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Levels of sex steroid and CVD measures in premenopausal and hormone-treated women at mid-life: implications for the "timing hypothesis"

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Abstract

Background—The "timing hypothesis", in addressing findings from the Women's Health Initiative trial, suggests that hormone therapy (HT) use should be initiated within six years of the menopause transition to extend a favorable estrogenic environment after menopause.

Methods—We compared sex steroid and cardiovascular profiles at visit 05 in a community-based, longitudinal study of the menopause transition (Study of Women's Health Across the Nation). Women, aged 47–57 years, were in one of four groups: premenopausal, using conjugated equine estrogen (CEE) with or without progestin, or postmenopausal (<5 years). Cardiovascular assays included low density lipoprotein cholesterol (LDL-c), oxidized LDL-c, high density lipoprotein cholesterol (HDL-c), triglycerides, apolipoproteins A-1 and B, F_{2a}-isoprostanes, C-reactive protein (CRP), and lipoprotein(a)-1. Sex steroid assays were for estradiol (E2), estrogen receptor ligand load (ERLL), 2-hydroxyestrone (2-OHE1), 16α-hydroxyestrone (16α-OHE1), total testosterone, and sex hormone-binding globulin (SHBG).

Results—HT users had 50% higher SHBG levels ($p<0.0001$ for both groups), which limits sex steroids binding to their receptors, and higher excreted estrone metabolites (more than 60%, $p<0.0001$ for both groups) than pre- or postmenopausal women. These were, in turn, associated with higher F_{2a}-isoprostanes, an oxidative stress measure, compared to premenopausal women. HT users had a more favorable HDL-c/LDL-c ratio than pre- or postmenopausal women ($p<0.01$), but higher triglyceride levels ($p<0.01$).

Conclusions—Though HT users had some more favorable lipid profiles than pre- and postmenopausal women, there was evidence of adverse HT effects even in women free of atherosclerosis evaluated within the approximate 6-year time period proposed with the "timing hypothesis".

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INTRODUCTION

Studies to understand underlying cardioprotection in women relative to men at mid-life[1] have focused on the importance of estrogens and their relation to lipids, especially high density lipoprotein cholesterol (HDL-c). Exogenous hormone therapy (HT) was the long-time paradigm thought to demonstrate that maintenance of estrogen levels following menopause contributed to heart health.[2,3] However, when the Women's Health Initiative (WHI) and the Heart and Estrogen/progestin Replacement Study (HERS) identified that widely-prescribed exogenous hormone products were not cardioprotective,[4,5] as had been originally hypothesized, alternative explanations were proposed.

One proposal, the "timing" hypothesis, is that a positive impact of HT on cardiovascular status is dependent extending a favorable estrogenic environment after menopause without a substantial time discontinuity.[6] Substantial time discontinuity between menopause and HT use could be permissive for the development of atherogenic lesions and vascular compromise that would be less responsive to positive elements associated with HT use. In instances of HT initiation around menopause, it could also be hypothesized that HT use would confer sex steroid status that would help sustain favorable lipid or cardiovascular profiles.

To determine if there were unappreciated changes in the endogenous sex steroid hormone environment among users of conjugated equine estrogen (CEE) (with and without progestin) that might compromise lipids or other cardiovascular measures, we evaluated estradiol (E2) levels and novel estrogen measures in four groups: 1) premenopausal women; 2) CEE users; 3) CEE + progestin users; and 4) women postmenopausal for less than 5 years without HT use. Novel measures included the amount of estrogen acting as a ligand to the estrogen receptor [estrogen receptor ligand load (ERLL)] and an estimate of estradiol bioavailability [free estrogen index (FEI)]. Selected estrogen metabolites have been hypothesized to contribute biological activity,[7] so we assayed 2-hydroxyestrone (2-OHE1) and 16α-hydroxyestrone (16α-OHE1) on the premise that 2-hydroxyestrogens may act as anti-oxidants[8] or that 16α-OHE1 activity may include covalent binding to the estrogen receptor.[9] We characterized the potential antioxidant associations of estrogens using F_{2a}-isoprostanes**,** a product of arachidonic acid oxidation and degradation.[10,11] We further characterized the endogenous sex steroid environment using measures of total testosterone (T) and sex steroid bioavailability with sex hormone binding globulin (SHBG).

