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Effect Modification of Obesity on Associations between Endogenous Steroid Sex Hormones and Arterial Calcification in Women at Midlife

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Abstract

Objective—To examine whether obesity modify the effects of endogenous steroid sex hormones on arterial calcification in women at midlife.

Methods—Associations between estradiol, testosterone, sex hormone binding globulin and free androgen index and the presence and extent of coronary and aortic calcification were evaluated in 187 obese (body mass index ≥ 30) and 281 non-obese (body mass index < 30) women from the Study of Women's Health Across the Nation. Logistic and linear regressions were used as appropriate.

Results—Prevalence rates of coronary and aortic calcification were significantly higher among obese compared to non-obese ($P < 0.001$, for both). In multivariable analyses, steroid sex hormones were not associated with presence of coronary calcification. However, for extent of coronary calcification, significant interactions were found between obesity and both sex hormone binding globulin ($P < 0.0001$) and free androgen index ($P = 0.008$). In non-obese women, higher sex hormone binding globulin ($P = 0.0006$) and lower free androgen index ($P = 0.01$) were associated with greater extent of coronary calcification while lower sex hormone binding globulin was associated with greater extent of coronary calcification in obese women ($P = 0.05$). For aortic calcification outcomes, higher sex hormone binding globulin was associated with presence of

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aortic calcification among non-obese (OR:1.64, 95%CI:1.16, 2.32, for each 1-SD greater sex hormone binding globulin).

Conclusions—Associations between endogenous steroid sex hormones and arterial calcification vary by obesity status among perimenopausal women. Further research is needed to better understand the possible mechanisms.

Keywords

atherosclerosis; coronary calcification; aortic calcification; hormones; obesity; sex hormone binding globulin

Introduction

The incidence of coronary artery disease (CAD) increases after the fifth decade of life among women.¹ This suggests that alterations of steroid sex hormones (SSHs) around this age may play a key role in the development of the disease.

Obesity significantly influences level of SSHs,² mainly after menopause.³ Lower SHBG and higher estrogen and androgen concentrations have been observed among obese postmenopausal women compared to non-obese.^{4–6} Further, obesity has been suggested to be related to CAD through its associations with several cardiovascular risk factors such as hypertension, lipid abnormalities, and impaired glucose metabolism.^{7–10} Therefore, it is possible that the associations between SSHs and the development of CAD vary by obesity status.

Arterial calcification, a marker of calcified atheroma and total plaque burden, has been associated with an increase risk of CAD.¹¹ Few studies have evaluated the relationship between endogenous SSHs and arterial calcification in women.^{12–14} Further, the relationships between SSHs and CAD^{12–15} are not completely clear. For testosterone (T), inconsistent findings have been reported with studies showing no relationship,^{12,14} a positive relationship (adverse effect)¹⁵ or a negative relationship (protective effect)^{13,16,17} with arterial calcification,^{12,13,14} other subclinical measures^{14,16,17} or CAD¹⁵ in young to middle aged,¹⁴ premenopausal¹⁶ or postmenopausal^{12,13,15–17} women.

The effect of endogenous estrogen on CAD is also not clear. Although estrogen is widely regarded as protective against atherosclerosis,¹⁸ no relationship was observed between endogenous estrogen and arterial calcification^{12,13} or CAD¹⁹ in postmenopausal women.

The relationship between sex hormone binding globulin (SHBG) and CAD has not been well investigated. Low plasma levels of SHBG were found to be independently associated with CAD in women.²⁰ Recently, two studies have evaluated the association between SHBG and arterial calcification with conflicting findings.^{13,14} Results from the Multi-Ethnic Study of Atherosclerosis (MESA), among postmenopausal women aged 45–84, showed that higher coronary artery calcification (CAC) scores were associated with higher SHBG.¹³ On the other hand, Calderon-Margalit et al reported an inverse relationship between SHBG and presence of CAC in young to middle-aged women.¹⁴

Despite the well-known metabolic effect of obesity on SSHs^{2,3} and the association of obesity measures with unfavorable CAD risk profiles,^{7–10} none of the studies that evaluated the relationship between SSHs and arterial calcification^{12–14} assessed the effect modification of obesity. To the best of our knowledge, only one study presented stratification analysis for T and carotid atherosclerosis by obesity status in postmenopausal women.¹⁷ Protective effect of testosterone was observed in non-obese group only.

