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Both Raloxifene and Estrogen Reduce Major Cardiovascular Risk Factors in Healthy Postmenopausal Women

A 2-Year, Placebo-Controlled Study

Gerdien W. de Valk-de Roo, Coen D.A. Stehouwer, Piet Meijer, Velja Mijatovic, Cornelis Klufft, Peter Kenemans, Fredric Cohen, Steven Watts, Coen Netelenbos

Abstract—Currently raloxifene, a selective estrogen receptor modulator, is being investigated as a potential alternative for postmenopausal hormone replacement to prevent osteoporosis and cardiovascular disease. We compared the 2-year effects of raloxifene on a wide range of cardiovascular risk factors with those of placebo and conjugated equine estrogens (CEEs). Analyses were based on 56 hysterectomized but otherwise healthy postmenopausal women aged 54.8 ± 3.5 (mean \pm SD) years who entered this double-blind study and who were randomly assigned to raloxifene hydrochloride 60 mg/d ($n=15$) or 150 mg/d ($n=13$), placebo ($n=13$), or CEEs 0.625 mg/d ($n=15$). At baseline and after 6, 12, and 24 months of treatment, we assessed serum lipids, blood pressure, glucose metabolism, C-reactive protein, and various hemostatic parameters. Compared with placebo, both raloxifene and CEEs lowered the level of low density lipoprotein cholesterol by 0.53 to 0.79 mmol/L (all $P < 0.04$) and lowered, at 24 months, the level of fibrinogen by 0.71 to 0.86 g/L (all $P < 0.05$). The effects of raloxifene and CEEs did not differ significantly. In contrast to raloxifene, from 6 months on CEEs increased high density lipoprotein cholesterol by 0.25 to 0.29 mmol/L and reduced plasminogen activator inhibitor-1 antigen by 30.6 to 48.6 ng/mL (all $P < 0.02$ versus both placebo and raloxifene). CEEs transiently increased C-reactive protein by 1.0 mg/L at 6 months ($P < 0.05$ versus placebo) and prothrombin-derived fragment F1+2 by 0.79 nmol/L at 12 months ($P < 0.001$ versus placebo). Finally, from 12 months on, CEEs increased triglycerides by 0.33 to 0.56 mmol/L (all $P < 0.05$ versus both placebo and raloxifene). Our findings suggest that in healthy postmenopausal women, raloxifene and estrogen monotherapy have similar beneficial effects on low density lipoprotein cholesterol and fibrinogen levels. These treatments differ, however, in their effects on high density lipoprotein cholesterol, triglycerides, and plasminogen activator inhibitor-1 and possibly in their effects on prothrombin fragment F1+2 and C-reactive protein. (*Arterioscler Thromb Vasc Biol.* 1999;19:2993-3000.)

Key Words: raloxifene ■ estrogen ■ lipids ■ coagulation ■ fibrinolysis

Observational studies among postmenopausal women have found that the use of estrogens with or without progestogens is associated with a reduction of the risk of osteoporosis,^{1,2} cardiovascular disease,³⁻⁵ and overall mortality.⁶ The results of the recently published HERS study (Heart Estrogen-progestin Replacement Study), the only randomized trial so far, were therefore disappointing, in that it showed no beneficial effect of postmenopausal hormone replacement therapy on cardiovascular morbidity and/or mortality.⁷ It did concern, however, a secondary prevention study of conjugated equine estrogens (CEEs) continuously combined with medroxyprogesterone acetate. Results of that study may therefore not be extrapolated to healthy postmenopausal women nor to other regimens of hormone replacement therapy. Disadvantages of the prolonged use of hormone

replacement with estrogens and progestogens include an increase in the risk of breast cancer,^{8,9} a possible increase in the risk of endometrial carcinoma,¹⁰ and the recurrence of vaginal bleeding.

