Title

Association Between Endogenous Sex Hormones and Liver Fat in a Multiethnic Study of Atherosclerosis

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(MESA AC 291) The Association of Endogenous Sex Hormones with Liver Fat - Multi-Ethnic Study of Atherosclerosis (MESA)

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Abstract

Background—Circulating sex hormone levels are associated with glucose metabolism and adiposity, but their association with ectopic fat deposition in the liver is not well understood.

Methods—We studied the association of the circulating levels of bioavailable testosterone (Bio-T), estradiol (E2), dehydroepiandrosterone (DHEA) and sex hormone binding globulin (SHBG) with fatty liver, defined as attenuation ≤ 40 Hounsfield Units by magnetic resonance imaging in 2835 postmenopausal women and 2899 men in the Multiethnic Study of Atherosclerosis baseline examination.

Results—In women, there was a significantly greater odds ratio of fatty liver prevalence in the highest tertile versus the lowest tertile of Bio-T (1.73, 95% CI 1.05 – 2.87) and E2 (2.42, 95% CI 1.37 – 4.29) adjusting for age, race/ethnicity, body mass index, hypertension, total and high density lipoprotein cholesterol, smoking, insulin sensitivity and hormone replacement therapy use. In men, there was a significantly greater odds ratio of fatty liver prevalence in the highest tertile versus the lowest tertile of E2 (1.96, 95% CI 1.21 – 3.18), but a significantly lower odds ratio for the highest versus lowest tertiles of SHBG (0.50, 95% CI 0.30 – 0.84). Other associations of hormones with fatty liver were not statistically significant.

Conclusions—A more androgenic internal mileu is associated with fatty liver in postmenopausal women. In men, lower levels of SHBG are associated with fatty liver. Higher levels of E2 are associated with fatty liver in both sexes. This pattern is consistent with the sex-specific associations of sex hormones with other cardiometabolic risk factors.

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Introduction

Ectopic deposition of fat in the liver in the absence of significant alcohol consumption is the early stage of non-alcoholic fatty liver disease (NAFLD), one of the most common chronic liver conditions that may progress to more serious clinical consequences including non-alcoholic steatohepatitis (NASH), fibrosis, liver failure and hepatocellular carcinoma.[1-5] Metabolic abnormalities are major drivers of NAFLD and include overweight and obesity,[6, 7] the metabolic syndrome [8, 9] and insulin resistance.[7, 10] Given that the population prevalence of overweight and obesity[11, 12] is increasing in the general US population, the prevalence of NAFLD is also reaching epidemic proportions.[13, 14] Circulating levels of endogenous sex hormones are associated with these metabolic abnormalities: higher levels of testosterone are associated with lower levels of central obesity cross-sectionally and longitudinally,[15-17] and with lower prevalence and incidence of diabetes in men but not in postmenopausal women.[18-20] Higher levels of estradiol and lower levels of sex hormone binding globulin are associated with greater central obesity, metabolic syndrome, diabetes and atherogenic lipid profile in both men and postmenopausal women.[21]

Reports of studies in small samples suggest that lower levels of sex hormone binding globulin are associated with NAFLD in men and menopausal women.[22, 23] Another study reported an association between low levels of DHEA and NAFLD.[24] However, no associations with estradiol or testosterone have been reported in US population based studies.

The aim of this study is to determine the cross-sectional associations of liver fat with circulating sex hormones in a large multiethnic US population sample and examine if this association is independent of cardiometabolic profile.

Materials and Methods

Sample population

This analysis was performed using data from the baseline examination of the Multiethnic Study of Atherosclerosis (MESA), which enrolled 3213 men and 3601 women free of clinical cardiovascular disease, aged 45-84 years of 4 US racial/ethnic groups (White, Black, Hispanic and Chinese) from 6 field centers.[25] The sex hormone ancillary study included 3009 postmenopausal women and 3164 men. Liver fat measurements derived from abdominal CT scans were available in 2835 women and 2899 men who were included in the current analysis. All study participants gave informed consent and the study was overseen by the Institutional Review Boards of all participating centers.