Therefore, the purpose of this study was to evaluate the cross-sectional effect of endogenous SSHs on the presence and extent of CAC and aortic calcification (AC) stratified by obesity in women at midlife age. We hypothesized that the associations between SSHs and arterial calcification would vary by obesity status.

Methods

Study population

Study of Women's Health Across the Nation (SWAN) is a multi-ethnic longitudinal study, designed to examine the physical, biological, psychological and social changes during the menopausal transition. Detailed study design for SWAN has been previously published. 21 Briefly, between 1996 and 1997, 3,302 participants aged 42 to 52 years were recruited from seven designated sites (Boston, MA; Detroit, MI; Oakland, CA; Los Angeles, CA; Pittsburgh, PA; Chicago, IL; and Newark, NJ).

The present study used data from the SWAN Heart ancillary study, which was conducted at the Pittsburgh and Chicago sites to examine the natural history of subclinical CAD. Between 2001 and 2003 (coincident with the 4th–7th annual SWAN visits) 608 SWAN participants (382 Caucasian, 226 African American) were enrolled in the SWAN Heart ancillary study. SWAN participants were only eligible for the ancillary study if they had not had a hysterectomy or bilateral oophorectomy subsequent to their enrollment in the SWAN study, were not pregnant, were free of self-reported CVD, were not diabetic, and did not report current hormone use.

At the Pittsburgh site only, limited SWAN Heart enrollment began before the 4th annual visit of SWAN. Those participants were deemed eligible for SWAN Heart if they were not pregnant at baseline even if they failed to meet the other eligibility criteria. Therefore, participants were excluded from the current analysis for the following reasons: 16 underwent hysterectomy, 65 reported using hormones and 3 developed CVD or diabetes. Data regarding body mass index (BMI) were missing in 25 participants who also were excluded from the analysis. Because of technical problems, 31 participants had no CAC and AC score and 2 had no AC score. The final samples were 468 women for CAC outcomes and 466 for AC outcomes. Sample size in the analytic models varied based on missing data for SSHs (n=27), low-density lipoprotein (LDL) (n=56), triglycerides (n=53) and homeostatic model assessment of insulin resistance (HOMA index) (n=78). Participants who were not included in the analysis had lower T, higher SHBG, lower FAI, waist circumference, weight, BMI and systolic blood pressure than those who were included. No significant differences were found between the two groups in age, race, CAC score, AC score, lipids and HOMA index.

Research protocols were approved by the institutional review board at each site and all the participants provided a written informed consent prior to enrollment.

Study measures

CAC and AC measures—CAC and AC were measured during the SWAN Heart baseline. Electron beam tomography studies were conducted using a GE-Imatron C-150 Ultrafast CT scanner (Imatron, South San Francisco, CA) administered by a trained technician. An initial scout scan was performed to identify anatomical landmarks. To evaluate the coronary arteries, 30–40 contiguous 3 mm thick transverse images were obtained from the level of the aortic root to the apex of the heart during maximal breath holding. To evaluate the aortic artery, 30–40 6 mm contiguous images were obtained from the aortic arch to the iliac bifurcation with a 300-millisecond exposure time. All scans were

read at the University of Pittsburgh with a DICOM workstation and software by AcuImage, Inc (South San Francisco, CA) using the Agatston method. 22 Presence of AC and/or CAC was determined in a given image if at least three contiguous pixels were present with >130 HU. The CAC score was the sum of scores for each of the 4 major epicardial coronary arteries while the AC was obtained from the single aortic score. The scoring system had a high reproducibility, with an intraclass correlation of 0.98 and 0.99 for aortic and coronary scores, respectively. 23

Physical measures—Waist circumference, weight and height were measured annually. BMI was calculated as weight/ height². BMI was categorized into two levels: BMI<30 (non-obese) and BMI≥30 (obese). Blood pressure was measured in the right arm with the participant seated after at least 5 minutes of rest. The average of two sequential measures was used.

