A Comparison of the Short-Term Effects of Oral Conjugated Equine Estrogens *Versus* Transdermal Estradiol on C-Reactive Protein, Other Serum Markers of Inflammation, and Other Hepatic Proteins in Naturally Menopausal Women

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Objective: Our objective was to compare the effects of oral *vs*. transdermal estrogen therapy on C-reactive protein (CRP), IL-6, E- and P-selectin, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule-1, serum amyloid A, transferrin, prealbumin, IGF-I, SHBG, thyroxine-binding globulin (TBG), and cortisol-binding globulin (CBG) in naturally menopausal women.

Design: This was a randomized, open-label crossover clinical trial. A 6-wk withdrawal from prior hormone therapy (baseline) was followed in randomized order by 12-wk oral conjugated equine estrogens (CEEs) (0.625 mg/d) and 12-wk transdermal estradiol (E2) (0.05 mg/d), with oral micronized progesterone (100 mg/d) given continuously during both regimens.

Results: A total of 27 women enrolled, and 25 completed both treatment periods. Nine parameters changed significantly during oral CEE (median percent change from baseline; *P* value): CRP (192%; $P<0.001$); E-selectin (−16.3%; $P = 0.003$); P-selectin (−15.3%; $P = 0.012$); ICAM-1 (−5%; $P = 0.015$); transferrin (5.3%; $P = 0.024$); IGF-I (−30.5%; $P < 0.001$); SHBG (113%; $P < 0.001$); TBG (38%; $P < 0.001$); and CBG (20%; $P < 0.001$). With transdermal E2, only three parameters changed significantly and to a lesser degree: ICAM-1 (−2.1%; $P = 0.04$); IGF-I (−12.5%; $P < 0.001$); and SHBG (2.6%; $P = 0.042$). During oral CEE the intrasubject changes in CRP correlated strongly with the changes in serum amyloid A ($r = 0.805$; $P < 0.001$), and were only weakly associated with the changes in SHBG ($r = 0.248$; nonsignificant), TBG (0.430; $P = 0.031$), and CBG ($r = 0.072$; nonsignificant). The log-log relationship between CRP and IL-6 observed at baseline showed a parallel shift during oral CEE, suggesting an amplified hepatic response or a greater sensitivity to IL-6 stimulation.

Conclusion: Compared with oral CEE, transdermal E2 exerts minimal effects on CRP and the other inflammation and hepatic parameters. **(*J Clin Endocrinol Metab* 93: 1702–1710, 2008)**

C-reactive protein (CRP), an acute phase reactant synthesized in the liver, as well as other inflammation factors in serum have been proposed as predictive cardiovascular risk markers in men and women (1, 2). Oral but not transdermal estrogen therapy (ET) has increased the level of CRP and altered certain other cardiovascular risk markers in postmenopausal women (3–6). Although plausible, a causal relationship between the changes in these cardiovascular risk markers and the cardiovascular out-

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Abbreviations: BMI, Body mass index; CAD, coronary artery disease; CBG, cortisol-binding globulin; CEE, conjugated equine estrogen; CRP, C-reactive protein; E2, estradiol; ET, estrogen therapy; HT, hormone therapy; ICAM, intercellular adhesion molecule; NS, nonsignificant; SAA, serum amyloid A; TBG, thyroxine-binding globulin; VCAM, vascular cell adhesion molecule.

comes associated with oral ET in the Women's Health Initiative clinical trials (7–9) has not been established and remains controversial. Moreover, the mechanism by which oral ET increases CRP levels and its relationship to changes in other inflammatory markers and other hepatic proteins are presently not well understood (3, 5, 6).

In a recently conducted, randomized crossover study in 27 naturally menopausal women, we compared the differential effects of oral *vs.* transdermal ET on the serum concentrations of SHBG, thyroxine-binding globulin (TBG), and cortisol-binding globulin (CBG) (glycoproteins synthesized and secreted by the liver), and the downstream consequences of these effects on androgen levels, thyroid parameters, and adrenal hormones (10). In conjunction with this study, we also investigated and report here a comparison of the effects of oral *vs.* transdermal ET on CRP, other inflammation markers, and other hepatic proteins. The inflammation markers included the cytokine IL-6, the endothelial and platelet-derived surface adhesion molecules E- and P-selectin, the intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, and serum amyloid A (SAA), another acute phase reactant. The hepatic proteins included the iron carrier protein transferrin, the secondary thyroxine carrier prealbumin (transthyretin), IGF-I, as well as the previously studied hormone-binding globulins. By analyzing the changes and interrelationships among these parameters, our study offers some new insights into the mechanisms by which oral ET modulates the levels of CRP, and the other inflammation markers and hepatic proteins. The possible relevance of these changes to the cardiovascular risk associated with oral *vs.* transdermal ET is also discussed.