SUBJECTS AND METHODS

Sampling and Study Population

Data are from the Study of Women's Health Across the Nation (SWAN) a multi-center, multi-ethnic longitudinal study of the menopausal transition. Data and specimens were from the fifth annual follow-up examination which took place in the year prior to the release of WHI trial cardiovascular findings.[4]

At baseline, SWAN study eligibility criteria included age 42–52 years; presence of an intact uterus and at least one ovary; no use of exogenous hormones; menses in the three months prior to enrollment; and, self-identification with a site's designated race/ethic group. Therefore, a Caucasian and non-Caucasian sample was recruited including African-American women in

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Boston, Chicago, the Detroit area, and Pittsburgh, and Japanese, Chinese, and Hispanic women in Los Angeles, Oakland, and Newark, respectively.[12]

There were 2606 women who participated in follow-up visit 05 (79% of the 3302 baseline participants). However, data from two of the seven sites (Chicago and Newark, NJ) were precluded from these analyses because their site protocols did not include urine collection for assay of estrogen metabolites. Excluded from data analyses were women with diabetes, heart disease, non-estrogen hormone use or having surgical menopause without HT use. Women in the early (n=670) and late perimenopause stages (n=196) were excluded to allow a more clear delineation of hormone and cardiovascular measures in premenopausal women, postmenopausal women (without HT use) and women using two types of conjugated equine estrogen (CEE) therapy, with and without a progestin. Institutional Review Board approval for the study protocol and repository storage was obtained at each study site.

Menopause stage and HT use

Premenopause was defined as the presence of menses within the previous three-month period, with no decrease in predictability. Early perimenopause was the presence of menses within the previous three months and less predictable menstrual frequency; late perimenopause was defined as having three to 11 months of amenorrhea.[13–15] Twelve consecutive months of amenorrhea without alternative explanation indicated postmenopause.[15] Medication and HT use were self-reported with corroborative visualization of the prescription container, when possible. Of 782 women available for this report, 12.5% were premenopausal without HT use, 8% used CEE only, 32% used CEE-progestin combinations, and 47% were postmenopausal without HT use. Eighteen women using transdermal formulations were excluded from analyses.

Specimens and assays

Annual morning blood and urine collections followed an overnight fast. Two attempts were made to obtain specimens in days 2–5 of a spontaneous menstrual cycle. If timed specimens could not be obtained, random fasting specimens were collected. Blood was refrigerated 1–2 hours after phlebotomy, centrifuged, aliquotted, frozen and then sent on dry ice to laboratories for assay.

Specimens were analyzed for hormones and SHBG at the University of Michigan (CLIA Certified) laboratory on the ACS-180 automated analyzer (Bayer Diagnostics, Tarrytown NY). E2 was assayed in duplicate using a rabbit anti-E2-6 ACS-180 immunoassay which was modified to reduce the lower limit of detection to 1.0 pg/mL and a coefficient of variation (CV) of 3–12%. The testosterone (T) competitive immunoassay used testosterone labeled with dimethylacridinuimester (DMAE), a polyclonal rabbit anti-testosterone antibody and a monoclonal mouse anti-rabbit antibody coupled to paramagnetic particles. The T reporting range was 0.4–3.5 nM (10–100 ng/dL) and assay range was 0.07–16.6 nM (2–478 ng/dL). The T assay was standardized analytically and confirmed by gas chromatography/mass spectrometry (GC-MS). Inter-assay and intra-assay CV were 13.8% and 6.6%, respectively. The competitive sex hormone-binding globulin (SHBG) assay was developed on-site with rabbit anti-SHBG antibodies, with a lower limit of detection of 1.95 nM. Total T was indexed to SHBG to calculate the Free Androgen Index (FAI = 100 × T (ng/dL)/28.84 × SHBG (nM)). Total E2 was indexed to SHBG [Free Estradiol Index (FEI = 100 × total estradiol/272.11 × SHBG] to estimate non-bound estradiol bioavailability. 10*E2/T was used to make the resulting ratio unit-free.

