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Differential Effects of Oral Versus Transdermal Estrogen Replacement Therapy on C-Reactive Protein in Postmenopausal Women

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OBJECTIVES We investigated whether the route of estrogen replacement therapy (ET) is the major determinant of C-reactive protein (CRP) in postmenopausal women.

BACKGROUND Recent studies demonstrated that oral ET causes a sustained increase in CRP, implicating a proinflammatory effect. Because CRP is synthesized in the liver, we hypothesized that estrogen-induced CRP elevation is related to first-pass hepatic metabolism.

METHODS In 21 postmenopausal women, we conducted a randomized, crossover, placebo-controlled study to compare the effects of transdermal versus oral ET on CRP and inflammatory cytokines. We measured CRP, interleukin (IL)-1-beta, IL-6, and tumor necrosis factor-alpha before and after eight weeks of transdermal estradiol (E_2) (100 μg/day), oral conjugated estrogen (CEE) (0.625 mg/day), or placebo. Insulin-like growth factor-1 (IGF-1), a hepatic-derived anabolic peptide, was also measured.

RESULTS Transdermal E_2 had no effect on CRP or IGF-1 levels. In contrast, eight weeks of oral conjugated estrogens caused a more than twofold increase in CRP and a significant reduction in IGF-1 ($p < 0.01$) in the same women. The magnitude of increase in CRP was inversely correlated to the decrease in IGF-1 ($r = -0.49$, $p = 0.008$). Neither transdermal E_2 nor oral CEE had any effects on the plasma concentrations of cytokines that promote CRP synthesis.

CONCLUSIONS In postmenopausal women, oral but not transdermal ET increased CRP by a first-pass hepatic effect. An increase in CRP levels is accompanied by a reduction in IGF-1, an anti-inflammatory growth factor. Because CRP is a powerful predictor of an adverse prognosis in otherwise healthy postmenopausal women, the route of administration may be an important consideration in minimizing the adverse effects of ET on cardiovascular outcomes. (J Am Coll Cardiol 2003;41:1358–63) © 2003 by the American College of Cardiology Foundation

The role of inflammation in the pathogenesis of cardiovascular disease has been well established. Among markers of systemic inflammation, C-reactive protein (CRP) is the strongest independent predictor of myocardial infarction and cardiovascular mortality in apparently healthy women (1). A recent randomized trial and cross-sectional study indicated that oral estrogen replacement therapy (ET) and combined hormone replacement therapy (HT) caused a sustained increase in CRP in postmenopausal women (2,3), which may explain the increased risk of cardiovascular events (4,5). In contrast, animal studies failed to demonstrate such proinflammatory effects of estrogen given by subcutaneous implantation or injection (6,7). Because CRP is produced mainly in the liver, we hypothesized that the increase in CRP after oral estrogen is due to exposure of the liver to a high concentration of estrogen following gastrointestinal absorption. However, a comparison of the effects of oral ET versus transdermal ET, which avoided first-pass hepatic metabolism, on CRP and inflammatory cytokines has not been performed in postmenopausal women.

Accordingly, the major aim of this study was to test whether transdermal ET obviates the increase in CRP produced by oral ET. In 21 postmenopausal women, we performed a head-to-head comparison of the effects of transdermal versus oral ET on plasma levels of CRP and proinflammatory cytokines, using a randomized, crossover design. To further determine whether changes in CRP during oral ET are related to first-pass hepatic metabolism, the serum levels of another peptide produced by the liver, insulin-like growth factor-1 (IGF-1), were also measured in the same women.

METHODS

The study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas. After informed, written consent was obtained, 21 postmenopausal women (11 normotensive and 10 hyperten-

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Abbreviations and Acronyms

ET	= estrogen replacement therapy
CEE	= conjugated equine estrogen
HT	= combined hormone replacement therapy
CRP	= C-reactive protein
E_2	= estradiol
hs-CRP	= highly sensitive C-reactive protein
IGF-1	= insulin-like growth factor-1
IL	= interleukin
TNF-alpha	= tumor necrosis factor-alpha

sive) participated in a randomized, double-blinded, crossover trial. The rational for using a crossover design is that the effect of multiple treatments can be assessed in a small group of subjects, with the same power as that of a larger parallel study. Generally, the total number of subjects needed for a parallel trial is *four times larger* than the total number of subjects needed for a two-period crossover trial (8). Thus, a two-group parallel trial that requires total of 80 subjects (40 per group) would only need 20 subjects in a two-period crossover design. In this regard, our three-period crossover study of 21 subjects has the same power as a parallel trial of 42 subjects per each treatment group or a total of >120 subjects.