Clinical examination and questionnaires

All participants completed demographic and medical history questionnaires. Resting seated blood pressure measurements were performed, using the average of the second and third of 3 measurements using automated oscillometric sphygomanometry. Height was measured without footwear, and weight was measured with participants wearing light clothing. Body mass index was calculated as weight in kg/(height in meters)^2. Fasting blood draws were used to assay total and HDL-cholesterol, triglycerides and glucose. LDL-cholesterol was

calculated using the Friedwald equation.[26] Hypertension was defined by JNC VI criteria (REF) as BP ≥ 140/90 mmHg or the use of antihypertensive medications. Diabetes was defined according to American Diabetes Association (2003) criteria as fasting blood glucose ≥ 126 mg/dL or the use of anti-diabetes medications. The homeostatic model assessment of insulin resistance was calculated using fasting plasma glucose and insulin using the following formula: formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5 and QUICKI, a normally distributed transformation of HOMA-IR.[27]

Sex hormone measurements

Blood drawn between 7:30 and 10:30 am was used for the assays. Serum stored at -70°C was assayed at the University of Massachusetts Medical Center at Worcester, MA. Total testosterone (T) and dehydroepiandrosterone (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. Bioavailable testosterone (BioT) was calculated using the Vermeulen method.[28]

Liver fat measurements

Images were acquired using either Electron Beam Tomography (EBT) or four-detector row computed tomography (MDCT) scanners. Images were obtained from the level of the carina to the level of the apex of the heart.[29, 30] Examples of images from a person with high liver attenuation (i.e., low liver fat content) and low liver attenuation (i.e., high liver fat content) with are shown in Figure 1, panels A and B, respectively. All images were analyzed at the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center reading center. Hepatic attenuation, and splenic attenuation (when the spleen was in the field of view) was assessed from 3 regions of interest (>100 mm^2) in the liver and one in the spleen. Liver attenuation in Hounsfield units (HU) and liver attenuation < 40 HU as a dichotomous variable, indicating fat content of >30% were used as assessments of liver fat. Calculated Liver/Spleen (L/S) attenuation ratios as well as L/S ratios <1.0 as a dichotomous variable were used as assessments of liver fat in secondary analysis. Based on the previous literature, we defined fatty liver as liver attenuation <40 HU for the main analyses, and liver/spleen attenuation ratio < 1 in individuals in whom the CT imaging included the spleen for the secondary analyses.[30, 31]

Statistical Analysis

Demographic and cardiometabolic risk characteristics, and sex hormone levels were tabulated separately for women and men by fatty liver status. Categorical variables were tabulated as numbers and percentages, continuous variables were tabulated as means (standard deviations), or as medians [interquartile ranges] if the distribution was skewed. Differences by fatty liver status were tested using t-tests for normally distributed variables, rank sum tests for skewed variables and chi-squared tests for categorical variables.

Association analyses were performed separately for men and women. Each of the sex hormone variables (BioT, E2, SHBG and DHEA) was divided into sex-specific tertiles for analysis. The linear regression estimated mean and 95% CI of liver attenuation levels by sex hormone tertile, adjusted for race/ethnicity distribution, age (centered at 65 years) and BMI (centered at 28.5 kg/m^2) were tabulated, and the significance of tertiles and the linear trend was tested.

The association of fatty liver (defined in separate analyses as liver attenuation <40 HU with hormone tertiles was assessed using logistic regression, adjusted for age, race/ethnicity, BMI, current use of hormone replacement therapy in women, hypertension, current smoking, fasting total and HDL-cholesterol. Models for SHBG were also further adjusted for QUICKI (a normally distributed inverse transformation of HOMA-IR), because SHBG is also a proxy for metabolic status. In supplementary analysis, we also defined fatty liver as liver/spleen attenuation ratio < 1 in individuals in whom the CT imaging included the spleen. This was considered secondary analysis because missingness of spleen in the image was associated with higher BMI, a known confounder with both liver fat and sex hormone levels. We also examined heterogeneity of association of hormones by race. For this exploratory analysis we used a Bonferroni-corrected level of significance for 8 models (4 hormones, 2 sexes) at 0.05/8 = 0.00625

Sensitivity analyses

We performed sensitivity analyses adjusting for education levels as a proxy for socioeconomic status. We also performed all of the descriptive and association analyses excluding individuals who were heavy alcohol users (i.e, males consuming >14 drinks/day and women consuming >7 drinks/day)[3].