Blood assays—A fasting blood specimen was obtained during the early follicular phase of the menstrual cycle (day 2–5) if participants were still menstruating. Participants were scheduled for venipuncture on day 2–5 of a spontaneous menstrual cycle occurring within 60 day of recruitment. Two attempts were made to obtain the day 2–5 sample; however, if a timed sample could not be obtained (as was especially the case for late perimenopausal and postmenopausal women), a random fasting sample was taken within the 90-day period of recruitment. Hormone assays were performed at the University of Michigan SWAN Endocrine Laboratory by the ACS-180 automated analyzer (Bayer Diagnostics Corp., Norwood, MA). Estradiol (E2) was measured using a modified, off-line ACS: 180 (E₂-6), with a lower limit of detection of 1.0 pg/ml and inter- and intra-assay coefficients of variation averaged 10.6% and 6.4%, respectively. Serum T concentration was evaluated with the ACS: 180 total T assay modified to increase precision in the low ranges with a lower limit of detection of 2 ng/dl and inter- and intra-assay coefficients of variation were 10.5% and 8.5%, respectively. SHBG assay was measured with a two-site chemiluminescent immunoassay, with a lower limit of detection of 1.95 nmol/L and inter- and intra-assay coefficients of variation of 9.9% and 6.1%, respectively. E2 assays were conducted in duplicate while T and SHBG were conducted in singular. The arithmetic mean for the duplicate measures was calculated and reported (coefficient of variation of 3% to 12%). Free androgen index (FAI) was used to estimate the amount of the free active (unbound to SHBG) testosterone. FAI was calculated as $(100 \times T) / (28.84 \times SHBG)$. 24 Hormone values below the lower limit of detection were replaced with a random value between zero and the lower limit of detection. Cycle day of blood draw was reported as during days 2 to 5 (for regularly menstruating women) or outside of that period (for irregularly menstruating and for non-menstruating women).

Lipids, glucose and insulin were assayed at the Medical Research Laboratories (Lexington, KY), certified by National Heart Lung and Blood Institute, Centers for Disease Control and Prevention Part III program. 25 Total cholesterol and triglycerides levels were analyzed using enzymatic methods on a Hitachi 747 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, Ind), 26 and high-density lipoprotein (HDL) was isolated using heparin-manganese. 27 LDL was calculated using the Friedewald equation. 28 Serum insulin was measured by a radioimmunoassay (DPC Coat-a-count) procedure and monitored as part of the monthly quality assurance program by the Diabetes Diagnostic Laboratory at the University of Missouri. Glucose was measured with hexokinase coupled reaction (Boehringer Mannheim Diagnostics). The HOMA index was calculated from fasting insulin and glucose as $(\text{insulin (mU/Liter)} \times \text{glucose (mmoles/Liter)}) / 22.5$. 29 30

Menopausal status—Menopausal status was defined as follows: 1) premenopausal: menstrual period in the past 3 months with no change in regularity in the past 12 months; 2)

early perimenopausal: menstrual period in the past 3 months with some change in regularity during the previous 12 months; 3) late perimenopausal: no menstrual period within the past 3 months, but some menstrual bleeding over the previous 12 months; 4) postmenopausal: no menstrual period within the past 12 months. Both early perimenopausal and late perimenopausal categories were collapsed for this analysis to maintain sufficient number in each category.

Statistical analysis

CAC and AC scores were right skewed since many participants had zero values (CAC: 52.3%, AC: 29.8%). In order to use all available data, each of the CAC and AC scores was analyzed as both categorical (presence of calcification) and continuous (extent of calcification) outcomes. Presence of calcification was a two levels variable: none; if Agatston score =0 and any; if Agatston score >0. Extent of calcification was the log-transformation of non-zero Agatston scores.