The clinical characteristics of normotensive and hypertensive women are shown in Table 1. As expected, more hypertensive women used diuretics than did normotensive women ($p = 0.04$). There were no statistically significant differences in the proportion of women using an angiotensin-converting enzyme inhibitor, angiotensin-receptor blocker, or calcium channel blocker between the two groups ($p > 0.05$). The duration of menopause tended to be longer in hypertensive than in normotensive women, but the difference did not reach statistical significant ($p = 0.08$). None of subjects used aspirin or vitamin E. All of the women had no history of any cardiovascular disease, were at least one year from their last menstrual period, had a serum estradiol (E_2) concentration <40 pg/ml before study enrollment, and had received no hormonal therapy for at least four weeks before the study. This duration was determined to be adequate to allow inflammatory markers to return to baseline in our pilot study.

All postmenopausal women received the following three regimens in random order: 1) transdermal E_2 (Climara; Berlex Laboratories, Wayne, New Jersey) at 100 μg/day plus oral placebo for eight weeks; 2) oral conjugated equine estrogen (CEE) (Premarin; Wyeth-Ayerst, Madison, New Jersey) at 0.625 mg plus a placebo patch for eight weeks; and 3) a placebo patch plus oral placebo for eight weeks. For blinding purpose, the placebo patch (Berlex Laboratories) and oral placebo with an identical appearance to the active patch and pills were used in the study. Each subject was instructed to take the medication at the same time of the day.

Laboratory assays. Serum and plasma samples were obtained at baseline and after each treatment period, at the same time of the day, and kept frozen at −70°C until analysis. The estradiol-17-beta level was measured using iodine-125-labeled radioimmunoassay kits (Mayo Clinic, Rochester, Minnesota). The IGF-1 levels were measured by radioimmunoassay after acid-ethanol extraction (Nichols Institute Diagnostics, San Juan Capistrano, California). Serum levels of CRP were measured with a highly sensitive latex-enhanced immunonephelometric assay (Dade Behring, Deerfield, Illinois). Both inter-assay and intra-assay coefficients of variation were <5%. Plasma interleukin (IL)-1-beta, IL-6, and tumor necrosis factor-alpha (TNF-alpha) were each processed as one batch and assayed in triplicate, using highly sensitive ELISA kits (Human Quantikine HS IL-1-beta, IL-6, and TNF immunoassays; R&D Systems, Minneapolis, Minnesota). The intra-assay coefficient of variation was <5%.

Statistical analysis. Repeated measures analysis of variance (ANOVA) was used to assess the differences between

Table 1. Baseline Characteristics of Postmenopausal Women Participating in the Study

	Normotensive Women (n = 11)	Hypertensive Women (n = 10)	p Value
Age (yrs)	53 ± 1	60 ± 3	0.10
Body weight (kg)	69.3 ± 2.8	71.9 ± 4.8	0.65
Body mass index (kg/m^2)	26.0 ± 1.2	27.7 ± 1.5	0.37
Fasting plasma glucose (mg/dl)	78 ± 6	84 ± 5	0.46
Years after menopause	7.0 ± 1.4	13.7 ± 3.2	0.08
LDL cholesterol (mg/dl)	124 ± 10	115 ± 14	0.63
HDL cholesterol (mg/dl)	44 ± 3	50 ± 7	0.46
Total cholesterol to HDL cholesterol ratio	4.7 ± 0.3	3.7 ± 0.6	0.17
Triglycerides (mg/dl)	165 ± 30	100 ± 14	0.10
Concomitant medications			
Statin	1 (9%)	2 (20%)	0.59
ACE inhibitor	0	2 (20%)	0.21
Angiotensin receptor blocker	0	1 (10%)	0.48
Diuretics	0	4 (40%)	0.04
Calcium channel blocker	0	3 (30%)	0.09

Data are presented as the mean value ± SEM or number (%) of subjects.
ACE = angiotensin-converting enzyme; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

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Table 2. Effects of Oral Versus Transdermal Estrogen Administration on C-Reactive Protein and Inflammatory Cytokines