To increase the benefit-risk ratio of hormone replacement therapy, so-called designer estrogens are being developed: nonhormonal agents that bind with high affinity to the estrogen receptor and exhibit tissue-specific, estrogen-agonist or -antagonist effects. The tissue specificity of designer estrogens may be related to the existence of (at least) 2 different isoforms of the estrogen receptor with distinct signaling properties, depending on the ligand and the response element.^{11,12} Raloxifene, a nonsteroidal benzothio-phenone derivative, is a designer estrogen of which relatively much experience has been gained. It possesses beneficial

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TABLE 1. General Characteristics at Baseline

	Total	Placebo (n=15)	Ral 60 mg/d (n=15)	Ral 150 mg/d (n=15)	CEEs 0.625 mg/d (n=15)
Age, y	54.8±3.5	54.9±4.5	54.0±3.5	54.6±2.8	55.7±3.2
Estradiol, pmol/L	11 (9; 14)	12 (7; 20)	11 (7; 19)	15 (8; 27)	8 (6; 11)
FSH, U/L	81±23	91±26	79±19	67±11	86±28
BMI, kg/m ²	26.6±3.5	27.0±4.2	26.5±4.2	26.2±2.3	26.8±3.4
Smoking, %	23	15	13	46	20
SBP, mm Hg	125±14	127±16	121±11	125±15	127±13
Hypertension,* %	11	8	13	15	7
TC, mmol/L	6.4±1.1	6.4±1.3	6.6±1.2	6.3±1.0	6.3±1.1
HDL-C, mmol/L	1.6±0.4	1.6±0.5	1.7±0.3	1.5±0.4	1.5±0.3
LDL-C, mmol/L	4.3±1.0	4.4±1.1	4.5±1.1	4.2±0.8	4.3±1.1
TG, mmol/L	1.0 (0.8; 1.1)	1.0 (0.8; 1.2)	0.9 (0.8; 1.1)	1.2 (1.0; 1.4)	0.8 (0.5; 1.2)
Glucose, mmol/L	5.0±0.6	4.8±0.5	5.0±0.3	5.4±1.0	5.1±0.5
Insulin, pmol/L	48 (42; 54)	42 (31; 58)	46 (36; 59)	53 (43; 65)	50 (43; 59)
CRP, mg/L	1.2 (0.9; 1.6)	1.2 (0.8; 1.8)	1.1 (0.6; 2.1)	1.1 (0.7; 1.7)	1.6 (1.0; 2.5)
Fibrinogen, g/L	3.6±0.8	3.6±0.6	3.6±0.9	3.3±0.5	3.8±0.8
Factor VII antigen, %	89±13	89±10	91±14	89±17	88±10
F1+2, nmol/L	1.3±0.4	1.2±0.4	1.3±0.5	1.4±0.4	1.4±0.4
TAT, μg/L	3.1 (2.6; 3.7)	3.6 (2.5; 5.0)	3.4 (2.2; 5.3)	2.5 (1.9; 3.3)	2.9 (2.1; 4.1)
tPA, μg/L	7.9±2.6	6.9±2.4	7.9±2.2	8.2±2.2	8.4±3.4
PAI-1, μg/L	52 (43; 63)	40 (26; 63)	56 (40; 78)	56 (44; 72)	58 (38; 90)
PAP, μg/L	446±139	451±177	447±114	425±147	460±130
D-dimer, μg/L	22 (17; 28)	26 (17; 40)	16 (11; 25)	18 (11; 31)	30 (18; 47)

Ral indicates raloxifene; FSH, follicle-stimulating hormone; BMI, body mass index; SBP, systolic blood pressure; and TAT, thrombin-thrombin complexes. Values are mean±SD or, for nonnormally distributed variables, the geometric mean with its 95% CI.

*As defined by World Health Organization criteria.

estrogen-agonist effects on bone¹³ and cardiovascular risk factors^{13,14} but estrogen-antagonist effects on the endometrium¹³ and breast tissue.¹⁵ However, in ovariectomized monkeys fed an atherogenic diet, long-term treatment with raloxifene in contrast to CEEs did not reduce coronary artery plaque size.¹⁶ The long-term effects of raloxifene on cardiovascular risk factors have not been extensively investigated. Therefore, we compared the effects of raloxifene on serum lipids, blood pressure, glucose metabolism, and hemostatic cardiovascular risk factors with those of placebo and CEEs in a group of hysterectomized but otherwise healthy postmenopausal women in a double-blind, randomized study of 2 years' duration.