Descriptive statistics were performed to summarize study variables by BMI groups. The differences between BMI groups in study variables were assessed using the Wilcoxon rank sums test and Chi-square test as appropriate. Data were presented as median/inter-quartile ranges or percentages.

Univariate and multivariable analyses were performed for the total sample and stratified by BMI groups. Logistic regression was used for presence of calcification outcomes and linear regression was used for extent of calcification outcomes. SSHs, triglycerides, insulin, glucose and HOMA index were log transformed in univariate and multivariable linear regression as their original distributions were not normal. Standardized estimates were presented for linear regression to facilitate interpretation of the estimated coefficients. Odds ratios were presented per 1-SD change of hormones.

For multivariable analyses, all variables found to be significantly associated with study outcomes in the univariate analyses were considered as potential covariates. Age, race, study site, cycle day, waist circumference and systolic blood pressure were included in all models (model 1 and model 2). Lipid/lipoprotein measures and glucose metabolism measures were added to the final models (model 2) to assess impact of these variables on the associations between SSHs and study outcomes. Because these measures were highly correlated, only covariates that found to impact the regression coefficient by 15% or more were included in the final models. Both variance inflation factor and condition index were used to test for multicollinearity.

All final models were refitted including all potential covariates found to be significantly associated with study outcomes in univariate analyses but did not change the regression coefficient by 15% or more (data not shown). Similar results were obtained. Only parsimonious models were presented.

Effect modifications by BMI groups were evaluated for all SSHs in models before stratification. All analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC) and STATA version 10 (Stata Corp, College Station, TX) and were two sided at $\alpha = 0.05$.

Results

Baseline characteristics and potential covariates

Characteristics of the study population are presented in Table 1. Obese participants had a worse cardiovascular risk profile in comparison to non-obese. SSHs significantly differed by BMI; in obese participants, androgens were significantly higher while SHBG was

significantly lower compared to non-obese. In Figure 1, prevalence of CAC and AC were significantly higher in the obese group compared to non-obese. Further, greater extent of AC was found in obese women in comparison to non-obese (median score=46.5 vs. 19.0, $P=0.001$, respectively). A similar pattern was seen for extent of CAC, however it was not significant (median score=8.2 vs. 6.7, $P=0.3$, respectively).

Age, body composition measures, triglycerides, LDL, HOMA index, and systolic blood pressure were significantly associated with calcification outcomes in univariate analyses. Being postmenopausal was associated with greater extent of CAC but not AC in comparison to premenopausal women. (data not shown)

Presence of CAC and AC

In unadjusted analyses of the total group, higher E2 (OR: 0.78, 95% CI: 0.63,0.96, for each 1-SD greater E2) and higher SHBG (OR: 0.66, 95% CI: 0.53,0.81, for each 1-SD greater SHBG) were associated with lower odds of CAC. These associations did not persist with multivariable adjustment either before or after stratification by BMI.

In unadjusted analyses of the total group, higher E2 (OR: 0.82, 95% CI: 0.68,0.99, for each 1-SD greater E2) was associated with lower odds of AC and higher T (OR: 1.26, 95% CI: 1.01,1.57, for each 1-SD greater T) was associated with higher odds of AC. These associations turned to not significant with further adjustment either before or after stratification by BMI.

SHBG was not associated with presence of AC in univariate analysis. After adjusting for potential covariates (model 2), SHBG was positively associated with presence of AC among the non-obese group (OR: 1.64, 95% CI: 1.16,2.32, for each 1-SD greater SHBG). The same results were obtained after including menopausal status in the final models (data not shown). We were not able to study the same association among the obese group as only 6 of them had no AC and therefore the models would not converge. (data not shown)

Extent of CAC and AC

Table 2 shows results for extent of CAC. In the univariate analysis, E2 had a significant negative association with extent of CAC only among the obese group ($\beta=-0.220$, $P=0.008$). In addition, an increase in SHBG was associated with a greater extent of CAC among non-obese ($\beta=0.285$, $P=0.02$) and a lesser extent of CAC among obese participants ($\beta=-0.205$, $P=0.01$). In adjusted analysis, the association between E2 and extent of CAC significantly differed by obesity status (P -interaction=0.03), model 1. High E2 was significantly associated with less extent of CAC among obese participants ($\beta=-0.193$, $P=0.02$). This negative association among obese was attenuated with further adjustment ($P=0.08$) as seen in model 2.