	Baseline	Oral CEE	Placebo	Transdermal Estradiol	p Value* by ANOVA
Estradiol (pg/ml)	35 ± 4	172 ± 45†‡	31 ± 4	168 ± 54†‡	< 0.01
IGF-1 (ng/ml)	130 ± 11	103 ± 9†‡§	128 ± 11	122 ± 9	< 0.01
hs-CRP (μg/ml)	1.50 (0.80–4.50)	3.70†‡§ (1.55–6.20)	1.15 (0.73–3.70)	1.40 (0.7–4.00)	< 0.001
IL-1-beta (pg/ml)	0.32 (0.23–0.43)	0.26 (0.19–0.33)	0.26 (0.18–0.71)	0.19 (0.08–0.39)	NS
IL-6 (pg/ml)	1.48 (0.93–2.29)	1.19 (0.84–2.54)	1.42 (1.15–1.65)	1.27 (0.97–2.03)	NS
TNF-alpha (pg/ml)	1.36 (0.65–2.31)	1.88 (0.66–2.64)	1.43 (0.81–2.39)	1.00 (0.74–2.36)	NS

*For comparison between baseline and three treatment groups. †$p < 0.01$ vs. baseline. ‡ $p < 0.01$ vs. placebo. §$p < 0.01$ vs. transdermal estrogen. Data are presented as the mean value ± SEM or median value (interquartile range).

ANOVA = analysis of variance; CEE = conjugated equine estrogen; hs-CRP = highly sensitive C-reactive protein; IGF-1 = insulin-like growth factor-1; IL = interleukin; NS = not significant; TNF = tumor necrosis factor.

baseline, transdermal E_2, placebo, and oral estrogen. Contrasts from these models were used for pair-wise comparisons. The CRP, IL-1-beta, IL-6, and TNF-alpha data were analyzed after a natural logarithmic transformation. Carryover effects were also tested using mixed linear models, including treatment period, and carryover factors. Various models were examined, and the carryover effect was highly nonsignificant ($p > 0.80$) in all models for log(e) CRP. The 0.05 level of significance was used for ANOVA, and the 0.01 level of significance was used for pair-wise tests to adjust for multiple testing. Comparisons of continuous variables between normotensive and hypertensive women and between large and small responders were performed with the unpaired *t* test at the 0.05 level of significance. Comparisons of categorical variables between normotensive and hypertensive women and between large and small responders were performed with the Fisher exact test at the 0.05 level of significance. The Pearson correlation coefficient was used to assess the association between IGF-1 and CRP levels. C-reactive protein, IL-1-beta, IL-6, and TNF-alpha are expressed as the median value and interquartile range. Estradiol and IGF-1 are expressed as the mean value ± SEM. Statistical analysis was performed with SAS version 8.0 (SAS Institute Inc., Cary, North Carolina).

RESULTS

As expected, oral CEE cause more than twofold increase in highly sensitive (hs)-CRP ($p < 0.01$ vs. baseline and placebo). However, in the same women, transdermal E_2 had no effect on hs-CRP, despite a similar increase in serum E_2 levels (Table 2, Fig. 1). When the analysis was limited to the effect of the first treatment each woman received, the CRP levels were still increased significantly in subjects who received oral CEE first (from 1.5 ± 0.5 vs. 3.2 ± 1.0 μg/ml, $p = 0.03$), but not in those who received transdermal E_2 first (from 2.45 ± 0.87 to 2.88 ± 0.89 μg/ml, p = NS) or placebo first (from 1.60 ± 1.30 to 1.60 ± 1.00 μg/ml, p = NS). This increase in hs-CRP after oral CEE was accompanied by a significant decrease in IGF-1 levels (from 130 ± 11 to 103 ± 9 ng/ml; $p < 0.01$ vs. baseline) (Table 1). Transdermal E_2 had no effect on IGF-1. Changes in IGF-1 levels from baseline were inversely correlated to changes in hs-CRP from baseline, regardless of the treatment each woman received ($r = -0.49$, $p = 0.008$) (Fig. 2). Neither transdermal E_2 nor oral CEE had any effect on plasma IL-1-beta, IL-6, or TNF-alpha concentrations (Table 2).

