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The Association of Endogenous Sex hormones with Lipoprotein Subfraction Profile in the Multi-Ethnic Study of Atherosclerosis (MESA)

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Abstract

There traditional lipid profile differs by sex hormone levels. However, associations of sex hormones with lipoprotein subfractions, which may more accurately represent metabolic pathways to atherosclerosis, are not well studied. We quantified the cross-sectional associations of endogenous sex hormones with lipoprotein subfractions in 3143 men and 2038 postmenopausal women who were not on hormone replacement therapy, aged 45–84 years, in the Multi-Ethnic Study of Atherosclerosis baseline examination. Particle sizes and numbers of Very Low Density (VLDL), Low Density (LDL) and High Density (HDL) Lipoproteins were measured by Nuclear Magnetic Resonance. In both men and women, after multivariable adjustment, higher Sex Hormone Binding Globulin (SHBG) levels are associated with smaller, fewer VLDL, larger, fewer LDL, and larger, more numerous HDL particles; while higher endogenous estradiol levels are associated with smaller VLDL, and smaller, more numerous HDL and LDL particles (all $p < 0.05$). Testosterone (adjusted for SHBG) is associated with a smaller VLDL particles in men but not women (sex difference $p = 0.040$). Higher dehydroepiandrosterone (DHEA) levels are associated with more numerous, smaller VLDL particles only in women (sex difference $p = 0.030$, 0.004, respectively). In conclusion, we found sex differences in the association of endogenous androgens with lipoprotein particle sizes and numbers. Higher endogenous estradiol, but lower SHBG is associated with a more atherogenic lipoprotein particle profile. These findings highlight the potential to improve the lipoprotein profile with sex hormones, but emphasize the intricacies of the interactions.

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Introduction

Circulating levels of the cholesterol content of lipoproteins are strong predictors of cardiovascular mortality (1). It has been suggested that for predicting cardiovascular risk, the composition of lipoprotein subfractions may be more important than the routinely measured levels of cholesterol in High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) fractions (2-5). Sex hormones levels are associated with levels of HDL and LDL cholesterol (HDL-C and LDL-C) in men (6) as well as premenopausal (7) and postmenopausal women (8). Menopause is associated with increasing total cholesterol, triglycerides, Low Density Lipoprotein cholesterol (LDL-C), and lower High Density Lipoprotein cholesterol (HDL-C) concentrations (9). Estrogen and androgen receptors are present in both visceral and subcutaneous adipocytes in humans, suggesting that endogenous sex hormones may affect lipid metabolism in adipocytes (10-12). Drugs affecting endogenous sex hormone metabolism affect total and LDL-C levels (13) and serum triglycerides (14). Lipoprotein lipase enzyme activity has been shown to be inversely correlated with estradiol levels and positively correlated with testosterone levels in obese women (15). Estrogens have been shown to be associated with a favorable lipid profile (16, 17) and androgens have been associated with an unfavorable lipid profile in some studies (18, 19). However, in cross-sectional analysis of the Atherosclerosis Risk in Communities cohort by Mudali et al (8), estrones were shown to be associated with a worse lipid profile especially in women within the upper 5th percentile of intimal-medial thickness, though the association of androgens with more atherogenic profile was consistent with previous studies. Endogenous sex hormones have been shown to be associated with lipoprotein subfractions in terms of their particle numbers and their cholesterol content. Women with polycystic ovary disease have endogenous hyperandrogenemia and a higher concentration of small dense LDL as compared to controls (20). In addition to lipid changes mentioned, menopause is associated with higher concentrations of cholesterol in the HDL3 subfraction and lower levels in the HDL2 subfraction measured using sequential precipitation methods (9).

There is a suggestion that the relationships of multiple metabolic factors (21), including HDL-C (22), with sex hormones differ between men and women. Rather than total lipoprotein levels, lipoprotein subfractions and particles sizes might more accurately reflect different stages in the synthesis and clearance of lipoproteins and lipids. However, the association between endogenous sex hormone levels and lipoprotein subfractions among men and women is not known. In this study, we have analyzed and contrasted these associations between men and postmenopausal women using the baseline examination data of the Multi-Ethnic Study of Atherosclerosis (MESA), a population-based cohort in the United States.