Subjects and Methods

Experimental subjects

The study was approved by the Partners Research Committee of Massachusetts General Hospital (Boston, MA). All women provided informed written consent. Eligible subjects were healthy, naturally menopausal women, aged 42–70 yr, who were currently using combination estrogen-progestin hormone therapy (HT). Women with contraindications to HT use were excluded, including those with unexplained vaginal bleeding, liver disease, a history of breast or endometrial cancer, or venous thromboembolic events. Women with known thyroid or adrenal disease also were excluded.

Study design

The study was a randomized, open-label, crossover design. After a 6-wk withdrawal period from prior HT, baseline measurements of CRP and the other parameters were performed. Subjects were then randomized to 12-wk treatment with oral conjugated equine estrogen (CEE) or transdermal estradiol (E2). At the end of treatment, CRP and the other parameters were measured a second time and subjects crossed over to 12 wk of the other treatment, at the end of which the measurements were performed a final time. Oral micronized progesterone was continuously administered throughout both ET treatments for endometrial protection. There was no washout period between the two ET periods. With very few exceptions, the serum samples were obtained between 0800 and 1100 h to minimize the effects of circadian variation. The use of concomitant medications, which could have influenced the main outcome parameters, did not change during the study.

Study drugs

During oral ET treatment, subjects ingested a 0.625 mg tablet of CEE (Premarin; Wyeth Pharmaceuticals Inc., Philadelphia, PA) daily for 12 wk. During transdermal ET treatment, subjects applied a 0.05 mg/d transdermal E2 matrix patch (Alora; Watson Pharmaceuticals, Inc., Corona, CA) twice weekly for 12 wk. During both ET treatments, subjects ingested a 100-mg capsule of oral micronized progesterone (Prometrium; Solvay Pharmaceuticals Inc., Marietta, GA) each night.

Measurement of CRP, other inflammation markers, and other hepatic proteins

The serum concentrations of CRP, IL-6, E- and P-selectin, ICAM-1 and VCAM-1, SAA, transferrin, prealbumin, IGF-I, and the hormone-binding globulins SHBG, TBG, and CBG were measured at baseline and at the end of each treatment period. As described previously the serum concentrations of SHBG, TBG, and CBG were measured by Esoterix Endocrinology (Calabasas Hills, CA) (10). The remaining parameters were measured in the Pathology Department of the Children's Hospital Medical Center (Boston MA) using highly sensitive and specific immunoassays described previously (11–13). Details on the assay methods, the limits of quantitation, and interassay coefficients of variation for each analyte are given in Table 1. Reference ranges for each analyte are given in Tables 2 and 3.

Statistical analyses

The sample size (n = 27) was based on power calculations corresponding to the expected changes in free testosterone concentration, the

TABLE 1. Type and performance specifications of assays used in the present study

Analyte (U)	Type of assay (manufacturer)	LOQ	Interassay CV (%)
CRP (mg/liter)	Ultrasensitive immunonephelometric assay (Dade Behring, Newark, DE)	0.15	<5.6
IL-6 (pg/ml)	Ultrasensitive ELISA (R&D Systems, Minneapolis, MN)	0.094	<12.2
E-selectin (ng/ml)	ELISA (R&D Systems)	2	<8.8
P-selectin (ng/ml)	ELISA (R&D Systems)	10	<9.9
ICAM-1 (pg/ml)	ELISA (R&D Systems)	40	<8.9
VCAM-1 (ng/ml)	ELISA (R&D Systems)	70	<6.1
SAA (mg/dl)	Immunonephelometric assay (Dade Behring)	0.08	<7.0
Transferrin (mg/dl)	Immunoturbidometric assay (Roche Diagnostics, Indianapolis, IN)	10	<2.4
Prealbumin (mg/dl)	Immunoturbidometric assay (Kamiya Biomedical Reagents, Seattle, WA)	1.5	<1.4
IGF-I (ng/ml)	ELISA (Diagnostic Systems Laboratories, Webster, TX)	0.3	<8.3
SHBG (nmol/liter)	IRMA (Esoterix)	10	<8.2
TBG (mg/dl)	RIA (Esoterix)	0.2	<10.8
CBG (mg/dl)	RIA (Esoterix)	0.5	<11

CV, Coefficient of variation; IRMA, immunoradiometric assay; LOQ, limit of quantitation.

