Asian populations) has been assembled, enabling future examination of sex steroids, adiposity, and lipid measures. The value of lipid subfractions and activity versus conventional CVD risk factor assessment is controversial, and lipid subfractions are not currently recommended for risk stratification purposes.[27–29] Future examinations will determine whether lipoprotein subfractions have additional discriminatory value for CVD risk prediction among this population with high levels of insulin resistance. Future investigations in larger subsets may help us understand the pathophysiology of atherosclerosis in men and women by examining whether sex steroids may influence CVD risk via alterations in lipid subfractions apart from ectopic fat deposition.

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Author Disclosure Statement

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