Methods

Study Population

The design of MESA, a multi-center, longitudinal cohort study of the prevalence and correlates of subclinical cardiovascular disease among persons without overt cardiovascular disease, has been described previously (23). The study was approved by the institutional review boards of all participating institutions, and all participants signed informed consent

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prior to study enrollment. Individuals were excluded if they had clinical cardiovascular disease at baseline, including physician-diagnosed angina, stroke, transient ischemic attack, or heart failure, use of nitroglycerine, current atrial fibrillation, or had undergone a procedure related to cardiovascular disease (coronary artery bypass surgery, angioplasty, valve replacement, pacemaker or defibrillator implantation, any surgery on the heart or arteries). In addition, for this analysis, women who were on current hormone replacement therapy were excluded. Thus, we included 3155 men and 2046 postmenopausal women from the baseline examination of MESA who had measurement of lipoprotein particle size and sex hormones. Women were considered postmenopausal if they were self-reported as having amenorrhea for one year or more, were > 55 yrs, or had reported hysterectomy. MESA baseline examination included medical and medication history questionnaires, blood pressure measurements and questionnaires assessing smoking, alcohol, and physical activity. Routine fasting blood profiles included total and HDL cholesterol and triglyceride concentrations, and glucose measurements were obtained. LDL cholesterol concentration was calculated using Friedewald's equation (24). Participants were considered to have Type II Diabetes if they had a fasting glucose 7.0 mmol/L (126 mg/dL) or reported use of hypoglycemic medication.

Sex hormone measurements

Fasting blood samples were drawn between 7:30 am and 10:30 am during the MESA baseline examination. Serum samples, extracted by centrifugation at 2000G for 15 min and 3000G for 10 minutes were immediately stored at −70°C, and serum sex hormone levels were assessed at the University of Massachusetts Medical Center in Worcester, MA. Total testosterone (T) and dehydroepiandrostendione (DHEA) were measured directly using radioimmunoassay kits and sex hormone binding globulin (SHBG) was measured by chemiluminescent enzyme immunometric assay using Immulite kits obtained from Diagnostic Products Corporation (Los Angeles, CA). Estradiol (E2) was measured by use of an ultra-sensitive radioimmunoassay kit from Diagnostic System Laboratories (Webster, TX). The intra-assay coefficient of variation for total T, SHBG, DHEA, and E2 were 12.3%, 9.0%, 11.2%, and 10.5%, respectively.

Lipoprotein Nuclear Magnetic Resonance (NMR) spectroscopy

Lipoprotein particle concentrations and size were measured on frozen plasma specimens (−70 °C) by proton NMR spectroscopy (LipoScience Inc., North Carolina)(25, 26). The amplitude of the spectroscopic lipid methyl group NMR signals that distinguish lipoprotein subclasses was measured to obtain lipoprotein concentrations. The weighted-average lipoprotein particle sizes were derived from the sum of the diameter of each subclass multiplied by its relative mass percentage as described previously (25).

The lipoprotein subclasses are: small LDL (diameter of 18.0–21.2 nm), large LDL (21.2–23.0 nm), intermediate-density lipoprotein or IDL (23.0–27.0 nm), large HDL (8.8–13.0 nm), medium HDL (8.2–8.8 nm), small HDL (7.3–8.2 nm), large very low-density lipoprotein or VLDL (>60 nm), medium VLDL (35.0–60.0 nm), and small VLDL (27.0–35.0 nm). Inter-assay reproducibility of these measures in MESA has been previously published (27).

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Statistical Analysis

Demographic and risk factor variables were tabulated for men and women. Sex differences in categorical variables were assessed using χ^2 tests, for normally distributed continuous variables t-tests were used, while for non-normally distributed variables rank sum tests were used. The medians levels of the concentrations of particles of various lipoprotein subclasses and their particle sizes were tabulated by sex. Differences were tested by rank sum tests.

Linear regression analyses were used to test the association of lipoprotein particle numbers and particle sizes with endogenous sex hormone levels, adjusted for age, race, Body Mass Index (BMI), diabetes status, current cigarette smoking, current alcohol use, lipid-lowering medication, and weekly reported moderate to vigorous physical activity. The particle number and size variables were non-normally distributed and could not be transformed to normality by standard mathematical transformations. Hence standard errors and 95% confidence intervals were empirically estimated using bootstrapped regressions with 100 iterations for every regression model that was estimated. Separate models were assessed for the particle numbers and size for each lipoprotein class (VLDL, LDL, HDL), as well as components of the traditional lipid profile (LDL-C, HDL-C and log-transformed triglycerides). All log-transformed hormones (T, E2, DHEA, and SHBG) were entered into the same model, but a separate model was estimated to test for interaction by sex with each of the hormones. All hormone variables were entered into the same model because total levels of the hormones include non-functional bound fractions, which are corrected by including SHBG into the same model. Sex-stratified models were estimated, and beta coefficients presented separately for men and women. For the above analyses $p < 0.05$ was the level of statistical significance. All analyses were also repeated after restricting the sample to individuals not currently taking lipid lowering medication. To illustrate the association of sex hormones with the mean particle size and particle number in a different manner, the particle numbers within different subclasses of size were regressed against sex hormones and covariates.