Results

Sample Characteristics

The demographic, cardiometabolic risk and sex hormone profile of men and women in the sample by fatty liver status are shown in Table 1. Both men and women with fatty liver were younger, less likely to be African-American, and more often Hispanic American, and had worse cardiometabolic profile in terms of hypertension, diabetes, BMI, fasting lipids, and HOMA-IR. Both men and women with fatty liver had lower SHBG levels and higher E2 levels. Men with fatty liver had lower levels of T and higher levels of DHEA, but there was no significant relationship for these hormones in women.

Association of Sex Hormone Tertiles with Liver Attenuation Signal

In table 2, the age-, race/ethnicity-, and BMI-adjusted means and 95% confidence intervals of liver attenuation signal are tabulated by sex-specific tertile of the sex hormone variables along with the p-value for a linear trend. In both men and women, higher tertiles of E2 were associated with lower attenuation (more fatty) liver signal, and higher tertiles of SHBG were associated with lower attenuation (more fatty) liver signal. Only among women, higher tertiles of BioT are associated with lower attenuation (more fatty) liver signal.

Association of Sex Hormones with Fatty Liver

Table 3 shows the association of sex-specific sex hormone tertiles with the odds of the presence of fatty liver, adjusted for age, race/ethnicity, BMI, current use of hormone replacement therapy in women, hypertension, current smoking, fasting total and HDL-cholesterol. Consistent with the quantitative findings, higher E2 tertiles were associated with greater odds of fatty liver in both men and women and higher SHBG tertiles were associated with lower odds of fatty liver. Higher tertiles of Bio-T in women were associated with higher odds of fatty liver. When SHBG models were further adjusted for QUICKI insulin sensitivity, the association in women became non-significant, however the odds ratio point estimates are not very different. Similar associations were found when analysis was restricted to individuals who were non-heavy alcohol users by self-report (Supplementary Table 1). Supplementary table 2 shows that in analysis restricted to individuals in whom spleen image was available, the central tendency of odds ratios using L/S ratio < 1 as the definition of fatty liver have a similar pattern, however not all associations remained significant. No interactions by race were significant at the Bonferroni-corrected level.

In sensitivity analyses, the adjusted findings were robust to further adjustment with education levels as a proxy for socioeconomic status.

Discussion

To our knowledge, this is the largest study to date showing the association of circulating multiple sex hormone levels with liver fat, as measured using CT scan, in a population-based multiethnic sample of both men and women. We have shown that higher levels of E2 and lower levels of SHBG are associated with higher levels of liver fat, in both men and women, after adjusting for age, sex and BMI. These associations with sex hormones remained with extreme levels, i.e., fatty liver in both men and women, adjusting for cardiometabolic risk factors. Higher levels of bioavailable testosterone were associated with higher quantitative fat measurement, and with higher odds of fatty liver in women.

Our findings are consistent with other metabolic associations of sex hormones that we and others have demonstrated in the MESA and other study populations. Higher levels of E2 are associated cross-sectionally with central obesity in terms of waist-to-hip ratio and to longitudinal increases in waist-to-hip ratio[17] . SHBG levels had the converse association in that study. Similar patterns of E2 and SHBG association were found for impaired glucose tolerance and prevalent diabetes in men and women[18, 19] and incident diabetes in women.[20] The association of E2 with fatty liver may reflect its actions in the liver increasing fatty acid synthesis, in addition its inhibition of gluconeogenesis and glycogenolysis leading to insulin resistance.[32] Indeed, the association we observed was partially diminished on adjustment for insulin sensitivity, but still remained significant.

In addition to being a carrier for sex hormones, SHBG is marker for nutritional and metabolic status.[33] The association of SHBG with fatty liver adjusted for insulin sensitivity, shows some attenuation in terms of odds ratio, and loss of statistical significance in women, but not men. This indicates that the metabolic status in terms of insulin sensitivity may explain the association of SHBG with fatty liver to a greater extent, but not fully.