Methods

Subjects

Sixty postmenopausal, hysterectomized but otherwise healthy women (mean±SD age 54.8±3.5 years) were included in the study. Hysterectomy had been performed because of a benign endometrial abnormality (n=50), uterine prolapse (n=8), and/or a benign ovarian abnormality (n=5). Postmenopausal status was defined as a serum estradiol level ≤73 pmol/L and a follicle-stimulating hormone level ≥40 IU/L. Exclusion criteria were a history of breast carcinoma and/or recent thromboembolism; evidence of liver disease and/or renal dysfunction; use of lipid-lowering drugs and/or anticonvulsives; and use of sex steroids and/or corticosteroids more recently than 6 months before the start of the study. Hypertension was not an exclusion criterion. Four participants dropped out of the study before the first postbaseline visit. Reasons for withdrawal were incorrect inclusion (placebo group), nausea (raloxifene 60-mg group), per-

sonal conflict (placebo group), and death due to a traffic accident (raloxifene 150-mg group). These subjects were excluded from the analyses. Analyses in this report are therefore based on 56 women randomly assigned to 1 of 4 treatment groups: placebo (n=13), CEEs 0.625 mg/d (n=15), and raloxifene hydrochloride 60 mg/d (n=15) and 150 mg/d (n=13). All women received 500 mg of elemental calcium per day. To monitor compliance, at each visit the subjects returned the unused medication, which was then counted. If a subject had missed >30% of the study medication during 2 separate time periods, she was regarded as severely noncompliant. The study was conducted on an outpatient basis according to the principles of the Declaration of Helsinki and was approved by the medical ethics committee of the Academic Hospital Vrije Universiteit of Amsterdam. Informed consent was obtained from all volunteers after oral and written information was supplied.

General Procedures

At baseline and at 12-month intervals, smoking status (yes or no), body mass index (weight/height²), and blood pressure were assessed. Blood pressure (mean of 4 readings) was measured on the left arm after 10 minutes of rest by using an automated device (BP-8800, Colin). Hypertension was defined as a systolic blood pressure ≥160 mm Hg and/or a diastolic blood pressure ≥95 mm Hg and/or the use of antihypertensive drugs. At baseline and after 6, 12, and 24 months of treatment, blood samples were collected between 8:30 and 11:30 AM after an overnight fast. Circulating levels of insulin, all markers of coagulation and fibrinolysis, and C-reactive protein (CRP) were assayed at the end of the study in a single run. A carefully standardized procedure¹⁷ was applied for samples to be used for determination of markers of coagulation and fibrinolysis.

Laboratory Variables

At baseline, we measured serum levels of estradiol (radioimmunoassay; Double Antibody, DPC) and of follicle-stimulating hormone (radioimmunoassay; IMX, Abbott Laboratories).

TABLE 2. Change From Baseline: Active Treatment Versus Placebo—Lipids

	Months	Ral 60 mg/d	<i>P</i>	Ral 150 mg/d	<i>P</i>	CEEs 0.625 mg/d	<i>P</i>
TC, mmol/L	6	-0.56 (-1.05, -0.08)		-0.30 (-0.80, 0.20)		-0.26 (-0.74, 0.22)	
	12	-0.52 (-0.98, -0.07)		-0.59 (-1.05, -0.12)		-0.24 (-0.69, 0.21)	
	24	-0.67 (-1.16, -0.18)	† §	-0.59 (-1.10, -0.09)	* §	-0.03 (-0.52, 0.46)	
LDL-C, mmol/L	6	-0.61 (-1.06, -0.16)	*	-0.52 (-0.99, -0.06)	*	-0.67 (-1.12, -0.23)	†
	12	-0.54 (-0.95, -0.13)	*	-0.65 (-1.08, -0.23)	†	-0.65 (-1.06, -0.23)	†
	24	-0.76 (-1.18, -0.34)	†	-0.79 (-1.23, -0.36)	†	-0.53 (-0.95, -0.11)	*
HDL-C, mmol/L	6	0.00 (-0.13, 0.14)	¶	0.12 (-0.01, 0.26)	§	0.29 (0.16, 0.42)	†
	12	-0.01 (-0.15, 0.13)	¶	0.04 (-0.11, 0.18)	¶	0.26 (0.12, 0.39)	†
	24	0.06 (-0.09, 0.21)	§	0.06 (-0.09, 0.22)	§	0.25 (0.10, 0.40)	†
TG, mmol/L	6	0.09 (-0.31, 0.49)		0.23 (-0.18, 0.64)		0.27 (-0.13, 0.67)	
	12	0.05 (-0.27, 0.38)	§	0.07 (-0.27, 0.41)	§	0.33 (0.00, 0.66)	*
	24	0.07 (-0.29, 0.42)	¶	0.30 (-0.07, 0.66)	§	0.56 (0.21, 0.91)	†

Ral indicates raloxifene. CEEs indicates conjugated equine estrogens. Values are mean changes (with 95% CIs) from baseline in the active-treatment groups relative to corresponding mean changes from baseline in the placebo group. Because simple back-transformation of a difference between 2 values for the same measure at 2 different times is not allowed, changes versus baseline are based on actual values.