Though for main analysis all sex hormones were entered into the same model, to address the issue of possible low level multicollinearity between sex hormone variables (Spearman correlation coefficients range between −0.21 to 0.52), secondary analyses included only single hormone variables with all other non-hormone covariates. Major qualitative differences in associations were noted. If an association was borderline significant ($p>0.05$ but <0.1) in these secondary analyses, this was not considered a qualitative difference from either a significant or non-significant result in the primary analysis.

We examined whether the association of traditional lipid profile and NMR lipid variables on one hand with sex hormone variables on the other hand where differed by race. These analyses were done separately by sex. Heterogeneity of each NMR variable and traditional lipid profile-hormone association by race was explored within each sex. For each particular lipoprotein variable (dependent variable)-hormone (independent variable)-sex (strata) combination, three interaction terms (for 3 races, one being designated as the reference race to avoid collinearity) were introduced into the regression model. All three interactions by race were assessed by a single omnibus test to reduce the number of p-values calculated. For a total of 72 exploratory tests (9 variables, 4 hormones, 2 sexes), we considered a

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Bonferroni-corrected p-value of <0.0007 as statistically significant. If the omnibus interaction test for any NMR variable-hormone-sex combination was significant, the interaction by individual race/ethnic group was examined. No post-hoc adjustment to p-value was made for the individual interactions.

Results

Sample population characteristics

Table 1 shows the sample characteristics of men and women included in the analyses. In MESA men and women are balanced with respect to age and race/ethnicity (23). However, as premenopausal women and those using current hormone replacement are excluded from this analysis, the women in this subsample are older and have higher levels of some cardiovascular risk factors than men. Women using HRT were excluded from this analysis, and since there was greater use of HRT in whites, the analysis sample has fewer white women.

Lipoprotein subfractions in men and women

The medians and interquartile ranges of lipoprotein particle numbers per liter and particle sizes are shown in Table 2. In terms of particle number, women have a lower concentration of VLDL particles and LDL particles, but a higher concentration of HDL particles as compared to men. This difference between men and women is further reflected by differences between lipoprotein subclass concentrations. Expressed in terms of mean particle size, the mean VLDL and LDL particle size is smaller, while the mean HDL particle size is larger in women compared to men.

Correlations between traditional lipid measures and NMR derived variables

The Spearman rank order coefficients of the correlations between the traditional lipid profile components, and the NMR derived measures (Table 3), show statistically significant relationships across the board. However, in the numerical value of the coefficients, the triglyceride concentration has a high correlation with VLDL particle number, but not the mean VLDL particle size, the LDL-C concentration has a strong positive correlation with the LDL particle number but a very weak negative correlation with mean LDL particle size, and the HDL-C concentration has moderately strong positive correlation with both HDL particle number and mean size.

Relationship between components of the traditional lipid profile and NMR related variables with sex hormone levels in men and women

In both men and women, in models adjusting for demographic and non-lipid cardiovascular risk factors, including all sex hormones, higher E2 levels, and lower SHBG levels are associated with greater triglyceride levels (Table 4). However, higher E2 levels are associated with larger VLDL particle size in both sexes, but with greater particle number only in men. SHBG is only associated with a lower VLDL particle number, but not particle size. Though the positive association of triglycerides with DHEA in women does not reach statistical significance, VLDL particle size is smaller and the VLDL particle number is greater in women with higher levels of DHEA. Similarly, though the negative association of

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triglyceride levels with Total T in men does not reach statistical significance, it reflects an underlying significant negative association with VLDL particle size but not number. The results regarding overall particle number and particle size are reflected in the secondary analysis of particle numbers within VLDL particle size categories. For example, the fact of the association of SHBG with a greater number of overall VLDL particles in the face of no difference in particle size is reflected in the association of SHBG with a greater number of particles with all subclasses of VLDL particles. In contrast, the association of E2 only with mean VLDL particle size (but not overall particle number) in women is reflected by the fact that there are a greater number of particles associated with E2 only in the large VLDL/ chylomicron size category.

Adjusted associations of LDL related variables with sex hormones are shown in Table 5. Higher DHEA levels, but lower SHBG levels are associated with greater LDL-C concentrations calculated from the routine lipid profile. E2 levels are positively associated with LDL-C concentrations only among women. However, in both men and women E2 levels are associated with smaller mean LDL particle size but greater LDL particle number, while the reverse association profile is seen for SHBG levels. DHEA levels are positively associated and Total T levels are negatively associated with particle number only in women. The secondary analysis of particle numbers in LDL particle size categories largely reflects the shifts in particle distributions associated with the hormones that give rise to the overall particle size and number associations. For example, the greater number of particles of smaller mean size associated with higher E2 levels is reflected in the lower number of particles in the large LDL category and the higher number of particles in the medium small and very small LDL categories.