Significant interactions between obesity and both SHBG ($P<0.001$) and FAI ($P=0.02$) were found in relation to extent of CAC (Figure 2, 3, respectively). High SHBG was associated with a greater extent of CAC in non-obese participants ($\beta=0.355$, $P=0.003$) and a lesser extent of CAC among obese group ($\beta=-0.175$, $P=0.03$) (Figure 2). These associations remained significant in final models.

In Figure 3, FAI was negatively associated with extent of CAC in the non-obese group ($\beta=-0.255$, $P=0.04$) (Table 2, model 1), while no significant association was seen in the obese group. These associations persisted in final models (Table2, model 2). Similar results were observed in models that included menopausal status (data not shown).

When evaluating AC, similar significant interaction between SHBG and obesity was observed (P -interaction=0.03), (Table 3, model 2). SHBG was positively associated with extent of AC in the final model.

Discussion

Our results provide a clear evidence for the importance of considering obesity status when assessing associations between SSHs and arterial calcification. The relationship between CAC and both SHBG and FAI was significantly different by obesity status independent of the other well-known risk factors. A higher SHBG was associated with less calcification in the obese group and with more calcification in the non-obese group. In addition, an increase in FAI was associated with less calcification in the non-obese group. No significant association was observed for FAI in the obese group.

In women, conflicting findings have been reported regarding the potential role of androgens as CAD risk factors. In a study that included postmenopausal women undergoing coronary angiography, free T was positively associated with severity of CAD. 15 On the other hand, higher, but physiological levels of androgens were found to be correlated with lower risk of carotid artery disease in pre- and postmenopausal women. 16 Lower levels of T and free T index were reported among women with CAD compared to those without. 31 Moreover, findings from MESA supported an independent negative association between free T and the extent of CAC in postmenopausal women. 13 Recent report from prospective Rancho Bernardo study in postmenopausal women concluded that low levels of T are associated with an increased risk of coronary heart disease events. 32 In addition, very recent results from a prospective cohort European study in women aged 57.9 ± 14.4 years showed that low baseline T was associated with increased all-cause mortality and incident CV events independent of traditional risk factors. 33

Our finding of a negative association between FAI and CAC among non-obese women is consistent with results from MESA 13 and other studies. 16·17·31–33 Interestingly, in non-obese postmenopausal women, the prevalence of carotid atherosclerosis was significantly higher with a low T level. 17 Because obesity substantially impacts CV risk profile, 7–10 it is possible that the mechanism by which T affects CAD will not be the same in non-obese compared to obese women. The relationships between androgens and CAD are complex and not completely understood. T provides an antiatherogenic effect by stimulating efflux of cellular cholesterol to HDL, and proatherogenic effects by facilitating the uptake of lipoproteins. 34·35 T impacts CV risk factors by decreasing serum levels of HDL, plasminogen activator type 1 (deleterious), lipoprotein a, fibrinogen, insulin, and leptin (beneficial). 36·37 Moreover, T inhibits the calcium channel of smooth muscle cells and suppresses the activation of pro inflammatory cytokines. 36·37 Therefore, it is possible that FAI associated with less extent of calcification in healthy non-obese women through the above suggested favorable mechanisms. 37

Our results showed different pattern of associations between SHBG and arterial calcification by obesity status. The positive association between SHBG and the extent of calcification among the non-obese group was consistent with other researchers. 13 In a sample of postmenopausal women (BMI grand mean (SD): 28.3 (5.33)) who were not on hormone therapy or insulin use, greater SHBG was associated with more extent of calcification ($\beta=0.298$, $P=0.03$, both SHBG and extent of calcification score were log transformed) in full adjusted model. 13 SHBG is a glycoprotein synthesized in the liver and binds, with higher affinity to androgens more than estrogens. 25 An increase in SHBG is associated with a decreased androgenic status. Therefore, one could argue that this positive association that we reported may be explained by the direct relationship between SHBG and androgen.