Our finding that Bio-T is associated with fatty liver in women is concordant with findings by others that an androgenic internal milieu is associated with worse cardiometabolic profile in women.[34-36]

Our results that lower levels of sex hormone binding globulin are associated with NAFLD men and postmenopausal women are consistent with small prior studies,[22, 23] although the association was marginal in our study, and was largely explained by adjusting for the insulin sensitivity index. Another small study in hospital based patients and controls reported an association between low levels of DHEA and NAFLD,[24] but this study did not present sex-specific analysis. In our population based study, we found no association of DHEA in men or women. No associations studies for fatty liver with estradiol or testosterone have been reported in US population based studies. One study in China found that was E2 associated with lower fatty liver prevalence in men,[37] however our study shows a strong opposite association. One Korean study found that lower levels of testosterone in men were associated with NAFLD.[38] We find a similar non-significant trend for greater liver attenuation, i.e., less fat, for men with higher Bio-T, but this does not translate to a significant difference in fatty liver.

A major strength of this study is its large population-based sample that includes both men and women with adequate representation of ethnicities particularly affected by fatty liver disease. These individuals have been extensively phenotyped with validated questionnaires, and imaging studies were rigorously read at reading centers. The population has well measured anthropometric and fasting blood biochemistry data allowing for the adjustment of confounding. A potential limitation is that the major postmenopausal estrogen, estrone, was not measured in this study. However, we have shown associations with the most potent circulating estrogen E2, thus our findings have validity. Another potential limitation stems from the fact the CT scans were not primarily designed for quantitating fatty liver, thus imaging in 51% of individuals did not include the spleen for comparison of non-fatty tissue attenuation, and missingness of spleen image was associated with higher BMI. However, our findings are essentially unchanged using from the full sample liver attenuation analysis, when we used liver-spleen attenuation ratio in the subset of persons in whom the ratio was available. We thus believe that our findings are robust to this limitation. Another potential limitation is that our main analysis does not distinguish alcoholic from non-alcoholic fatty liver. However, our supplementary data analysis excluding self-reported heavy alcohol users show that our finds remain unchanged.

In conclusion, we have shown that there is a strong association of fatty liver with high E2 levels in men and women and low SHBG levels in men independent of demographic, anthropometric and cardiometabolic risk factors. Higher androgenic milieu is associated with fatty liver in women only. These associations may partially, but not fully be explained by insulin sensitivity. Future studies with measurement of liver inflammation and fibrosis are necessary to determine whether these associations may give rise to clinically significant liver-related morbidity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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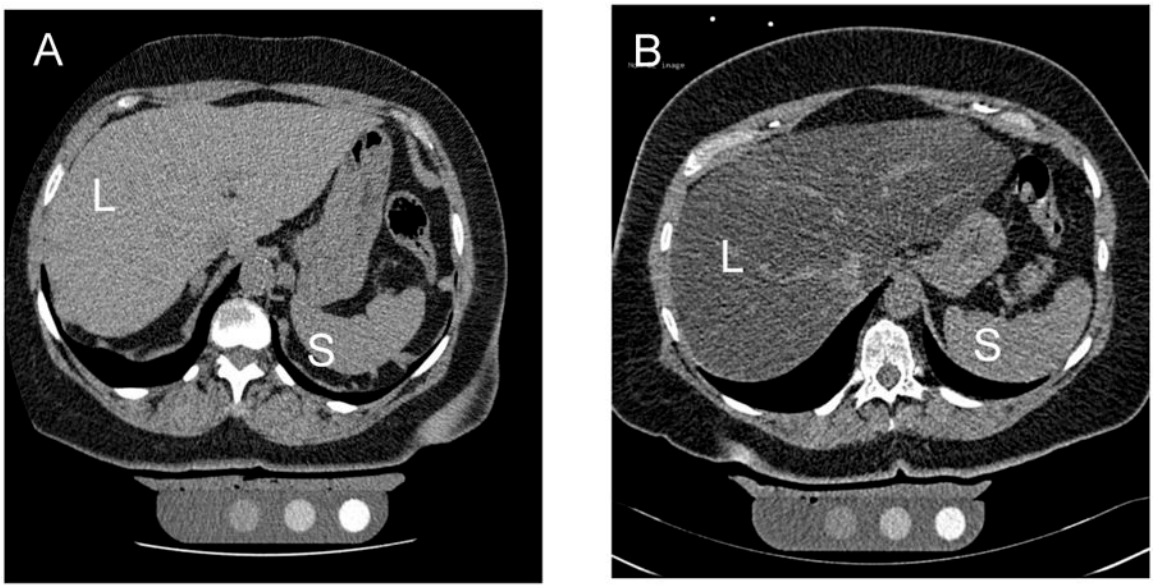

Figure 1.