SHBG levels are positively associated with HDL-C from the routine lipid profile in both men and women, while Total T is negatively associated only in women, and DHEA is positively associated only in men (Table 6). Though no association is seen with E2 in the routine lipid profile HDL-C, there is a negative association with mean HDL particle size and a positive association with particle number. Greater SHBG levels are associated with a larger mean HDL particle size in both men and women, but also greater particle number in men. Total T is negatively associated with HDL particle number in both sexes, while DHEA is positively associated with HDL particle number only in men. The associations of sex hormones with the particle numbers in HDL particle subclasses are presented as secondary analysis, and reflect the associations of sex hormones with overall mean particle size and number.

Secondary analyses restricted to the sample population not taking lipid lowering medication

All significant associations of the traditional lipid profile, overall lipoprotein particle numbers and mean sizes mentioned above were in the same direction, and all but one retained at least borderline statistical significance ($p<0.1$) when the sample was restricted only to those not taking lipid-lowering medications. Only the association of E2 with calculated LDL-C from the routine lipid profile in women lost significance entirely ($p=0.23$).

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Secondary analyses with sex hormones included one at a time in regression models

In terms of differential association of lipoprotein variables with sex hormones variables in men and women, no qualitative differences were seen for E2, DHEA or SHBG. Only among men, for triglycerides, HDL-cholesterol, particle numbers of VLDL, LDL, HDL, and particle sizes of LDL and HDL as dependent variables, the qualitative relationship of T as the independent variable differed from the primary analysis. In each of these cases, the direction of the association was concordant with that of the SHBG variable in the primary analysis, suggesting that SHBG is an important independent confounder of the relationship between lipoprotein variables (dependent) and T (independent).

Interactions by race/ethnicity

We investigated if the association of lipoprotein variables (dependent) with sex hormone variables (independent) differed by race either among men or women. Only in men, some exploratory hormone-by-race interactions were significant at the Bonferroni-corrected level. In Hispanic men, but not others, higher T levels were associated with higher serum triglycerides. In Black men, SHBG is strongly positively associated with VLDL particle size ($p<0.001$ vs. whites), however, there is no pooled association of SHBG with VLDL particle size in men. In Chinese men, T levels are positively associated with LDL particle size ($p = 0.031$ vs. whites), a weak positive association is seen between T and LDL particle size in whites and blacks, while no association is seen among Hispanics ($p=0.002$ vs. whites). T is also negatively associated with HDL particle size only in Hispanic men ($p<0.001$).

Discussion

We have examined the lipoprotein particle sizes, numbers and subfractions in men and women in a multi-ethnic cohort and shown them to be related to endogenous sex hormone levels in these middle aged and older individuals. In our analysis, the association of SHBG with a favorable traditional lipid profile confirms findings in previous studies (6, 8, 28). E2 was associated with higher triglyceride concentrations as shown previously in men (6) and other studies have shown a similar association between estrone and triglycerides in women (8). T, which was adjusted in our analysis for all other sex hormones (thus represents the association of bioavailable T), was associated with lower levels of HDL-C unlike many previous studies (reviewed by Barrett-Connor (29)), however these previous findings may be confounded by the fact that most T is bound to SHBG. DHEA was associated with mixed favorable and unfavorable differences in the traditional lipid profile. We found no major qualitative interactions by sex of the relationships between hormones and the traditional lipid profile.

Studies show that lipoprotein particle size and number provide independent additional information about atherogenicity of circulating lipids. Large VLDL particle sizes have been shown to be related to greater severity of coronary artery disease assessed by angiography in men (2), and also to coronary artery calcification in non-diabetic women (3). Greater VLDL particle numbers (greater number of particles of all subfractions) were associated with incident coronary artery disease among diabetics (4). In this study, higher E2 levels and lower SHBG levels are associated with more atherogenic VLDL profile in both men and

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women, whereas T (adjusted for SHBG) is associated with less atherogenic VLDL profile only in men. Small LDL particle sizes and greater numbers of particles are associated with incident cardiovascular events (5). Small LDL particle subfraction concentrations and also small and intermediate HDL subfractions were associated with greater coronary disease severity in men (2). Sex hormones may appear to be associated with lipoproteins due to a confounding association with adiposity and insulin resistance (30). In this study, higher E2 levels and lower SHBG levels are associated with more atherogenic LDL and HDL profiles in both men and women. T (adjusted for SHBG) is associated with fewer HDL particles in both men and women, but the particle sizes are similar across T levels, suggesting no difference in the atherogenicity of the HDL lipoprotein fraction. DHEA is associated with greater HDL particle number in men, a greater number of smaller VLDL particles, and a greater number of larger LDL particles in women. While neither of these suggests a clear-cut atherogenicity of lipoprotein profiles associated with DHEA, they do suggest that DHEA differentially affects lipoprotein metabolism in men and women. The adrenal secreted steroids are the principal sources for steroids in postmenopausal women and are peripherally converted to both androgenic and estrogenic products (31). This protean role may underlie the significant but mixed relationship of DHEA levels with the lipoprotein particle profile in our analysis. However, the associations found in our analyses are adjusted for adiposity. In earlier studies there is conflicting information about the relationship of lipoprotein lipase activity in relation with estrone in women, suggesting that estrogens may mobilize lipoproteins (15, 32).