2-OHE1 and 16α-OHE1 were assayed by enzyme immunoassay (ESTRAMET™) in triplicate.[16] Inter- and intra-assay CV were less than 10% for each analyte. Because urinary 2-OHE1

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and 16α-OHE1 are found as 3-glucuronides or 3,3,16-glucuronides, these sugars were removed to achieve recognition sites for the monoclonal antibodies using a mixture of β-glucuronidase and arylsulphatase enzyme isolated from *Helix Pomatia*. The assay range was 0.6 to 40.0 ng/ml.

At the University of California, Davis, archival serum specimens were analyzed for the estrogen receptor ligand load (ERLL) a cell-based bioassay for measuring signal transduction activity of total circulating bioactive estrogens and detecting activation of estrogen-dependent gene expression.[17,18] Procedures are available in Appendix I or from the authors.

Specimens were assayed for cardiovascular measures at MRL/GCL which is certified by the National Heart Lung and Blood Institute, Centers for Disease Control Part III program. Lipid fractions were determined from EDTA plasma. Total cholesterol and triglycerides were analyzed by enzymatic methods on a Hitachi 747 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). HDL-c was isolated using heparin-2M manganese chloride.[19,20] LDL-c was calculated using the Friedewald equation.[21] Lipoprotein(a) [Lp(a)-1] was quantified by competitive ELISA.[22] Plasma oxidized LDL-c (oxLDL-c) concentrations were measured by sandwich ELISA. Apolipoproteins A-I (ApoA-1)and B (ApoB) were measured by immunonephelometry (BNA-100-Behring Diagnostics, Westwood, MA) calibrated with a World Health Organization traceable standard.[23] C-reactive protein (CRP) was quantified using an ultra-sensitive rate immunonephelometric method (hs-CRP on BN 100, Dade-Behring, Marburg, Germany). The lower limit of detection of the CRP assay was 0.03 mg/dL, the CV was 10–12% (0.05 mg/dL) and 5–7% (2.2 mg/dL). Plasma plasminogen activator inhibitor-1 (PAI-1) was measured with a sandwich procedure using a solid phase monoclonal antibody and enzyme-labeled goat antiserum (IMUBIND plasma PAI-1 ELISA, American Diagnostica, Greenwich, CT). Monthly inter-assay CV percents were 5–9% and 4–9% at mean concentrations of 7 and 22.5 ng/dL, respectively.

Samples analyzed for F_{2a}-isoprostanes were pre-purified on affinity columns (Cayman Chemical, Ann Arbor, MI), washed, eluted with 95% ethanol, evaporated, dried and then diluted 1:10 with 0.1 M phosphate buffer, assayed using an F_{2a}-isoprostane enzyme immunoassay (EIA) (Cayman Chemical, Ann Arbor, MI) and read at 405 nm. The standard curve range was 3.9–500 pg/mL. Post-extraction intra- and inter-assay CV% were 14.4% (51.2 pg/mL, n=83 pairs) and 17.5% (51.2 pg/mL, n=85), respectively; the intra-assay CV% was 5.8% (n=1707 pairs).

Physical and interview-based measures

Heights (cm) and weights (kg) were measured to estimate BMI (kg/m^2) as weight divided by the square of height. Waist circumference (cm) was measured about 3 cm above the umbilicus following a relaxed expiration.

Based on self-report of race/ethnicity, the analytical sample was 20% African-American, 58% Caucasian, 12% Japanese, and 10% Chinese. Exogenous hormone users in the analytical sample were 17% African American, 62% Caucasian, 12% Chinese and 8% Japanese. Type of hormone delivery system did not vary according to race/ethnicity. Smoking and physical activity data were from self-administered questionnaires incorporating American Thoracic Society questions[24] and a modified Baecke instrument,[25,26] respectively.