However, including both T and SHBG in the same model (data not shown) did not attenuate the significant positive effect of SHBG on the extent of CAC among non-obese women. Probably there are other pathways by which SHBG impacts calcification that we do not know. SHBG may have biologic functions beyond simply regulation of the levels of free sex hormones. It has been reported that SHBG is part of a signal transduction system for steroids at the cell membrane.³⁸ Further studies are needed to understand the mechanism of this positive association that we and others¹³ observed.

The negative relationship between SHBG and calcification among the obese group could be explained by its association with androgens. When both T and SHBG included in model for obese group, the negative relationship between SHBG and extent of CAC was no longer significant (data not shown). Higher SHBG is associated with higher HDL levels.^{12,39} Increasing levels of HDL are associated with less subclinical atherosclerosis.¹² Hepatic lipase, involved in HDL catabolism, is known to be stimulated by androgens independently of insulin and abdominal fat. Therefore, high SHBG would result in low free androgen which maintains a low hepatic lipase activity and a high level of HDL.³⁹

In this study, none of the SSHs was associated with presence of CAC in final models. Our results were similar to those of Ouyang et al who reported null effects of SSHs on presence of CAC among a multiethnic sample of postmenopausal women.¹³ In contrast, Calderon-Margalit et al reported a prospective significant inverse association between SHBG and presence of CAC among young to middle-aged women (OR for women with SHBG above the median=0.59, 95% CI: 0.4, 0.87; $P=0.008$).¹⁴ The inconsistency between our findings and Calderon-Margalit et al findings could be related to differences in study design (prospective vs. cross-sectional) and study population's menopausal status. It is also possible that we did not find any significant association between SSHs and presence of CAC due to the lack of sufficient power when evaluating the outcome variable as a binary variable (presence of CAC) given our small sample size. Despite the well-known advantages of dichotomizing a continuous variable, one main disadvantage is the loss of power.⁴⁰

The main limitations of the current study include: 1) The cross-sectional design which limited any causal inference. A second measure for calcification is now available within SWAN and can be used for a longitudinal assessment; 2) Hormone assays were collected during the early follicular phase of the menstrual cycle, which might not be optimum for all hormones that were measured. All our analyses were adjusted for cycle day; 3) Biologically active T was not measured directly. However, our estimation of FAI is a valid measure of free T;⁴¹ 4) Obese participants' scans may have more artifact than non-obese that artificially raising the calcium scores. This is very difficult to control for and still under investigations.⁴²

The main strengths of our study are: 1) The first to consider evaluating roles of SSHs on arterial calcification by obesity status; 2) The study population are well-characterized women followed up throughout the menopausal transition; 3) Current analysis adjusted for most of the major and potential covariates that may impact the assessed associations.

Conclusions

In conclusion, our findings suggested that obesity may have significant impact on how SSHs associated with arterial calcifications. Both SHBG and FAI are strongly associated with extent of CAC. The directions of these associations significantly differed by obesity status. These data may explain why results from previous reports are inconsistent. Future studies of SSHs and other subclinical measures should consider assessing effect modification by BMI.

Summary

Associations between endogenous steroid sex hormones and arterial calcification vary by obesity status in women at midlife. These data may explain why results from previous reports are inconsistent.

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Figure 1. Presence of Coronary Arterial Calcification (CAC) and Aortic Calcification (AC) by BMI Groups (n=468 for CAC, n=466 for AC)

Abbreviations: BMI: body mass index; CAC: coronary arterial calcification; AC: aortic calcification.