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, significantly different from baseline.

§ $P < 0.05$, ¶ $P < 0.01$, # $P < 0.001$, significantly different from CEEs.

||Logarithmically transformed values.

At baseline and after 6, 12, and 24 months of treatment, we measured serum total cholesterol (TC), HDL cholesterol (HDL-C), and triglycerides (TGs; all with enzymatic methods from Boehringer Mannheim). LDL cholesterol (LDL-C) was calculated by the Friedewald formula. Serum insulin was measured by an immunoradiometric assay (Biosource Diagnostics; intra-assay CV 5%). Coagulation activity was assessed by measuring fibrinogen (thrombin time method according to Clauss; STA fibrinogen kit, Boehringer Mannheim), factor VII antigen (enzyme immunoassay; Asserchrom, Diagnostica Stago), the prothrombin-derived fragment 1+2 (F1+2), and thrombin-antithrombin complexes (both by enzyme immunoassay; Enzygnost, Behringwerke, with detection limits of 0.02 nmol/L and 0.5 $\mu\text{g/L}$, respectively). Fibrinolytic activity was determined by measuring tissue-type plasminogen activator antigen (tPA; ELISA Imulysse t-PA, Biopool, with a detection limit of 1.5 $\mu\text{g/L}$), plasminogen activator inhibitor-1 antigen (PAI-1 enzyme immunoassay; Innostest PAI-1, Innogenetics, with a detection limit of 2.5 $\mu\text{g/L}$), and plasmin-antiplasmin complexes (PAP) and D-dimer (both by enzyme immunoassay; Enzygnost, Behringwerke, with a detection limit of 10 and 0.5 $\mu\text{g/L}$, respectively). Intra-assay CVs were all <10% except for tPA, for which they were <12%. Because inflammation may play a role in the pathogenesis of atherosclerotic disease,¹⁸ we also measured levels of CRP with a sensitive (detection limit of 0.01 mg/L) in-house enzyme immunoassay with antibodies supplied by Dako. The intra-assay CVs were <10%.

Statistical Analyses

The statistician did not have any contact with the participants, and the physicians did not know the randomization codes. Data are given as mean \pm SD. For variables for which the residuals of the fitted least-squares model were not normally distributed, logarithmic transformation gave approximately symmetrical distributions that were used for all statistical analyses described below. For these variables, geometric means with their 95% CIs are presented. At baseline, we assessed the general characteristics for the 4 different treatment groups and compared these by either 1-way ANOVA or by Fisher's exact test. Additionally, at 12 and 24 months, the prevalence of hypertension in each of the treatment groups was assessed and compared by Fisher's exact test. Missing values were replaced by carrying forward the last available postbaseline value. Analyses of particular importance were those comparing any raloxifene group to either the placebo group or the CEE group. Pairwise comparisons were carried out within the framework of the ANOVA by using the treatment group least-square means (LSMEANS) and the pooled

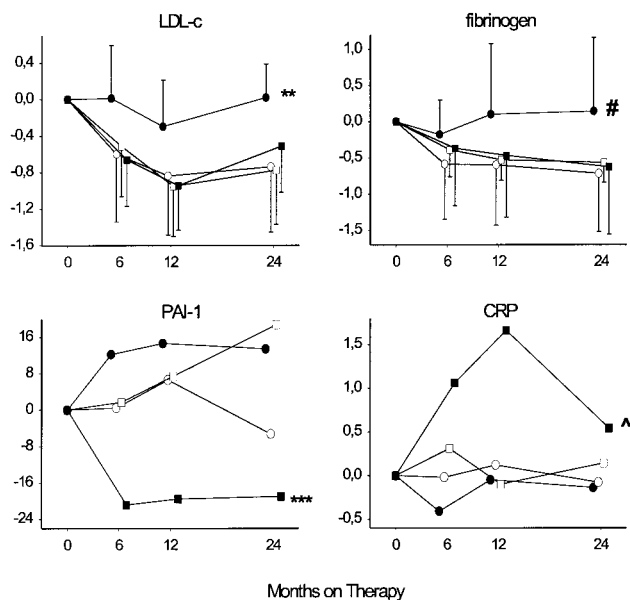
variance of the LSMEANS; pairwise differences between any 2 treatment groups were established by contrasting the differences in the treatment LSMEANS by using the pooled variance. Tests of within-group changes versus baseline were carried out with Student's *t* tests. Any pairwise differences were examined for significance only after an overall treatment group difference had been established. Overall changes from baseline were evaluated by using repeated-measures ANOVA. This was accomplished by using the Statistical Analysis System MIXED procedure, in which the model included effects for therapy, visit, and therapy-by-visit interaction. These analyses assumed an autoregressive covariance structure. Finally, Pearson correlations were used to assess associations between markers of fibrinolysis. Statistical tests resulting in $P < 0.05$ were judged to be significant. All analyses were performed with SAS version 6.08 running under the MVS operating system.