The sex differences in our primary analyses (including all sex hormones in the same regression models) of E2, DHEA, and SHBG do not differ qualitatively from the secondary analyses (with one sex hormone included with other covariates). However, the results for T in men (if SHBG is not included) are concordant with those of SHBG were it included. Thus the results of our primary analyses must be interpreted as the association of bioavailable testosterone with the various lipoprotein variables.

Overall, we found that higher levels of SHBG are associated with a less atherogenic profile, while the higher E2 levels are associated with a more atherogenic profile in both men and women. For VLDL and LDL particle numbers and LDL and HDL particle sizes, there is a quantitative interaction, with E2 being associated with significantly worse profiles in men as compared to women. T is associated with a better VLDL profile in men but not in women.

Strengths and Limitations

A significant strength of this study is that it has been conducted in a large multiethnic population cohort. We were able to adjust for many confounders because of the thorough data collection within MESA. However, certain limitations must be noted. This is a cross-sectional study, and the causality of the detected associations cannot be assessed. In this study estradiol measurements were available, though estrone has the highest circulating levels in postmenopausal women. However, estradiol is the more potent estrogen, and we believe that the associations we report are valid.

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Conclusion

In older men and women, higher SHBG levels are associated with a less atherogenic lipoprotein subclass profile, while higher endogenous estradiol levels are associated with a more atherogenic profile. T (adjusted for SHBG) is associated with a less atherogenic profile in men but not women, suggesting that androgen deficiency may have adverse lipoprotein metabolism consequences only in men. DHEA has different associations with the various lipoprotein subclasses in men and women, suggesting a complex but strong involvement in lipoprotein metabolism. These findings highlight the potential to improve the lipoprotein profile with sex hormones, but emphasize the intricacies of the interactions. Depending on the sex, there are cautionary implications for the use of T, estrogens and adrenal androgens to replace low hormone levels in aging individuals. More studies are needed to evaluate the effect of HT in men and women on lipoprotein subfractions, size, and particle number.

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Table 1

Demographics characteristics of the population sample

	Men	Women	p
N	3143	2038	
Age (years)	62.2±10.2	65.6±9.2	<0.001
Race			
White (%)	39.4%	30.3%	<0.001
Chinese (%)	12.3%	13.6%	
Black (%)	25.7%	31.0%	
Hispanic (%)	22.6%	25.1%	
Systolic BP (mmHg)	126±19	131±24	<0.001
Diastolic BP (mmHg)	75±9	70±10	<0.001
Diabetes (%)	15.5%	15.8%	0.768
Total cholesterol (mg/dL)	188±35	203±37	<0.001
LDL cholesterol (mg/dL)	117±31	122±32	<0.001
HDL cholesterol (mg/dL)	45±12	55±15	<0.001
Triglycerides (mg/dL)	113 [79 to 166]	110 [78 to 157]	0.027
BMI (kg/m^2)	27.9±4.5	29.0±6.1	<0.001
Lipid-lowering medication use (%)	17.6%	20.5%	0.011

Tabulated as mean±standard deviation, p-value by t-tests; or median [25th to 75th percentile], p-value by rank sum tests; or percentage (%) of group with characteristic, tested by χ^2 tests.

Conversion factor for cholesterol measures 1 mg/dL = 0.0259 mmol/L.

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Table 2

Lipoprotein subclass particle numbers (in nmol/L) and mean sizes (nm) in men and women