Examples of abdominal computed tomography images showing the liver (L) and spleen (S) from a person with high liver attenuation, i.e., low liver fat content (panel A) and one with low liver attenuation, i.e., high liver fat content (panel B). Liver attenuation in Hounsfield units was used as a quantitative variable and also to define fatty liver (attenuation < 40 HU) in the main analysis. In images where spleen image was available liver-to-spleen attenuation ratio was calculated, and ratio <1 was defined as fatty liver for supplementary analysis.

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Table 1

Demographic Characteristics, Cardiometabolic Measurements and Sex Hormone Distributions in the Study Sample

	Women			Men		
	No Fatty liver (≥ 40 HU)	**Fatty liver (< 40 HU)**		**No Fatty liver (≥ 40 HU)**	**Fatty liver (< 40 HU)**	
N	2675	160		2746	153	
Age (years)	64.7 (9.13)	61.2 (8.02)	<0.001	62.3 (10.2)	58.7 (8.5)	<0.001
Race/Ethnicity (column adds to 100%)						
Caucasian-Americans	987 (37%)	55 (34%)	<0.001	1062 (39%)	66 (43%)	0.001
Chinese-Americans	306 (11%)	17 (11%)	<0.001	314 (11%)	18 (12%)	0.001
African-Americans	805 (30%)	28 (18%)	<0.001	746 (27%)	20 (13%)	0.001
Hispanic Americans	577 (22%)	60 (38%)	<0.001	624 (23%)	49 (32%)	0.001
Diabetes	311 (12%)	46 (29%)	<0.001	376 (14%)	35 (23%)	0.002
Hypertension	1367 (51%)	96 (60%)	0.029	1169 (43%)	82 (54%)	0.007
Current Smoker	295 (11%)	17 (11%)	0.87	379 (14%)	28 (18%)	0.12
Non-heavy alcohol users*	2032 (76%)	130 (81%)	0.13	1906 (69%)	101 (66%)	0.38
Body Mass Index (kg/m^2)	28.6 (5.93)	32.9 (6.5)	<0.001	27.8 (4.22)	31.9 (4.98)	<0.001
HOMA IR (HOMA units)	1.2 [0.8, 2.0]	2.7 [1.7, 4.3]	<0.001	1.3 [0.8, 2.1]	2.6 [1.8, 4.1]	<0.001
Total Cholesterol (mg/dL)	202 (35.7)	198 (33.1)	0.20	188 (34.6)	194 (41.6)	0.041
HDL Cholesterol (mg/dL)	56.9 (15.3)	46.7 (10.3)	<0.001	45 (11.8)	41.6 (11)	<0.001
LDL Cholesterol (mg/dL)	119 (32.2)	114 (27.6)	0.058	117 (31.1)	114 (30.2)	0.34
Triglycerides (mg/dL)	112 [79, 158]	161 [130, 221]	<0.001	112 [78, 165]	162 [118, 236]	<0.001
Total Testosterone (nmol/L)	0.9 [0.6, 1.3]	1.1 [0.7, 1.4]	0.004	14.2 [11.4, 17.8]	12.7 [8.8, 15.2]	<0.001
Bioavailable Testosterone (nmol/L)	0.2 [0.1, 0.3]	0.3 [0.2, 0.5]	<0.001	5.2 [4.2, 6.5]	5.1 [4.0, 6.1]	0.16
Estradiol (nmol/L)	0.07 [0.05, 0.16]	0.10 [0.07, 0.22]	<0.001	0.11 [0.09, 0.14]	0.13 [0.10, 0.16]	<0.001
Dehydoepiandrosterone (nmol/L)	10.2 [7.0, 14.5]	11.4 [7.9, 16.2]	0.010	12.5 [9.2, 17.0]	12.7 [8.7, 19.7]	0.31
Sex Hormone Binding Globulin (nmol/L)	59.8 [41.2, 94.3]	40.8 [27.2, 62.6]	<0.001	40.9 [31.5, 52.8]	32.7 [23.8, 43.8]	<0.001
Spleen image available	1400 (52%)	97 (61%)	0.041	1239 (45%)	73 (48%)	0.53