TABLE 2. Inflammation markers

Analyte (U)	Reference range[a]	BL	Oral CEE % change from BL *P* (CEE *vs.* BL)	TD E2 % change from BL *P* (TD E2 *vs.* BL)	*P* (CEE *vs.* TD E2)
CRP (mg/liter)	0.6–3.5	1.2; 2.3 (3.3)	2.3; 3.8 (4.3) 192.0; 243.7 (269.1) % <0.001 (LT)	1.2; 2.3 (3.1) 13.7; 40.9 (120.3) % NS (LT)	<0.001 (LT)
IL-6 (pg/ml)	0.094–11.5	1.11; 1.59 (1.21)	1.13; 1.25 (0.61) −4.0; −5.7 (36.0) % NS (LT)	1.13; 1.69 (1.55) 0.6; 6.4 (54.7) % NS (LT)	NS (LT)
E-selectin (ng/ml)	29.1–63.4	46.6; 48.1 (21.0)	38.7; 40.3 (16.1) −16.3; −10.10 (17.5) % 0.003	49.1; 49.0 (17.9) −2.9; 8.8 (41.4) % NS	0.001
P-selectin (ng/ml)	45–241	101.3; 102.8 (27.3)	90.7; 90.3 (21.3) −15.3; −8.5 (17.9) % 0.012 (LT)	93.9; 100.4 (45.0) −11.9; −2.3 (32.0) % NS (LT)	NS (LT)
ICAM-1 (ng/ml)	115–306	249.0; 248.0 (33.8)	236.9; 229.4 (42.2) −5.0; −7.3 (15.0) 0.015	238.1; 237.3 (36.6) −2.1; −4.0 (9.2) % 0.040	NS
VCAM-1 (ng/ml)	237–872	740.5; 731.9 (161.2)	700.3; 730.6 (161.0) −2.5; 2.2 (18.7) % NS	719.4; 723.2 (166.9) −3.1; 0.0 (15.0) % NS	NS
SAA (mg/dl)	0.08–0.97	0.31; 0.70 (1.15)	0.41; 0.63 (0.53) 23.5; 41.8 (89.2) % NS (LT)	0.33; 0.54 (0.48) 1.7; 24.2 (120.4) % NS (LT)	NS (LT)

Data are presented as median, and mean (SD) of the measured concentrations and of the percent change from baseline (BL) during oral CEE and transdermal (TD) E2 for 25 completers. *P* values are derived from mixed effects models of the measured concentrations. LT, Log-transformed data. NS, $P > 0.05$.

[a] Reference range for CRP is the interquartile range (25th-75th percentile) for adult women not on HT (11). Reference range for SAA is 95% confidence interval (12). Reference ranges for remaining parameters represent mean ± 1 SD (data from assay kit manufacturer).

primary hormone parameter in the study (10). Descriptive analysis of the data from the subjects who completed the study included calculation of the median, mean, SD, maximum and minimum, and the use of box and whisker plots (14). The means and variances of the baseline distributions of CRP and the other parameters were compared between subjects who received oral ET in the first treatment period, and those who received transdermal ET first using the Levene test and Student's *t* tests. The Shapiro-Wilk test for normality was performed on the on-treatment data

TABLE 3. Hepatic proteins

Analyte (U)	Reference range[a]	BL	Oral CEE % change from BL *P* (CEE *vs.* BL)	TD E2 % change from BL *P* (TD E2 *vs.* BL)	*P* (CEE *vs.* TD E2)
Transferrin (mg/dl)	200–400	273.7; 274.6 (35.2)	273.0; 288.7 (50.4) 5.3; 5.2 (10.2) % 0.024 (LT)	272.1; 299.0 (90.1) 1.1; 6.8 (21.2) % NS (LT)	NS (LT)
Prealbumin (mg/dl)	12–42	32.3; 32.4 (4.7)	31.5; 32.2 (3.9) −0.4; 1.2 (14.6) % NS	32.6; 34.0 (6.0) 2.7; 6.2 (20.0) % NS	NS
IGF-I (ng/ml)	40–258	133.0; 129.4 (33.1)	86.4; 89.3 (25.1) −30.5; −30.7 (13.0) % <0.001	112.6; 111.7 (26.0) −12.5; −11.9 (16.9) % <0.001	<0.001
SHBG (nmol/liter)	40–120	99; 108 (46.5)	231; 243 (112) 112.9; 132.2 (74.5) % <0.001	117; 122 (66) 2.6; 12.0 (25.0) % 0.042	<0.001
TBG (mg/dl)	1.8–4.2	2.2; 2.3 (0.3)	3.1; 3.2 (0.6) 38.1; 39.9 (20.1) % <0.001 (LT)	2.1; 2.3 (0.4) 0; 0.4 (11.1) % NS (LT)	<0.001 (LT)
CBG (mg/dl)	2.3–3.9	3.4; 3.5 (0.5)	4.1; 4.1 (0.8) 20.0; 18.0 (19.5) % <0.001	3.5; 3.4 (0.5) −5.1; −2.2 (11.3) % NS	<0.001

Data are presented as median, and mean (SD) of the measured concentrations and of the percent change from baseline during oral CEE and transdermal (TD) E2 for 25 completers. The data on SHBG, TBG, and CBG concentrations have been previously published in Ref. 10. *P* values are derived from mixed effects models of the measured concentrations. LT, Log-transformed data; NS, ($P > 0.05$).