	Men	Women	p
	Particle numbers (in nmol/L)		
VLDL (total)	75.3 [51.2 to 102.6]	70.1 [45.4 to 98.8]	<0.001
Large VLDL/ Chylomicrons>60nm	1.8 [0.4 to 5.7]	1.6 [0.4 to 4.7]	0.051
Medium VLDL 35-60nm	30.4 [15.5 to 49.6]	24.5 [12.8 to 42.3]	<0.001
Small VLDL 27-35 nm	38.0 [26.8 to 50.7]	39.4 [26.4 to 53.7]	0.040
LDL (total)	1316 [1093 to 1571]	1256 [1039 to 1522]	<0.001
IDL 23-27 nm	12.8 [1.4 to 32.9]	13.3 [0 to 35.1]	0.643
Large LDL 21.2-23nm	306 [183 to 443]	448 [305 to 593]	<0.001
Medium small LDL 19.8-21.2nm	200 [146 to 260]	151 [101 to 222]	<0.001
Very small LDL 18-19.8nm	773 [564 to 1010]	602 [419 to 887]	<0.001
HDL (total)	28.6 [25.7 to 31.8]	32.9 [28.8 to 35.3]	<0.001
Large HDL 8.8-13nm	4.8 [3.0 to 7.2]	7.3 [4.9 to 10.4]	<0.001
Medium HDL 8.2-8.8 nm	2.9 [1.0 to 5.6]	3.9 to [0.9 to 7.2]	<0.001
Small HDL 7.3-8.2nm	19.7 [17.0 to 22.4]	19.4 [16.6 to 22.5]	0.095
	Mean particle sizes (nm)		
VLDL particle size	49.5 [45.3 to 54.6]	48.8 [44.8 to 54.1]	0.014
LDL particle size	20.5 [20.0 to 21.0]	21.0 [20.4 to 21.5]	<0.001
HDL particle size	9.0 [8.7 to 9.2]	9.2 [9.0 to 9.5]	<0.001

Tabulated as median [25th to 75th percentile], tested using rank sum tests

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Table 3

Pairwise Spearman rank order correlation of traditional lipid variables with each other and with NMR-derived lipoprotein variables

	Spearman correlation coefficients (p-value)		
	Triglycerides	**LDL-C**	**HDL-C**
Traditional Lipid Profile			
Triglycerides	1.00	-	-
LDL-C	0.11 (<0.001)	1.00	-
HDL-C	−0.50 (<0.001)	0.03 (0.019)	1.00
NMR Particle Number			
VLDL	0.82 (<0.001)	0.34 (<0.001)	−0.47 (<0.001)
LDL	0.47 (<0.001)	0.69 (<0.001)	−0.38 (<0.001)
HDL	−0.08 (<0.001)	−0.02 (0.083)	0.63 (<0.001)
NMR Particle Size			
VLDL	0.26 (<0.001)	−0.30 (<0.001)	−0.08 (<0.001)
LDL	−0.62 (<0.001)	−0.04 (0.008)	0.74 (<0.001)
HDL	−0.56 (<0.001)	−0.05 (<0.001)	0.76 (<0.001)

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Table 4

Regression coefficients (95% confidence intervals) for routine lipid profile triglycerides and VLDL variables (as dependent variables) vs. sex hormone levels in men and women

	Men	Women	Sex Difference p
	Routine lipid profile Triglycerides (% difference)*		
Total T	−2.90 (−5.99 to 0.30)%	−1.23 (−3.43 to 1.03)%	0.322
E2	12.07 (8.24 to 16.03)%†	5.09 (2.88 to 7.35)%†	<0.001
DHEA	−1.53 (−4.41 to 1.44)%	2.17 (−0.54 to 4.96)%	0.296
SHBG	−20.7 (−23.7 to −17.5)%†	−21.5 (−23.9 to −19.0)%†	0.027
	NMR Mean Particle size (pm)		
Total T	−804.0 (−1514.3 to −93.9)†	1.7 (−445.7 to 449.2)	0.040
E2	743.3 (77.4 to 1409.4)†	897.5 (419.2 to 1375.8)†	0.760
DHEA	89.0 (−419.8 to 597.7)	−1031.7 (−1620.1 to −443.4)†	0.004
SHBG	−328.2 (−1084.0 to 427.6)	−699.9 (−1376.5 to 23.4)	0.718
	NMR Particle number (nmol/L)		
	All VLDL particles		
Total T	−0.80 (−3.40 to 1.79)	−1.18 (−2.98 to 0.62)	0.862
E2	5.49 (3.07 to 7.92)†	0.45 (−1.21 to 2.02)	<0.001
DHEA	0.14 (−2.52 to 2.59)	4.91 (2.48 to 7.34)†	0.030
SHBG	−15.42 (−18.37 to −12.48)†	−15.5 (−18.1 to −13.0)†	0.108
	Large VLDL/ Chylomicrons>60nm		
Total T	−0.34 (−0.67 to 0.00)	−0.21 (−0.45 to 0.03)	0.492
E2	1.16 (0.82 to 1.51)†	0.47 (0.27 to 0.67)†	<0.001
DHEA	−0.02 (−0.44 to 0.40)	0.04 (−0.28 to 0.35)	0.899
SHBG	−2.18 (−2.66 to −1.70)†	−2.29 (−2.65 to −1.93)†	0.098
	Medium VLDL 35-60nm		
Total T	−0.68 (−2.41 to 1.04)	−1.14 (−2.22 to −0.06)†	0.722
E2	2.92 (1.19 to 4.65)†	0.48 (−0.46 to 1.43)	0.002
DHEA	−0.35 (−2.17 to 1.46)	2.34 (1.02 to 3.65)†	0.038
SHBG	−9.52 (−11.53 to −7.49)†	−7.83 (−8.82 to −6.03)†	<0.001
	Small VLDL 27-35 nm		
Total T	0.22 (−1.14 to 1.58)	0.17 (−0.74 to 1.07)	0.958
E2	1.41 (0.31 to 2.51)†	−0.55 (−1.56 to 0.46)	0.005
DHEA	0.51 (−0.84 to 1.86)	2.54 (1.20 to 3.87)†	0.190
SHBG	−3.72 (−5.30 to −2.15)†	−5.83 (−7.13 to −2.53)†	0.208