* $P < 0.001$

Figure 2. Association of SHBG and Extent of Coronary Arterial Calcification (CAC) by Obesity

a

Abbreviations: BMI: body mass index; CAC: coronary arterial calcification; SHBG: sex hormone binding globulin.

* P value for interaction <0.001

^a Adjusted for age, race, systolic blood pressure, waist circumference, study site, cycle day and triglycerides; (n=200)

Figure 3. Association of FAI and Extent of Coronary Arterial Calcification (CAC) by Obesity a
Abbreviations: BMI: body mass index; CAC: coronary arterial calcification; FAI: free androgen index.

* P value for interaction =0.008

^a Adjusted for age, race, systolic blood pressure, waist circumference, study site, cycle day and triglyceride; (n=200)

Table 1

Baseline Characteristics of Participants in Analytic Sample by BMI Groups^a

Characteristics	Total (n=468)	Non-obese (BMI < 30) (n=281)		Obese (BMI ≥ 30) (n=187)		P-value for BMI groups differences ^b
	Median	I.Q. Range	Median	I.Q. Range	Median	I.Q. Range
Age, Yr	50.0	48.0–52.0	49.0	47.0–52.0	50.0	48.0–53.0
Race						
African American (%)	38.9		31.3		50.3	
White (%)	61.1		68.7		49.7	
Site						
Pittsburgh (%)	36.1		36.6		35.3	
Chicago (%)	63.9		63.3		64.7	
Hormones						
E2, pg/mL	29.5	16.2–75.9	36.5	16.0–89.3	25.3	16.7–51.3
T, ng/dL	36.2	27.4–49.5	35.4	26.3–46.7	37.4	29.6–52.5
SHBG, nM	41.7	28.3–59.6	48.0	34.7–65.6	33.7	22.0–48.8
FAI ^a	3.1	1.8–5.2	2.4	1.6–4.4	3.8	2.6–6.5
Body Composition						
Waist circumference, cm	87.2	78.2–97.6	79.8	75.0–86.6	100.2	94.4–108.0
Body weight, kg	75.2	65.3–88.6	68.0	61.2–73.5	92.6	84.6–104
BMI, kg/m ²	28.1	24.6–33.0	25.3	23.2–27.4	34.4	32.0–37.9
Lipids/Lipoprotein						
Cholesterol, mg/dL	194.5	173.5–224.0	192.0	169.0–219.0	199.0	180.0–226.0
Triglyceride, mg/dL	97.0	73.0–135.0	86.0	67.0–115.0	117.5	87.5–170.0
LDL, mg/dL	116.0	97.0–139.0	112.0	94.0–133.0	120.0	101.0–146.0
HDL, mg/dL	55.0	48.0–65.0	58.0	50.0–68.0	51.0	44.0–60.0
Glucose Metabolism						
Insulin, uIU/mL	9.2	7.0–13.8	7.9	6.1–10.6	13.5	9.3–19.7
Glucose, mg/dL	88.0	83.0–96.0	86.0	81.0–92.0	94.0	87.0–102.0
HOMA index ^a	2.1	1.4–3.2	1.6	1.2–2.3	3.1	2.1–4.9

Characteristics	Total (n=468)	Non-obese (BMI < 30) (n=281)		Obese (BMI ≥ 30) (n=187)		P-value for BMI groups differences ^b
	Median	I.Q. Range	Median	I.Q. Range	Median	I.Q. Range
Blood Pressure						
Systolic, mmHg	118.0	108.0–128.0	113.0	106.0–125.0	123.0	113.0–136.0
Diastolic, mmHg	75.0	69.0–82.0	72.0	68.0–79.0	78.0	72.0–85.0
Stage of Menopause						
Pre (%)	11.5		12.1		10.7	0.8
Peri (%)	61.7		61.9		61.5	
Post (%)	26.8		26.0		27.8	

Abbreviations: BMI: body mass index; E2: estradiol; FAI: Free androgen index; HDL: high density lipoprotein; HOMA: insulin resistance index; LDL: low density lipoprotein; SHBG: sex hormone binding globulin; T: testosterone.