[a] Reference ranges for transferrin and prealbumin are 95% confidence intervals in adults (data from assay kit manufacturer). Reference range for IGF-I is mean ± 1 SD (data from assay kit manufacturer). Reference range for SHBG is for premenopausal women not receiving HT (Esoterix). Reference ranges for TBG and CBG are for adults (Esoterix).

at the 0.001 significance level to detect marked departures from normality; log transformations were used in the case of nonnormal data. A mixed effects model (15) was fit to each parameter to evaluate carryover, period, and treatment effects. The model included treatment, period, and treatment sequence as fixed effects and subjects as random effects. A test for carryover effect is equivalent to a test for the treatment sequence effect. Based on F values, all but one of the 13 parameters (E-selectin) had nonsignificant (NS) carryover effects ($P > 0.05$). Further analyses suggested that anomalous P value (0.015) for E-selectin was most likely a spurious finding. In the absence of carryover effects, a test for period effect is equivalent with the test for the period main effect. A similar model was used to assess the change from baseline associated with each treatment. No adjustments were made for multiple comparisons because this part of the study was considered to be exploratory. The percent change from baseline associated with each treatment was computed for each parameter as a descriptive measure but was not used for inferential comparisons between treatments or between the treatment and baseline. Pearson correlation coefficients (r values) were computed for the percent change from baseline (during oral CEE) between all pairs of inflammation markers (CRP, IL-6, E-selectin, P-selectin, ICAM, VCAM, and SAA) and between all pairs of hepatic proteins (CRP, transferrin, prealbumin, IGF-I, SHBG, TBG, and CBG). Pearson r values were also used to explore possible associations between the percent change of the inflammation markers during oral CEE with the subject's current age, age when ET/HT was initiated, and the number of years on ET/HT, as well as the possible association between body mass index (BMI) and the baseline and percent change of the parameters during oral CEE. The slopes and intercepts of the log-log relationships between CRP and IL-6 at baseline and during oral CEE were estimated simultaneously and compared by a mixed effects model.

Results

Subjects

A total of 27 women enrolled in the study, and 25 completed both treatment periods. The two discontinuations occurred for personal reasons during the first treatment period (with both subjects on oral ET). The mean (SD) age of the subjects was 57.5 yr (5.9), weight was 69.6 kg (11.4), and BMI was 25.8 kg/m^2 (4.2); all were Caucasians. The subjects had started HT at an average age of 50.0 yr (4.7) and had used HT for 7.5 yr (4.9). At enrollment, 24 of the women used oral continuous combined HT regimens, one used an oral sequential regimen, one used a transdermal continuous combined regimen, and one used a transdermal sequential regimen.

CRP and other inflammation markers in serum

Descriptive and inferential statistical analyses of CRP and the other inflammation markers are summarized in Table 2. Box and whisker plots of the CRP levels at baseline, during oral CEE, and transdermal E2 are shown in Fig. 1. A logarithmic scale was used to compensate for the positive skewness of the distributions. As anticipated, the serum levels of CRP increased significantly during oral CEE (median percent change from baseline 192%; $P < 0.001$) but did not change significantly during transdermal E2 (15.7%; NS) ($P < 0.001$ between treatments). In contrast, the levels of IL-6 did not change significantly during either treatment. Small but significant decreases in E-selectin (−16.3%; $P = 0.003$) and P-selectin (−15.3%; $P = 0.012$) were observed during oral CEE, but not during transdermal E2. ICAM-1 and VCAM-1 levels showed small decreases during both oral CEE and transdermal E2 that were marginally significant for ICAM-1. SAA levels exhibited small but insignificant increases during both oral CEE and transdermal E2.

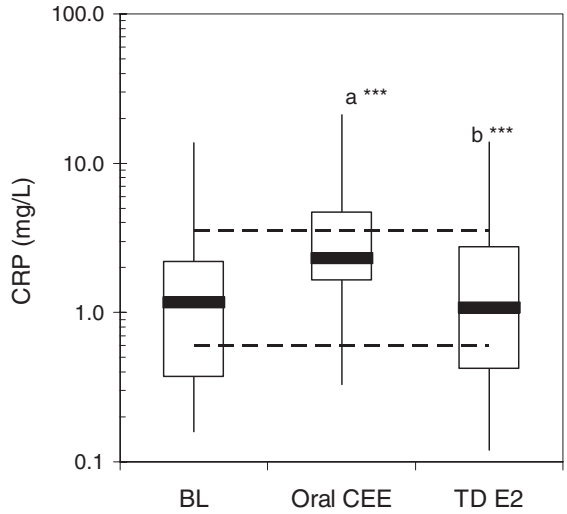

FIG. 1. Box and whisker plot of CRP levels measured at baseline (BL), after 12-wk 0.625 mg/d oral CEE, and after 12-wk 0.05 mg/d transdermal (TD) E2 in a crossover study of naturally menopausal women (n = 25 completers). Data are plotted on a logarithmic scale to reduce positive skewness in the distributions. *Dashed horizontal lines* enclose the interquartile range (25th-75th percentile) in women not receiving HT (11). Statistically significant comparisons to baseline are denoted by "a" and to oral CEE are denoted by "b." ***, $P < 0.001$.