Compared with baseline, there were no significant changes in the ratio of total cholesterol to high-density lipoprotein cholesterol after eight weeks of transdermal E_2, placebo, or oral CEE (4.3 ± 0.3 vs. 4.5 ± 0.3, 4.6 ± 0.3, and 4.0 ± 0.3, respectively). Women who developed large increases in CRP after oral CEE (arbitrarily defined as an increase in CRP above the median level of 224% from baseline) did not significantly differ from those who developed small (≤224%) increases in CRP, in terms of age, body mass index, plasma glucose, duration of menopause, cigarette or alcohol use, previous hormone use, or concomitant use of other medications that may influence CRP levels (Table 3).

A subset analysis of nine of 21 postmenopausal subjects who had never received ET or HT at the time of enrollment indicated that the average baseline CRP levels were similar to those after eight weeks of placebo (1.6 ± 0.5 vs. 1.7 ± 0.6 μg/ml, respectively), suggesting that the carryover effect, if any, was minimal or nonexistent with this duration of treatment.

DISCUSSION

Many previous studies have shown that estrogen increases CRP (2,3,9), a proinflammatory marker that may contribute to the increased risk of adverse cardiovascular events in postmenopausal women receiving HT in large clinical trials (4,5). In contrast, the major new finding from our study is that an increase in CRP is *not* an obligatory property of estrogen, but rather is directly related to first-pass hepatic metabolism, and thus may be avoided by a transdermal route of administration.

There is a paucity of direct experimental support for a proinflammatory effect of estrogen in animals or humans. A large body of published data indicates that, if anything, estrogen exerts anti-inflammatory action. In female rats, a subcutaneous injection of estrogen reduced serum CRP levels (7). In male rabbit recipients of a cardiac allograft, subcutaneous estrogen implantation reduced infiltration of macrophage and T lymphocytes in the transplanted coronary vessel and attenuated coronary allograft atherosclerosis