Statistical methods

Based on examination of distributions, log or square root-transformations were applied to measures of lipids and hormones for use in statistical modeling and backtransformed (including variances with a Taylor series) for presentation. Primary analytical approaches included

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analysis of covariance (ANCOVA), correlation analyses, and multivariable regression modeling, testing main effects and interactions (e.g., the interaction between SHBG and group status or interactions between 5-year difference in CVD measures and group status). ANCOVA was used to evaluate if there were significant mean different levels in lipid or hormone levels according to menopause stage or HT group designation, after adjusting for site, age, BMI, physical activity, race/ethnicity, and smoking status. Duration of HT use and difference in CVD measures between baseline and FU-05 were evaluated and did not change the reported relationships. Pearson and partial correlations ($r_{Y|X}$) were obtained, respectively, before and after adjustment for covariates. Type III sums of squares were used to estimate the adjusted partial correlations. Data are shown with ninety-five percent confidence intervals (95% CI) or p-values (two-sided with $\alpha < 0.01$). SAS version 9.1 statistical software (SAS Institute, Cary, NC) was used.

RESULTS

Mean ages of women in the premenopause group, the CEE group, the CEE + progestin group and the postmenopause group were 48.9, 52.1, 51.9, and 52.6 years, respectively; age was not different according to race/ethnicity. The number of women and duration of their CEE use were: 5 women for 1 year, 11 for 2 years, 12 for 3 years, 17 for 4 years and 8 women for 5 years. The number and duration of CEE + progestin use were: 40 women for 1 year, 55 for 2 years, 58 for 3 years, 50 for 4 years, and 40 women for 5 years.

Mean E2 values were similar in premenopausal women and the two HT groups and more than double the value in postmenopausal women (see Table 1). There were no statistically significant mean differences by group in ERLL levels. Also, there were no differences in mean levels of total T in the premenopausal women compared to the two HT groups though T levels were significantly higher in the postmenopausal women (Table 1).

There were pronounced estrone metabolite differences according to group (Table 1). Though mean 2-OHE1 levels were significantly higher the premenopausal group compared to the postmenopausal group, values in HT users were more than double those in either premenopausal or postmenopausal women (Table 1). Further, mean 16α-OHE1 levels were more than 60% higher in HT users compared to premenopausal or postmenopausal women.

Mean SHBG was more than 50% higher among the HT users compared to premenopausal or postmenopausal women (Table 1).

Intermediate cardiovascular markers

Mean oxLDL-c levels were similar in HT users and premenopausal women (Table 2). However, the LDL-c values in both premenopausal women and HT users were, on average, 11–12% lower than the mean value in postmenopausal women. The mean HDL-c values were approximately 10% lower in premenopausal women compared to the other groups (Table 2). Collectively, there was a more favorable HDL/LDL ratio in women using HT (Table 2). Further, the ApoB to ApoA-1 ratio was more favorable in premenopausal women and HT users as compared to postmenopausal women.

The triglyceride profile was less favorable in HT users compared to either premenopausal or postmenopausal women (Table 2). Further, mean Lp(a)-1 levels were approximately 10% higher in women using HT compared to premenopausal and postmenopausal women.

Mean CRP was significantly higher in women using the CEE preparations compared to postmenopausal women (Table 2). Mean $F_{2\alpha}$-isoprostanes, a measure of oxidative stress, was about 10% higher in those women using the CEE + progestin preparation compared to the other

three groups, a difference significant at the $p<0.05$ level. In contrast, mean PAI-1, an inhibitor of fibrinolysis, was almost 50% lower in HT users compared to mean values in premenopausal and postmenopausal women (Table 2). Adjusting for duration of CEE product use did not alter these associations.