Other hepatic proteins

Descriptive and inferential statistical analyses of the other hepatic proteins are summarized in Table 3. Transferrin and prealbumin concentrations changed negligibly from baseline during oral CEE and transdermal E2. In contrast, IGF-I decreased significantly from baseline during both oral CEE (−30.5%; $P < 0.001$) and transdermal E2 (−12.5%; $P < 0.001$), the decrease being greater during the oral treatment ($P < 0.001$ between treatments). As previously reported (10), all three hormone-binding protein concentrations increased significantly during treatment with oral CEE, the increase being greatest for SHBG (112.9%; $P < 0.001$), intermediate for TBG (38.1%; $P < 0.001$), and smallest for CBG (20.0%; $P < 0.001$). During transdermal E2 the small increase in SHBG (2.6%) achieved marginal significance ($P = 0.042$), whereas the small changes in TBG (0%) and CBG (−5.1%) did not.

Correlations between the intrasubject changes in CRP and the other parameters during oral CEE

Correlations between the intrasubject changes in CRP and the other parameters were based on pairwise analysis of the percent changes from baseline during oral CEE. Tables 4 and 5 present the Pearson correlation coefficients (r values) for these changes between all pairs of inflammation markers (CRP, IL-6, E-selectin, P-selectin, ICAM, VCAM, and SAA) and between all pairs of hepatic proteins (CRP, transferrin, prealbumin, IGF-I, SHBG, TBG, and CBG), respectively. Among the inflammation markers, the intrasubject changes in CRP (during oral CEE) correlated most strongly with the changes in SAA (r = 0.805; $P < 0.001$).

TABLE 4. Interrelationships (r values) between CRP and other inflammation markers (percent change during CEE)

	IL-6	E-selectin	P-selectin	ICAM	VCAM	SAA
CRP	0.420[a]	−0.236	−0.114	−0.085	−0.111	0.805[c]
IL-6		0.092	−0.069	−0.090	−0.117	0.522[b]
E-selectin			0.306	0.356	0.313	−0.098
P-selectin				0.095	−0.166	0.048
ICAM					0.451[a]	−0.039
VCAM						−0.127

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

Smaller positive correlations were also noted between the changes in IL-6 and SAA (r = 0.522; $P = 0.007$), IL-6 and CRP (r = 0.420; $P = 0.036$), and ICAM-1 and VCAM-1 (r = 0.451; $P = 0.026$). Among the other hepatic proteins, including CRP, the strongest associations were between the changes in SHBG and TBG (r = 0.500; $P < 0.01$), SHBG and CBG (r = 0.498; $P = 0.010$), and TBG and CBG (r = 0.460; $P = 0.020$). The changes in CRP exhibited a weaker correlation to the changes in TBG (r = 0.430; $P = 0.031$) and NS correlations to all other hepatic proteins.

Associations with current age, age when ET/HT was initiated, years on ET/HT, and BMI

The associations between the percent changes in CRP and the other inflammatory markers with the subject's current age, age when ET/HT was initiated, and the number of years on ET/HT were evaluated by Pearson r values. None of the associations was found to be statistically significant.

The r values between BMI and the baseline values of CRP and the other parameters, and the percent changes during oral CEE are given in Table 6. BMI was positively associated with the baseline values of CRP, IL-6, and SAA ($P < 0.01$), and negatively associated with the baseline value of SHBG ($P < 0.05$). None of the percent changes during oral CEE treatment was significantly associated with BMI.

Relationship between CRP and IL-6 concentrations

Because IL-6 is a known regulator of CRP production (2), the quantitative relationships between CRP and IL-6 concentrations at baseline, during oral CEE, and during transdermal E2 were investigated. As illustrated in Fig. 2, the logarithm of the CRP levels showed a distinct linear dependence on the logarithm of the IL-6 levels at baseline (open circles) and during oral CEE (filled circles). The relationship during transdermal E2 was virtually the same as the baseline data (data not shown). Modeling the relationships corresponding to the baseline and oral CEE data simultaneously with a mixed effects model indicated that the slopes of the lines were not significantly different from each other ($P = 0.1$), whereas the intercepts were ($P < 0.001$). Therefore, a simplified model with a constant slope and different intercepts was used to describe the baseline and oral CEE data together, as reflected by the dashed and solid lines in Fig. 2 (slope = 1.296; $P < 0.001$). The parallel shift of the line during oral CEE treatment can be interpreted as either an amplified hepatic response (vertical shift) or as a greater sensitivity to IL-6 stimulation (left shift). From the intercepts of the two lines, the vertical shift during oral CEE corresponds to a multiplicative factor of 2.79.

Tolerability and safety

As reported previously the oral and transdermal ET regimens were both well tolerated, and there were no clinically relevant changes in body weight, heart rate, or blood pressure during the study (10).