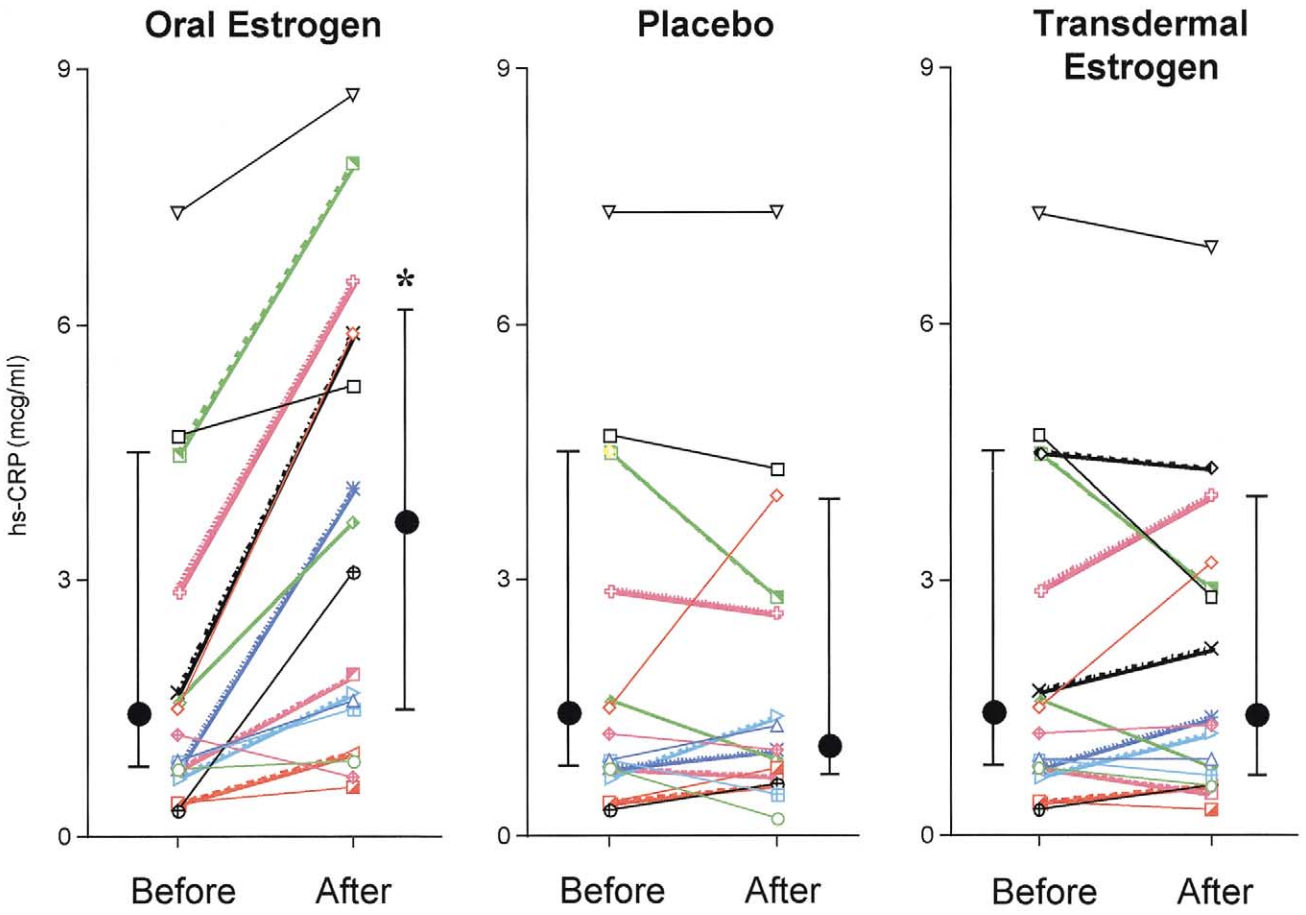

Figure 1. Individual and summary data of all subjects showing C-reactive protein (CRP) levels before and after oral estrogen **(left panel)**, placebo **(middle panel)**, and transdermal estrogen **(right panel)**. Transdermal estrogen had no effect on CRP. Oral estrogen administration for eight weeks evoked a more than twofold increase in CRP (*$p < 0.01$ vs. baseline, placebo, and transdermal estrogen).

(6). In young transsexual men (10), transdermal E_2 had no effect on CRP, and it had either no effect (11) or decreased CRP in postmenopausal women (12). However, in these human studies, concomitant use of oral progestins, which have been recently shown to attenuate the increase in CRP during oral estrogen administration (13,14), may be responsible for the failure of transdermal ET to increase CRP.

By using *unopposed* estrogen, we were able to provide clear-cut evidence that the route of administration and first-pass hepatic metabolism are the major determinants of CRP. Our ability to show that oral CEE at 0.625 mg/day (equivalent to transdermal E_2 at 50 μg/day) caused a robust increase in CRP, whereas transdermal E_2 at the twice larger dose of 100 μg/day had no effect on CRP in the same women, further confirming the hypothesis. Because only oral CEE was tested as oral ET in our study, we consider the possibility that CRP may not increase with other oral preparations. However, this is unlikely because oral estradiol-17-beta and E_2 valerate have also been shown to increase CRP levels in postmenopausal women to a similar extent (9,14). We also consider the possibility that oral estrogen acts at the extrahepatic sites, such as monocytes, T lymphocytes, and adipocytes, to stimulate release of proinflammatory cytokines, which promote hepatic synthesis of CRP. Because a recent observational study (15) and our randomized study demonstrate that levels of these inflammatory cytokines were unaffected by oral estrogen, we believe that oral estrogen acts directly in the liver to increase CRP.

Indeed, our hypothesis is supported by the concomitant reduction in IGF-1, a polypeptide primarily produced by the liver under the control of growth hormone. Previous studies have indicated that oral ET decrease IGF-1 levels through a first-pass hepatic effect, resulting in reduced feedback inhibition and elevated levels of growth hormone in postmenopausal women (16). These effects were not seen with transdermal ET (16). The linear correlation between the increase in CRP and the reduction in IGF-1 from baseline in our study provides further support for a common mechanism linked to first-pass hepatic metabolism, governing responses of these two proteins.

Mechanisms by which first-pass hepatic metabolism of oral ET increases CRP are also unknown. It is unlikely to be direct hepatic stimulation of CRP synthesis, because even supraphysiologic levels of estrogen had no effect on hepatic CRP messenger ribonucleic acid expression in female rats