Relating sex steroid measures to CVD markers

In exogenous hormone users, the partial correlations between E2 and HDL-c and ApoA-1 were 0.17 (95% CI, .05–.29) and 0.19 (95% CI, .06–.31), respectively. There were no other notable associations of CVD measures with E2, FEI or ERLL.

In premenopausal women, estrone metabolites were positively associated with LDL-c levels and with ApoB. Partial correlations between LDL-c and the 2-OHE1 and 16α-OHE1 metabolites were .25 (95% CI, .02,–.47) and .33 (95% CI, .09–.54), respectively. Partial correlations between ApoB and 2-OHE1 and 16α-OHE1 metabolites were .20 (95% CI, .05–.43) and .32 (95% CI, .08–.53), respectively. However, in HT users, estrone metabolites and lipid measures were not statistically associated although mean metabolite levels were almost 50% higher in HT users.

Higher estrone metabolites levels were associated with higher the F_{2a}-isoprostanes in all groups, indicating estrone metabolites were associated with greater oxidative stress. In premenopausal women, the partial correlations between the $_{log}F_{2a}$-Isoprostanes and $_{log}$2-OHE1 and $_{log}$16α-OHE1 metabolites were .57 (95% CI, .38–.72) and .63 (95% CI, .46–.76), respectively. In CEE only users, partial correlations between $_{log}F_{2a}$-isoprostanes and $_{log}$2-OHE1 and $_{log}$16α-OHE1 metabolites were .30 (95% CI, .05–.58) and .43 (95% CI, .10–.68), respectively. In CEE + progestin users, partial correlations between $_{log}F_{2a}$-isoprostanes and $_{log}$2-OHE1 and $_{log}$16a-OHE1 metabolites were .44 (95% CI, .31–.55) and .49 (95% CI, .37–.59), respectively.

In premenopausal women, higher SHBG levels were associated with lower LDL-c, ApoB, triglyceride, CRP and PAI-1 levels (Table 3), suggesting a potential protective effect for SHBG. However, these relationships were not replicated in HT users whose SHBG levels were remarkably higher than levels in premenopausal women. In women using CEE only, SHBG levels were positively correlated with HDL-c, ApoA-1 and CRP; in women using the CEE + progestin, associations were mixed (negative associations with LDL-c and PAI-1, but positive associations with CRP). SHBG was negatively associated with CRP levels in premenopausal women but positively associated with the CRP levels in HT users.

DISCUSSION

The WHI and HERS trials identified that widely-prescribed CEE-based products were not identified as cardioprotective[4,5] in women remote from their final menstrual period, thereby generating debate about explanatory mechanisms. This debate is currently centered about the "timing hypothesis"[6] which speculates that HT use should be initiated within six years of the menopause transition (based on data extrapolated from ovarietomized monkeys)[27] so that optimal sex steroid and CVD profiles observed in the premenopause stage can be sustained before CVD progresses to an irreversible state. We related a panel of usual and more novel estrogen and androgen measures with cardiovascular markers in HT users, premenopausal women and postmenopausal women under the theory that cardiovascular values observed in premenopausal women were an expression of the desirable CVD environment during HT use.

Traditionally, studies of HT use have focused on the HDL-c levels, citing the greater cardioprotective aspects with this lipid fraction.[2,3] We too found that HDL/LDL-c ratios and their apolipoprotein ratios were favorable in both CEE groups. However, triglyceride levels

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were much less favorable in the HT users compared to premenopausal women. This previously reported finding motivated the recommendation for using HT agents that do not require "first pass" hepatic metabolism.[28] The markedly higher SHBG levels, which affect sex steroid binding, may be a reflection of altered hepatic metabolism that indirectly affect cardiovascular measures. There were higher levels in Lp(a)-1 and PAI-1 levels among HT users compared to the other two groups. Lipoprotein(a) is thought to recruit inflammatory cells through interaction with Mac-1 integrin.[29] The Lp(a) structure is similar to plasminogen and tissue plasminogen activator (tPA) and it competes with plasminogen for its binding site, leading to reduction in fibrinolysis.