^aData are medians and interquartile ranges (I.Q.range), unless otherwise indicated. FAI= (100 × T)/(28.84 × SHBG); HOMA=(fasting insulin × fasting glucose)/22.5.

^bWilcoxon rank sums test for continuous variables, χ^2 test for categorical variables.

Unadjusted and Adjusted Linear Regression Models of the Association of Each Steroid Sex Hormone and the Extent of Calcification in the Coronary Arteries (CAC)^a

Table 2

	Non-obese (BMI < 30) (n=72)		Obese (BMI ≥ 30) (n=151)		Total (n=223)
	Standardized estimate	P value	Standardized estimate	P value	P value for interaction with obesity
Unadjusted Models					
E2, pg/mL	0.032	0.8	-0.220	0.008	----
T, ng/dL	0.023	0.8	-0.046	0.6	----
SHBG, nM	0.285	0.02	-0.205	0.01	----
FAI ^b	-0.192	0.1	0.156	0.06	----
Adjusted Models					
Model 1 ^c					
E2, pg/mL	0.261	0.07	-0.193	0.02	0.03
T, ng/dL	0.031	0.8	-0.119	0.1	0.3
SHBG, nM	0.355	0.003	-0.175	0.03	<0.001
FAI ^b	-0.255	0.04	0.092	0.3	0.02
Model 2 ^{c d}					
E2, pg/mL	0.225	0.1	-0.148	0.08	0.09
T, ng/dL	-0.011	0.9	-0.116	0.2	0.8
SHBG, nM	0.393	<0.001	-0.163	0.05	<0.001
FAI ^b	-0.315	0.01	0.081	0.3	0.008

Abbreviations: BMI: body mass index; E2: estradiol; FAI: Free androgen index; SHBG: sex hormone binding globulin; T: testosterone.

^a All steroid sex hormones were log transformed.

^b FAI = (100 × T)/(28.84 × SHBG).

^c Model 1: Adjusting for age, race, systolic blood pressure, waist circumference, study site and cycle day; model 2: Model 1 plus triglycerides.

^d Total n = 200, BMI < 30 n = 64, BMI ≥ 30 n = 136.

Unadjusted and Adjusted Linear Regression Models of the Association of Each Steroid Sex Hormone and the Extent of Calcification in the Aorta (AC)^a

Table 3

Non-obese (BMI < 30) (n=149)		Obese (BMI ≥ 30) (n=178)		Total (n=327)	
Standardized estimate	P value	Standardized estimate	P value	P value for interaction with obesity	
Unadjusted Models					
E2, pg/mL	0.068	0.4	-0.101	0.2	----
T, ng/dL	0.038	0.6	0.123	0.1	----
SHBG, nM	0.153	0.07	- 0.109	0.1	----
FAI ^b	-0.081	0.3	0.165	0.03	----
Adjusted Models					
Model 1 ^c					
E2, pg/mL	0.067	0.5	-0.039	0.5	0.07
T, ng/dL	0.047	0.6	0.030	0.6	0.9
SHBG, nM	0.146	0.1	0.001	0.9	0.004
FAI ^b	-0.059	0.5	0.016	0.8	0.08
Model 2 ^{c d}					
E2, pg/mL	-0.001	0.9	-0.037	0.6	0.4
T, ng/dL	0.054	0.6	0.021	0.8	0.6
SHBG, nM	0.167	0.09	0.016	0.8	0.03
FAI ^b	-0.092	0.3	-0.001	0.9	0.2

Abbreviations: BMI: body mass index; E2: estradiol; FAI: Free androgen index; SHBG: sex hormone binding globulin; T: testosterone.

^a All steroid sex hormones were log transformed.

^b FAI = (100 × T)/(28.84 × SHBG).

^c **Model 1:** Adjusting for age, race, systolic blood pressure, waist circumference, study site and cycle day; **model 2:** Model 1 plus triglycerides and HOMA (insulin resistance index).

^d Total n = 273, BMI<30 n=121, BMI≥30 n=152