Discussion

Relationship to prior studies in postmenopausal women

The regimens of oral CEE (0.625 mg/d) and transdermal E2 (0.05 mg/d) we evaluated were the most common estrogen therapies used in the United States at the time of this study and have been similarly efficacious in the treatment of vasomotor symptoms (10). As previously reported the comparable reductions of LH and FSH observed with both regimens are consistent with a similar degree of systemic estrogen action. However, it should be

TABLE 5. Interrelationships (r values) between CRP and other hepatic proteins (percent change during CEE)

	Transferrin	Prealbumin	IGF-I	SHBG	TBG	CBG
CRP	0.388	0.238	0.290	0.248	0.430[a]	0.072
Transferrin		0.335	0.308	0.375	0.135	0.084
Prealbumin			0.167	−0.118	−0.079	−0.095
IGF-I				−0.040	−0.289	−0.177
SHBG					0.500[b]	0.498[a]
TBG						0.460[a]

[a] $P < 0.05$.

[b] $P < 0.01$.

TABLE 6. Pearson correlation coefficients (r values) between BMI and the baseline values of CRP, other inflammation markers and hepatic proteins, and their percent changes during oral CEE treatment

	Baseline	% Change CEE
CRP	0.616[a]	−0.282
IL-6	0.661[a]	−0.364
E-selectin	0.242	−0.119
P-selectin	0.361	0.017
ICAM	0.238	−0.084
VCAM	−0.037	0.098
SAA	0.529[a]	−0.057
Transferrin	0.148	0.074
Prealbumin	−0.035	−0.118
IGF-I	0.039	−0.198
SHBG	−0.465[b]	−0.073
TBG	0.152	−0.154
CBG	−0.107	−0.259

[a] $P < 0.01$.

[b] $P < 0.05$.

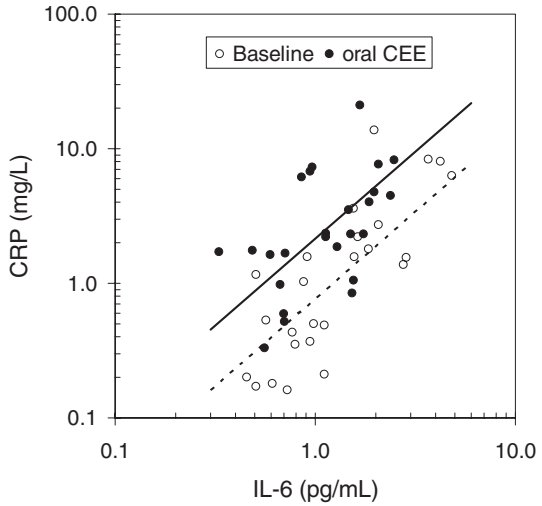

FIG. 2. Dependence of CRP levels on the corresponding levels of IL-6 at baseline (*open circles*) and after 12-wk treatment with oral CEE (*filled circles*). Data points are plotted on a log-log scale. The *dashed line* (baseline) and *solid line* (oral CEE) are based on a linear regression analysis of the ln-transformed data using a mixed effects model in which the slopes were assumed to be the same, and the y-intercepts were allowed to differ. The regression line for the baseline data is: ln CRP = −0.266 + 1.296 ln IL-6. The regression line for the oral CEE data is: ln CRP = 0.763 + 1.296 ln IL-6. The common slope and the difference between intercepts were both significantly different from zero ($P < 0.001$).

appreciated that a variety of steroidal compounds contained in CEE may contribute to its various biological activities (16).

Consistent with many prior studies of oral ET/HT regimens in postmenopausal women, we found that 0.625 mg CEE given daily with 100 mg oral micronized progesterone was associated with a marked increase (192%) in CRP concentration (3, 4, 17–23), small but significant decreases in the concentrations of E-selectin, P-selectin, and ICAM-1 (3, 4, 18, 19, 22–26), and little or no effect on IL-6, VCAM-1, and SAA (5, 6, 22–24, 27–30). Prior studies of the effects of oral ET/HT on other hepatic proteins are also consistent with our findings of a moderate decrease (30%) in IGF-I levels (5, 31), and the marked increases in SHBG, TBG, and CBG described previously (10). Likewise, previous studies of transdermal ET/HT are also consistent with our findings that 0.05 mg/d transdermal E2 given daily with 100 mg oral micronized progesterone produced little or no effect on CRP and other inflammation markers (4, 5, 23, 27, 28), and had minimal effects on the hepatic proteins that were studied (5, 10, 31). Although quantitative differences may be noted between our findings and some of the literature cited, these may be due in part to the different ET/HT dosing regimens and the androgenicity of the progestins that were used (6, 21, 30). In comparison to medroxyprogesterone acetate, norethisterone acetate, and levonorgestrel, oral micronized progesterone would be expected to have minimal androgenicity, and a negligible effect on the inflammation and hepatic parameters in this study (32, 33).