While HT use on some, but not all, lipids appeared favorable, users had a less favorable oxidative environment and more pronounced inflammatory response. Premarin™ or the Premarin-progestin combination, Prempro™, which comprise the preponderance of formulations used by SWAN (and all WHI) enrollees, include conjugated estrogens of which more than 50% are estimated to be estrone. Consistent with this, we identified much greater estrone metabolite excretion in HT users. Recent work suggests that higher estrone metabolite levels were highly associated with higher F_{2a}-isoprostane levels,[30] a product of the free radical-induced peroxidation of arachidonic acid.[31,32] F_{2a}-isoprostanes have been characterized as a superior marker of the *in vivo* oxidative stress response.[10,33–35] The mean F_{2a}-isoprostane level was highest in women using the CEE+ progestin combination. Notably, this treatment arm of the WHI was terminated early for excess cardiovascular risk.[4]

There were higher levels of CRP, an inflammation marker, in women using CEE formulations compared to postmenopausal women. Elevated CRP levels in hormone users have been reported previously by ourselves and investigators.[36] In the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study, hormone use was associated with 50% higher levels of CRP compared with non-users.[37] CRP was higher in each treatment group including estrogen alone or in combination with micronized progesterone or medroxyprogesterone acetate.[37] PEPI investigators suggested that a mechanism for adverse cardiovascular events with HT use is through increasing inflammatory response, with the potential for accelerated atherosclerosis, plaque destabilization, or thrombosis.

Markedly higher SHBG levels were observed in women using HT. SHBG is synthesized in the liver with concentrations being regulated by the androgen/estrogen balance, thyroid hormones, and insulin. Other publications have identified that SHBG levels were significantly correlated with intermediate markers of CVD risk[36,38] including positive associations with HDL-c concentrations and negative associations with LDL-c, oxLDL-c, triglyceride, and ApoB levels during the menopause transition.[39] Traditionally, descriptions of SHBG activity have focused on its binding capacity with testosterone and estradiol in determining bioavailability so that less than 2–3% of these sex steroids are available for hormone receptor-mediated action.[40] Expanded action of SHBG action are also being identified.[40] It remains to be determined if the elevated 2-OHE1 and 16α-OHE1 metabolites bind with SHBG and disrupts the favorable role of SHBG seen in premenopausal women. It also remains to be determined if alterations in liver metabolism alters the impact of sex steroids on CVD metabolism via the increased SHBG production.

We observed no significant associations of the ERLL assay. Potentially, estrogen receptor binding is a minor mechanism in the lipid synthesis and degradation system relative to other relevant receptors such as the ApoB receptor.

Strengths of this report include infrequently characterized lipid measures (oxidized LDL-c and the ApoB/ApoA-1 ratio) for a more complete revelation as to how hormone measures relate to CVD measures in HT uses and non-users. An expanded battery of sex steroid markers,

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especially the estrone metabolites, was also associated with CVD markers. Women were all pre-or early perimenopausal at study inception so that transitions to postmenopause occurred within the five years of study observation and all study enrollees were free of hormone use at study inception so that hormone therapy was initiated early in the menopausal transition and women with heart disease at baseline were excluded from analysis. Data also reflect relationships observed prior to the release of WHI findings. However, there are limitations. While we evaluated two specific formulations (CEE and CEE + progestin) and duration of use, we did not specifically address dose. This cross-sectional evaluation precludes experimentally-assigned HT use. Further, SWAN includes measures of cardiovascular intermediates but not hard endpoints.

Collectively, our findings demonstrate positive and negative aspects of CEE product use relative to cardiovascular intermediate endpoints observed in women during the menopause transition. The evidence suggests that CEE product use may provide positive effects via some, but not all, lipids; however, there were less favorable aspects of CEE product use including higher estrone metabolite and SHBG levels and increased propensity for greater oxidative stress, thrombotic activity, and inflammation even in mid-aged women free of heart disease. From a research perspective, this study indicates that there should be a much closer examination of liver metabolism of SHBG and the estrone metabolites during CEE use in relation to potential CVD risk. From a practice perspective, these results suggest very thoughtful consideration in the clinical use of HT as there was evidence of adverse HT effects even in women free of atherosclerosis who were evaluated in a time frame that was well within the approximate 6-year time period proposed with the "timing hypothesis".