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Regressions were performed with all log-transformed sex hormones in the same model, adjusted for age, race, Body Mass Index (BMI), diabetes status, current cigarette smoking, current alcohol use, lipid-lowering medication, and weekly reported moderate to vigorous physical activity.

[*]Triglycerides were analyzed on the log-scale. Beta coefficients represent the differences in mean triglycerides (in percent, i.e. proportional difference), or variable levels in pm or nmol/L, between a person with a given level of hormone and another person with double that level of hormone, keeping all other covariates constant, and 95% confidence intervals obtained from empirical bootstrapped standard errors.

[†]$p<0.05$.

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Table 5

Regression coefficients (95% confidence intervals) for routine lipid profile and NMR derived LDL variables (as dependent variables) vs. sex hormone levels in men and women

	Men	Women	Sex Difference p
	Calculated LDL-C from routine lipid profile (mg/dL)		
Total T	0.89 (−1.43 to 3.21)	−0.82 (−2.36 to 0.73)	0.228
E2	0.87 (−1.23 to 2.97)	−1.49 (−2.95 to −0.03)[†a]	0.062
DHEA	2.94 (1.02 to 4.86)[†]	5.42 (3.34 to 7.50)[†]	0.133
SHBG	−3.03 (−5.56 to −0.50)[†]	−3.68 (−5.87 to −1.48)[†]	0.599
	NMR Mean Particle size (pm)		
Total T	12.9 (−35.1 to 60.8)	17.8 (−17.8 to 53.3)	0.962
E2	−100.6 (−146.1 to −55.1)[†]	−50.3 (−81.3 to −19.2)[†]	0.002
DHEA	7.4 (−30.1 to 45.0)	−34.4 (−81.4 to 12.6)	0.206
SHBG	381.8 (327.5 to 436.1)[†]	432.9 (386.0 to 479.8)[†]	0.970
	NMR Particle number (nmol/L)		
	All LDL particles		
Total T	−6.25 (−35.1 to 22.6)	−20.4 (−37.0 to −3.8)[†]	0.431
E2	38.1 (13.0 to 63.3)[†]	18.7 (4.3 to 33.0)[†]	0.017
DHEA	11.3 (−10.9 to 33.5)	34.0 (10.8 to 57.1)[†]	0.435
SHBG	−106.2 (−134.3 to −78.1)[†]	−150.0 (−174.5 to −125.5)[†]	0.592
	IDL 23-27 nm		
Total T	−2.80 (−4.35 to −1.24)[†]	0.32 (−0.93 to 1.58)	0.001
E2	3.25 (1.80 to 4.70)[†]	0.85 (−0.15 to 1.85)	0.002
DHEA	1.12 (−0.49 to 2.73)	−0.14 (−1.19 to 1.64)	0.086
SHBG	−4.34 (−5.87 to −2.81)	−8.45 (−10.29 to −6.60)[†]	0.008
	Large LDL 21.2-23nm		
Total T	7.44 (−3.13 to 18.03)	−2.66 (−11.79 to 6.48)	0.144
E2	−18.36 (−30.63 to −6.10)[†]	−8.87 (−16.40 to −1.34)[†]	0.019
DHEA	4.56 (−3.94 to 13.07)	−0.91 (−13.24 to 11.43)	0.457
SHBG	80.23 (68.03 to 92.43)	87.27 (74.11 to 100.43)	0.832
	Medium small LDL 19.8-21.2nm		
Total T	−1.85 (−8.67 to 4.96)	−3.16 (−7.41 to 1.10)	0.779
E2	10.82 (4.91 to 16.73)[†]	5.12 (1.45 to 8.79)[†]	0.008
DHEA	0.94 (−4.06 to 5.94)	7.09 (1.03 to 13.15)[†]	0.317
SHBG	−38.70 (−44.77 to −32.63)[†]	−47.37 (−53.34 to −41.39)[†]	0.871