Mechanistic interpretation

The contrasting effects of oral *vs.* transdermal ET/HT on CRP and other hepatic proteins (IGF-I, SHBG, TBG, and CBG) have generally been attributed to the high local concentration of estrogens in the portal circulation after oral administration and their subsequent first-pass metabolism in the liver (4, 5, 10, 20). After an oral dose of 0.625 mg CEE, the portal estrogen concentration has been estimated to be approximately 3000 pg/ml, about 6-fold greater than after transdermal application of a 0.05 mg/d E2 patch (34). In this regard the hepatic effects of oral estrogen are qualitatively similar to the effects of pregnancy in which the systemic (and portal) concentrations of E2 and estrone reach 30,000 pg/ml by the third trimester, and result in even greater increases in SHBG, TBG, and CBG. Interestingly, longitudinal studies of CRP levels during normal uncomplicated pregnancy have not shown a consistent increase from the first to third trimesters, although the levels during pregnancy appear to be 3- to 5-fold higher than in nonpregnant women (35, 36). Moreover, during normal pregnancy the levels of the nonhepatic inflammation factors, *e.g.* IL-6, ICAM-1, VCAM-1, and E-selectin, do not vary markedly over time and appear to be comparable to those in nonpregnant women (37–39), whereas the level of the platelet-derived factor, P-selectin, increases (39). These trends clearly differ from the effects of oral ET/HT and suggest that factors other than estrogen modulate inflammation during pregnancy.

Further insights into the mechanism(s) by which oral ET/HT affects CRP, other inflammation markers, and other hepatic proteins may be inferred from the correlation analysis given in Tables 4 and 5. Among the inflammation markers (Table 4), the intrasubject changes in CRP correlated most strongly with the intrasubject changes in SAA and IL-6, which were also highly correlated with each other, suggesting that the mechanisms by which the acute phase reactants are coupled and modulated by IL-6 are maintained during oral CEE with daily oral micronized progesterone (2, 16, 40). This view is further reflected by the parallel shift (up-regulation) of the log-log relationship between CRP and IL-6 concentrations observed at baseline and during oral CEE (Fig. 2). At present we cannot distinguish whether the up-regulation is achieved by a multiplicative enhancement of CRP production, *i.e.* an upward shift of the IL-6 "dose-re-

sponse" curve, or is due to increased sensitivity to IL-6, *i.e.* a left-shift of the "dose-response" curve. Because the relationship between CRP and IL-6 during transdermal HT was virtually the same as the baseline data, it appears that the shift from baseline with oral HT was primarily due to the effect of oral CEE, and not to oral progesterone. The constant slope model we derived from our data is also consistent with a recent analysis of the changes in CRP and IL-6 levels obtained with oral CEE and different progestins in the Postmenopausal Estrogen/Progestin Interventions study (6). In that analysis the relationships between the logarithmic changes of the CRP and IL-6 levels, *i.e.* log(treatment) − log(baseline), were found to be linear for the three HT regimens, with positive slopes that were quite comparable to our value of 1.30. Although a negative slope was obtained for the subgroup of Postmenopausal Estrogen/Progestin Interventions subjects treated with oral CEE alone, the statistical significance of that finding was marginal (6).

Among the other inflammation markers (Table 4), only the r value between ICAM-1 and VCAM-1 was significant, suggesting that a common mechanism in the periphery lowers the expression of these adhesion markers under the influence of oral ET and to a much smaller degree with transdermal ET. It does not appear that this is a response to the changes in CRP levels because the r values with CRP were NS.

A key finding from the correlation analyses of the hepatic proteins (Table 5) is the apparently stronger pairwise correlations between the intrasubject changes in SHBG, TBG, and CBG than between each of these variables and CRP. This suggests that different estrogen-dependent mechanisms in the liver mediate these changes. Other recent studies have also found poor correlation between the changes in SHBG and CRP levels (6, 25, 26). SHBG, TBG, and CBG have similar glycoprotein structures with oligosaccharide chains containing sialic acid (41, 42). Estrogen-induced increases in the sialic content of these glycoproteins slow their metabolism, resulting in longer circulating half-lives, lower metabolic clearance rates, and higher serum concentrations. As a nonglycoprotein member of the pentraxin family, CRP cannot be altered or affected in this manner (40).

Finally, we found that the intrasubject changes in IGF-I levels during oral CEE were not correlated with the intrasubject changes in CRP or with any other hepatic protein (Table 5). This differs from a previous report (5) in which a significant inverse (negative) correlation between the changes in IGF-I and CRP was found in an 8-wk crossover study of 21 subjects treated with oral and transdermal ET. The basis for this difference is unclear.

Clinical relevance

Despite much speculation it is presently unknown if the elevations in CRP that we and others have observed during oral CEE (with and without progestin) pose an increased risk for cardiovascular events in postmenopausal women (6, 20, 27, 43). It is likewise uncertain if the presumably favorable reductions in ICAM-1, E-selectin, P-selectin, the decreases in IGF-I, and the effects on lipid profile, coagulation markers, and fibrinolysis that have been reported in other studies (27, 29) alter this risk. Although the finding that IL-6 levels did not change with oral ET/HT suggests that systemic inflammation has not changed in the periphery, it remains possible that the elevated CRP levels, themselves, may exert untoward effects on atherosclerotic plaques and stimulate other atherogenic processes (2, 44). The reduction in IGF-I levels could also be a pro-inflammatory factor (5).