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This report is based on samples from the SWAN Core Repository. If scientists are interested in developing studies based on this resource, a description of the SWAN Core and DNA Repositories and how to obtain access to the resources can be found at www.swanrepository.org.

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Table 1

Age, BMI, and hormones values according to menopause status or hormone therapy group, SWAN Follow-up 05*

	Premenopause	Conjugated equine hormone therapy (HT)estrogen		Postmenopause
		without progestin	with progestin	
Number	98	53	243	370
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
Age (y)	48.9 (0.3)	**52.1 (0.3)** †	**51.9 (0.2)**†	**52.6 (0.2)** †‡§
Body mass index (BMI, kg/m^2)	27.2 (0.6)	27.2 (0.8)	26.5 (0.4)	27.0 (0.3)
Waist circumference (cm)	83.8 (0.6)	83.9 (0.8)	84.8 (0.4)	84.9 (0.3)
Estradiol (E2, pg/mL)	**40.8 (4.3)**//	**34.5 (4.2)**//	**41.5 (2.5)**//	14.8 (0.7)
Estrogen receptor ligand load (ERLL, pg/mL)	37.2 (5.5)	48.0 (8.4)	33.6 (2.7)	38.6 (2.7)
2-hydroxyestrone (2-OHE1, pg/mL)	**9.57 (1.09)**//	**23.0 (3.12)**†	**18.8 (1.25)**†//	5.64 (0.30)
16α-hydroxyestrone (16α-OHE1, pg/mL)	**6.3 (0.6)**//	**11.4 (1.3)**†//	**10.0 (0.6)**†//	4.4 (0.20)
Testosterone (T, ng/dL)	29.8 (1.8)	26.0 (1.8)	29.8 (1.0)	**34.6 (1.0)** ‡§
E2/Testosterone ratio	**14.1 (1.6)**//	**13.8 (1.8)**	**14.5 (0.9)**//	4.7 (0.2)
Sex hormone binding globulin (SHBG, nM)	42.8 (3.2)	**61.4 (4.4)**†	**69.9 (2.4)** †	42.0 (1.5)
Free estradiol index (FEI, E2/SHBG)	**0.36 (.04)**§//	**0.24 (.03)**	**0.23 (.02)**//	0.14 (.007)
Free androgen index (FAI, T/SHBG)	**2.55 (0.24)**‡§	1.73 (0.19)	1.58 (0.09)	**3.07 (0.13)** ‡§
Current smoker (N, %)	8 (8%)	4 (7.5%)	25 (10%)	56 (15%)

* Data adjusted for age, site, smoking behavior, body size, and race/ethnicity and back-transformed.

Group-wise comparisons, $p \leq 0.01$

† > Premenopause

‡ > HT (conjugated equine estrogen)

§ > HT (conjugated equine estrogen + progestin)

// > Postmenopause

** Statistically significant data are bolded

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Table 2

A comparison of cardiovascular intermediate markers according to menopause state or conjugated equine estrogen (CEE)group, adjusted for age, site, smoking behavior, BMI, and race/ethnicity *