Data are presented as count (%) for categorical variables, mean (standard deviation) for non-skewed continuous variables and median [25^{th}, 75^{th} percentile] for skewed variables; with p-values for χ^2 tests, t-tests and rank sum tests, respectively. HOMA IR: homeostatic model for insulin resistance, HDL: high density lipoprotein, LDL: low density lipoprotein.

Table 2

Computed Tomography Liver Attenuation Levels by Sex and Sex Hormone Tertiles Adjusted for Age, Race and Body Mass Index

	Sex-Specific Hormone Tertiles			
	T1	**T2**	**T3**	**Linear trend p**
Women				
Bio-T	61.4 [60.8 - 62.1]	61.3 [60.6 - 62.0]	59.2 [58.5 - 59.9]	<0.001
E2	62.1 [61.5 - 62.8]	60.8 [60.1 - 61.5]	58.9 [58.2 - 59.6]	<0.001
DHEA	61.1 [60.4 - 61.7]	60.7 [60.0 - 61.3]	60.2 [59.6 - 60.9]	0.261
SHBG	58.8 [58.1 - 59.5]	62.3 [61.7 - 63.0]	60.8 [60.1 - 61.5]	<0.001
Men				
Bio-T	59.5 [58.8 - 60.1]	59.3 [58.7 - 59.9]	60.2 [59.4 - 60.9]	0.174
E2	60.7 [60.1 - 61.4]	59.6 [59.0 - 60.3]	58.3 [57.7 - 59.0]	<0.001
DHEA	59.2 [58.6 - 59.9]	60.4 [59.7 - 61.0]	59.3 [58.5 - 60.0]	0.015
SHBG	58.0 [57.3 - 58.7]	60.0 [59.3 - 60.6]	60.7 [60.0 - 61.3]	<0.001

Bio-T: Bioavailable testosterone, E2: Estradiol, DHEA: dehydroepiandrosterone SHBG: sex hormone binding globulin

Table 3

Association of Fatty Liver (Attenuation < 40 HU) by Sex and Sex Hormone Tertiles Using Adjusted Logistic Regression

	Sex-Specific Hormone Tertiles			
	T1	**T2**	**T3**	**Linear trend p**
Women				
Bio-T	Ref	1.34 (0.81 - 2.23)	2.12 (1.31 - 3.45)	0.001
Bio-T*	Ref	1.21 (0.72 - 2.04)	1.73 (1.05 - 2.87)	0.023
E2	Ref	2.12 (1.30- 3.47)	2.79 (1.58 - 4.92)	<0.001
E2*	Ref	1.81 (1.10 - 2.98)	2.42 (1.37 - 4.29)	0.002
DHEA	Ref	1.08 (0.68 - 1.70)	1.26 (0.82 - 1.97)	0.30
SHBG	Ref	0.54 (0.35 - 0.83)	0.70 (0.41 - 1.18)	0.056
SHBG*	Ref	0.69 (0.44 - 1.08)	0.90 (0.52 - 1.55)	0.43
Men				
Bio-T	Ref	1.17 (0.77 - 1.78)	0.82 (0.51- 1.32)	0.42
E2	Ref	1.65 (1.01 - 2.68)	2.05 (1.27 - 3.31)	0.004
E2*	Ref	1.61 (0.98 - 2.63)	1.96 (1.21 - 3.18)	0.007
DHEA	Ref	0.70 (0.45 - 1.10)	0.90 (0.57 - 1.40)	0.67
SHBG	Ref	0.67 (0.45 – 1.00)	0.49 (0.29 - 0.82)	0.004
SHBG*	Ref	0.72 (0.48 - 1.08)	0.50 (0.30- 0.84)	0.006

Regression was adjusted for Age, Race/Ethnicity, Body Mass Index, Hypertension, Total and high Density Lipoprotein Cholesterol, Current Smoking and

*Insulin Sensitivity Index.