To the extent that transdermal ET/HT does not produce similar adverse changes in CRP, IGF-I, or procoagulant factors, it may be associated with a smaller risk for the progression of coronary artery disease (CAD) and/or the occurrence of thrombotic events in postmenopausal women (44). In this regard, recent case-control studies have shown a 4-fold smaller risk of venous thromboembolism with transdermal compared with oral ET (45, 46). In addition to the route of ET/HT administration, recent secondary analyses of the Women's Health Initiative clinical trials suggest that the age at which ET/HT was initiated, and/or the years since menopause may determine whether it increases or decreases the risk or progression of CAD (9, 47). The so called "timing hypothesis" (43, 48) states that initiation of ET/HT at or near the time of menopause or before the development of atherosclerotic lesions will have a protective effect, whereas initiation 10 or more years from the time of menopause or after the development of atherosclerotic lesions may have a harmful effect. The lack of efficacy of oral and transdermal ET/HT reported in studies of the secondary prevention of CAD is consistent with this hypothesis (49, 50). Although we did not observe any relationship between the changes in CRP or the other inflammation markers with any of these "timing variables," including the subject's current age, this does not exclude a more downstream effect on the atherosclerotic process that would be dependent on the timing variables. Large prospective randomized clinical trials, such as the Kronos Early Estrogen Prevention Study trial (43), will be needed to assess the issues of timing and route of estrogen administration on the risk of developing coronary heart disease.

Finally, it may be noted that the mean BMI of the subjects in our study, 25.8 kg/m^2, is smaller than the current average for women of similar age and ethnicity in the United States (51). The difference may reflect the "healthy user bias" associated with HT use in observational studies (52). Although we and others (25, 26, 53) have observed that BMI has a positive association with certain inflammation markers, *e.g.* CRP, IL-6, and SAA, and a negative association with the baseline SHBG values, we did not observe any significant association between BMI and the percent changes of these or the other variables during treatment with oral CEE or with transdermal E2 (data not presented). This suggests that our findings may be applicable to obese as well as nonobese postmenopausal women, including those with metabolic syndrome who appear to have improved insulin sensitivity with transdermal *vs.* oral E2 (54).

Limitations of the study

A number of limitations of the present study should be noted. First, the study was designed as an open-label crossover study to compare the short-term effects of oral and transdermal ET on serum hormone concentrations, inflammation markers, and hepatic proteins rather than as a placebo-controlled, parallel group, study to evaluate clinical efficacy or safety endpoints.

Second, although we assayed more than 25 serum parameters per subject, including the previously reported hormone data (10), we did not evaluate the effects of oral *vs.* transdermal ET/HT on fasting lipid profiles, coagulation, or fibrinolytic markers because these have extensively been studied by others (4, 27, 29). Finally, although the study began with a 6-wk withdrawal period from prior HT to assess baseline status, a second withdrawal period and baseline assessment were not included between the two 12-wk treatment periods because they would have lengthened the study, and generally increased subject burden and distress. Furthermore, we expected that the initial 6-wk withdrawal period and 12-wk treatment duration would be sufficiently long relative to the biological half-lives of the measured parameters (as well as the estrogenic CEE components) to mitigate carryover effects from the prior treatment periods and enable the direct effect of each treatment regimen to be manifested.

Conclusions

We have compared the short-term effects of two commonly used regimens of oral and transdermal HT on the serum concentrations of CRP, other inflammation markers, and other hepatic proteins in naturally menopausal women. Our results confirm previous findings that oral ET/HT substantially increases the level of CRP, lowers ICAM-1, E- and P-selectin, and does not significantly alter IL-6, VCAM-1, or SAA levels. In contrast, transdermal ET/HT exerts minimal effects on these variables.

By quantitatively analyzing the interrelationships between the changes in CRP, IL-6, and the other measured parameters, we suggest that as a consequence of the high portal concentrations of estrogens, oral ET/HT elevates CRP levels by an up-regulation of the IL-6 dependent modulation of CRP production by the liver, rather than a nonspecific effect on hepatic protein synthesis or glycosylation. Further studies will be needed to clarify and confirm this interpretation.

From a clinical perspective, our study suggests that transdermal ET/HT may be preferable to oral ET/HT in minimizing the hepatic estrogen exposure that elevates CRP, increases coagulation markers, and reduces IGF-I. Whether the differential effects of oral *vs.* transdermal ET/HT on these risk factors will influence clinically important outcomes, such as cardiovascular disease, is presently unknown. Randomized clinical trials using direct measures of cardiovascular events, and/or surrogate measures of plaque formation or intima-media thickness will be needed to test this hypothesis in appropriately selected populations of postmenopausal women.

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