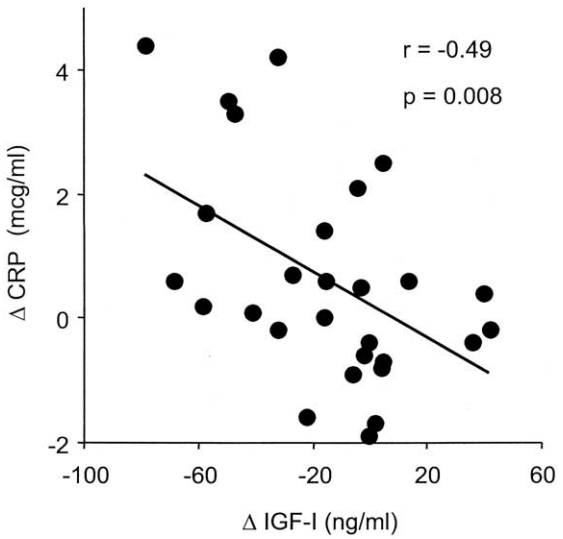

Figure 2. Scatter plots showing the inverse correlation between changes in serum insulin-like growth factor-1 (IGF-1) from baseline and changes in C-reactive protein (CRP) from baseline, regardless of the treatment each woman received.

(7). Alternatively, an estrogen-induced reduction in IGF-1, a peptide recently shown to have an anti-inflammatory property (17,18), may trigger a secondary increase in CRP production. Levels of CRP have been shown to be elevated in patients with hypopituitarism (19), and adult-onset growth hormone deficiency and growth hormone replacement in these patients restored IGF-1 and reduced CRP levels (17). In contrasts, CRP levels are reduced in patients with acromegaly, and the growth hormone receptor antagonist decreased IGF-1 and increased CRP (20). Furthermore, in children with severe burns, administration of exogenous IGF-1 attenuated overproduction of CRP (18).

Regardless of the mechanism by which oral ET increases CRP, this observation may have important clinical implications. C-reactive protein is not simply a strong independent predictor of future cardiovascular events without a specific pathogenetic role. It has also recently been shown to decrease the expression of endothelial nitric oxide synthase enzyme and production of the potent vasodilator nitric oxide in human endothelial cell cultures (21,22). In addition, CRP has been shown to exert a direct proatherogenic effect, promoting monocyte chemotaxis (23) and tissue factor expression in monocytes, as well as endothelial shedding of adhesion molecules in an ex vivo cellular model (24). Whether CRP elevation during oral CEE directly promotes atherosclerosis in postmenopausal women remains to be established. Because a recent prospective, observational study indicated that CRP is an independent predictor of future cardiovascular events, even in healthy women who used hormone replacement therapy (25), the elevation in CRP induced by oral CEE is likely to have clinical significance. Similarly, the lack of positive findings and the presence of cardiovascular harm observed in several recent multicenter trials of ET or HT on cardiovascular outcomes or coronary artery dimensions may be partly due to the use of an oral rather than transdermal route of administration (4,26). Large prospective studies are needed to determine the role of transdermal ET on coronary heart disease or atherosclerosis prevention after menopause.

Table 3. Characteristics of Women With Large Versus Small Increases in C-Reactive Protein After Oral Conjugated Equine Estrogen

	Large Responders (n = 9)	Small Responders (n = 12)	p Value
Age (yrs)	58 ± 3	55 ± 2	0.41
Body weight (kg)	67.1 ± 2.7	73.2 ± 4.1	0.23
Body mass index (kg/m^2)	25.9 ± 0.8	27.4 ± 1.5	0.40
Fasting plasma glucose (mg/dl)	78 ± 6	82 ± 6.3	0.79
Years after menopause	7.8 ± 1.5	13.3 ± 3.5	0.17
LDL cholesterol (mg/dl)	122 ± 9	117 ± 14	0.78
HDL cholesterol (mg/dl)	47 ± 4	48 ± 5.2	0.99
Total cholesterol to HDL cholesterol ratio	4.7 ± 0.3	4.5 ± 0.5	0.35
Triglycerides (mg/dl)	153 ± 31	120 ± 28	0.43
Estradiol levels during oral CEE (pg/ml)	125 ± 26	183 ± 55	0.36
Cigarette use	0	4 (33%)	0.10
Alcohol use	3 (33%)	5 (42%)	1.00
Previous hormone use	4 (44%)	8 (67%)	0.40
History of hypertension	3 (33%)	5 (42%)	1.00
Concomitant medications			
Statin	2 (22%)	1 (8%)	0.55
ACE inhibitor	1 (11%)	1 (8%)	1.00
Angiotensin receptor blocker	0	1 (8%)	1.00
Diuretics	2 (22%)	2 (17%)	1.00
Calcium channel blocker	1 (11%)	2 (17%)	1.00

Data are presented as the mean value ± SEM or number (%) of subjects.
Abbreviations as in Tables 1 and 2.

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