	Premenopause	Conjugated equine estrogen (CEE) hormone therapy (HT)		Postmenopause
		without progestin	with progestin	
Number	98	53	243	370
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
Oxidized low density lipoprotein (oxLDL-c, mg/dL)	39.1 (1.7)	38.0 (2.1)	39.3 (1.2)	38.6 (1.0)
Low density lipoprotein (LDL-c, mg/dL)	108.8 (3.8)	105.1 (4.4)	106.7 (2.2)	**121.8 (2.0)** †‡§
High density lipoprotein (HDL-c, mg/dL)	55.6 (1.5)	**64.3 (2.1)** †	**62.6 (1.0)** †	**60.7 (0.8)** †
HDL/LDL ratio	0.51 (.02)	**0.62 (.03)**//	**0.59 (.02)** †//	0.50 (.01)
Apolipoprotein B (ApoB, mg/dL)	103.8 (3.2)	110.2 (4.0)	108.4 (1.9)	112.0 (1.6)
Apolipoprotein A-1 (ApoA-1, mg/dL)	157 (3.1)	**181 (4.3)**†//	**180 (2.1)** †//	**167 (1.6)** †
ApoB/ApoA-1 ratio	0.70 (.03)	0.64 (.03)	0.63 (.02)	**0.70 (.01)** §
Triglycerides (mg/dL)	98 (5.2)	**122 (7.6)**†//	**122 (3.8)** †//	102 (2.5)
Lipoprotein a-l (Lp (a)-1, mg/dL)	50.0 (1.7)	**59.4 (2.4)**†//	**55.5 (1.1)** †//	51.1 (0.8)
F_{2a}-isoprostanes (pg/ml)	423 (43)	403 (49)	499 (30)	434 (21)
C-reactive protein (mg/L)	**2.0 (0.24)**//	**2.3 (0.33)**	**2.7 (0.19)**//	1.3 (0.07)
Plasminogen activator inhibitor-1 (PAI-1, mg/dL)	**16.6 (1.8)** ‡§	10.0 (1.3)	9.6 (0.6)	**14.4 (0.7)** ‡§

* Data adjusted for age, site, smoking behavior, body size, and race/ethnicity and back-transformed.

Group-wise comparisons, $p \leq 0.01$:

† > Premenopause

‡ > HT (conjugated equine estrogen)

§ > HT (conjugated equine estrogen + progestin)

// > Postmenopause

** Statistically significant data are bolded

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Table 3

Partial correlations (95% CI)* of cardiovascular measures with serum sex hormone binding globulin ($_{sqrt}$SHBG, nM)

		Conjugated equine estrogen (CEE)		
	Premenopause	**without progestin**	**with progestin**	**Postmenopause**
	ρY|X (95% CI)	**ρY|X (95% CI)**	**ρY|X (95% CI)**	**ρY|X (95% CI)**
$_{log}$HDL-c (mg/dL)	.19 (−.04, .41)	**.36 (.04, .61)**	.10 (−.03, .24)	**.24 (.13, .34)**
$_{log}$LDL-c (mg/dL)	**−.33 (−.53, −.11)**	−.17 (−.47, .16)	**−.15 (−.29, −.01)**	−.07 (−.18, .04)
oxLDL-c (mg/dL)	**−.38 (−.58, −.14)**	−.18 (−.50, .17)	−.02 (−.16, .13)	−.10 (−.21, .02)
$_{log}$ ApoA-1 (mg/dL)	.13 (−.11, .36)	**.37 (.05, .62)**	**.18 (.04, .31)**	**.18 (.07, .29)**
$_{log}$ApoB (mg/dL)	**−.36 (−.55, −.13)**	−.03 (−.35, .30)	−.06 (−.20, .08)	**−.12 (−.23, −.01)**
$_{log}$Triglycerides (mg/dL)	**−.30 (−.50, −.07)**	−.07 (−.39, .25)	.01 (−.13, .15)	**−.24 (−.34, −.13)**
$_{log}$C-reactive protein (mg/L)	**−.26 (−.47, −.01)**	.24 (−.08, .53)	**.17 (.03, .31)**	**−.21 (−.32, −.10)**
$_{log}$ PAI-1 (mg/dL)	**−.44 (−.61, −.22)**	**−.52 (−.73, −.22)**	**−.48 (−.58, −.36)**	**−.49 (−.57, −.40)**

* Adjusted for age, site, smoking behavior, body size, and race/ethnicity

** Statistically significant data are bolded