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	Men	Women	Sex Difference p
	Very small LDL 18-19.8nm		
Total T	−9.05 (−32.17 to 14.08)	−14.92 (−30.62 to 0.78)	0.747
E2	42.43 (21.24 to 63.62)†	21.57 (6.41 to 36.42)†	0.004
DHEA	4.68 (−13.10 to 22.46)	27.93 (5.00 to 50.86)†	0.327
SHBG	−143.41 (−165.9 to −120.9)†	−181.44 (−204.6 to −158.3)†	0.660

Regressions were performed with all log-transformed sex hormones in the same model, adjusted for age, race, Body Mass Index (BMI), diabetes status, current cigarette smoking, current alcohol use, lipid-lowering medication, and weekly reported moderate to vigorous physical activity. Beta coefficients represent the differences in mean LDL cholesterol levels (in mg/dL), or in variable levels in pm or nmol/L between a person with a given level of hormone and another person with double that level of hormone, keeping all other covariates constant, and 95% confidence intervals obtained from empirical bootstrapped standard errors.

†p<0.05.

[a]p=0.23 in sample restricted to those not on lipid-lowering therapy. Conversion factor for cholesterol: 1 mg/dL = 0.0259 mmol/L.

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Table 6

Regression coefficients (95% confidence intervals) for routine lipid profile and NMR derived HDL variables (as dependent variables) vs. sex hormone levels in men and women

	Men	Women	Sex Difference p
	HDL-C from routine lipid profile (mg/dL)		
Total T	−0.18 (−1.02 to 0.67)	−0.79 (−1.48 to −0.10)†	0.292
E2	−0.72 (−1.48 to 0.04)	−0.33 (−0.92 to 0.27)	0.111
DHEA	1.43 (0.73 to 2.12)†	−0.17 (−1.08 to 0.73)	0.014
SHBG	4.50 (3.58 to 5.42)†	5.73 (4.82 to 6.64)†	0.241
	NMR Mean Particle size (pm)		
Total T	6.4 (−18.8 to 31.5)	−1.4 (−20.5 to 17.6)	0.589
E2	−54.9 (−78.7 to −31.1)†	−20.8 (−37.8 to −3.8)†	<0.001
DHEA	12.4 (−8.7 to 33.5)	−17.8 (−44.7 to 9.1)	0.128
SHBG	184.3 (154.8 to 213.8)†	225.1 (198.6 to 251.4)†	0.685
	NMR Particle number (nmol/L)		
	All HDL particles		
Total T	−0.53 (−0.90 to −0.17)†	−0.37 (−0.62 to −0.11)†	0.503
E2	0.67 (0.32 to 1.02)†	0.72 (0.47 to 0.98)†	0.899
DHEA	0.71 (0.40 to 1.02)†	−0.16 (−0.52 to 0.20)	<0.001
SHBG	0.66 (0.28 to 1.03)†	0.17 (−0.18 to 0.53)	0.131
	Large HDL 8.8-13nm		
Total T	0.06 (−0.19 to 0.30)	−0.10 (−0.27 to 0.08)	0.324
E2	−0.39 (−0.58 to −0.19)†	−0.06 (−0.23 to 0.11)	0.001
DHEA	0.24 (0.06 to 0.42)†	−0.11 (−0.32 to 0.11)	0.060
SHBG	1.59 (1.37 to 1.80)†	1.91 (1.70 to 2.11)†	0.258
	Medium HDL 8.2-8.8 nm		
Total T	−0.41 (−0.68 to −0.14)†	−0.16 (−0.37 to 0.05)	0.085
E2	0.44 (0.16 to 0.72)†	0.14 (−0.10 to 0.39)	0.056
DHEA	0.23 (0.00 to 0.46)	−0.27 (−0.57 to 0.03)	<0.001
SHBG	−0.37 (−0.65 to −0.10)†	−0.94 (−1.23 to −0.65)†	<0.001
	Small HDL 7.3-8.2nm		
Total T	−0.18 (−0.47 to 0.11)	−0.12 (−0.32 to 0.09)	0.980
E2	0.61 (0.32 to 0.91)†	0.65 (0.43 to 0.86)†	0.756
DHEA	0.24 (−0.05 to 0.52)	0.22 (−0.10 to 0.54)	0.729
SHBG	−0.56 (−0.86 to −0.26)†	−0.80 (−1.12 to −0.47)†	0.699

Regressions were performed with all log-transformed sex hormones in the same model, adjusted for age, race, Body Mass Index (BMI), diabetes status, current cigarette smoking, current alcohol use, lipid-lowering medication, and weekly reported moderate to vigorous physical activity. Beta coefficients represent the differences in mean HDL cholesterol levels (in mg/dL), or in variable levels in pm or nmol/L between a person with a given level of hormone and another person with double that level of hormone, keeping all other covariates constant, and 95% confidence intervals obtained from empirical bootstrapped standard errors.

† $p<0.05$. Conversion factor for cholesterol: 1 mg/dL = 0.0259 mmol/L.

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