Results

Table 1 shows the baseline values of all variables measured for all women included in the study. At baseline there were no significant differences among the 4 groups except for the mean follicle-stimulating hormone level, which was $\approx 25\%$ lower in the raloxifene 150-mg group than in the placebo and CEE groups. Fifty-two women completed the 2-year follow-up. Four women withdrew before the first postbaseline visit and 4 after this visit. For the latter 4, who remained in the analyses, the reasons for leaving the study were development of a deep venous thrombosis (after 6.5 months; raloxifene 60-mg group), carcinoma of the bladder (after 9.5 months; CEE group), and personal conflict (after 9.5 and 12.1 months; raloxifene 60-mg and placebo groups). There was no severe noncompliance, as defined in Methods, in any of the treatment groups.

Lipid Levels

At 24 months, raloxifene at 60 and 150 mg/d decreased the mean serum TC by 0.67 and 0.59 mmol/L, respectively ($P < 0.03$ versus both placebo and CEE groups; Table 2). CEEs did not affect the serum TC. For LDL-C, the overall change from baseline in the placebo group differed significantly from baseline in both the CEE



Change from baseline for LDL-C, fibrinogen, PAI-1, and CRP. Values are either mean±SD (upper panels) or medians (lower panels). For PAI-1 and CRP, significance is based on logarithmically transformed ANOVA for repeated measurements over time. ● indicates placebo; ○, raloxifene 60 mg; □, raloxifene 150 mg; ■, CEEs 0.625 mg. #P=0.06; ^P=0.07; **P<0.01; ****P<0.001.

and the raloxifene groups ($P=0.002$; the Figure). From 6 months onward, both raloxifene and CEEs decreased the mean LDL-C to a similar extent (by 0.52 to 0.79 mmol/L, $P<0.05$ versus placebo). Compared with placebo, raloxifene did not change HDL-C, whereas CEEs increased the mean HDL-C ($P<0.02$ versus both placebo and raloxifene). Compared with placebo, TG serum levels were not affected by raloxifene; in contrast, from 12 months on, TG levels were raised by CEEs ($P<0.05$ versus both placebo and raloxifene).

Blood Pressure

Compared with placebo, neither raloxifene nor CEEs significantly affected systolic or diastolic blood pressure (data not shown). Two participants started antihypertensive therapy during the study. One participant stopped her antihypertensive medication. At none of the time points measured,

however, did the prevalence of hypertension differ significantly among the groups.

Biochemical Markers of Coagulation

The overall change in fibrinogen from baseline in the placebo group tended to be different from the overall change from baseline in both the CEE and raloxifene groups ($P=0.06$; the Figure). Compared with placebo, at 24 months raloxifene and CEEs decreased the mean fibrinogen level by 0.71 to 0.86 g/L ($P<0.04$; Table 3). At none of the follow-up measurements was the effect between raloxifene and CEEs significantly different. Compared with placebo, neither raloxifene nor CEEs affected factor VII antigen or thrombin-antithrombin complex. Compared with placebo, raloxifene did not significantly affect the mean F1+2; at 12 months, CEEs transiently increased the mean F1+2 by 0.79 nmol/L ($P<0.001$).

Biochemical Markers of Fibrinolysis

In the placebo group, an increase in PAI-1 (by 8.4, 13, and 5.3 $\mu\text{g/L}$, respectively; all $P<0.08$; the Figure) and a decrease in the tPA-PAI-1 ratio (all $P<0.05$; data not shown) were seen at all 3 time points [Table 4]. Compared with placebo, the levels of tPA and PAI-1 did not change significantly with raloxifene therapy, nor did the tPA-PAI-1 ratio. In contrast, compared with placebo, at 6 and 24 months CEEs reduced the mean tPA level by 2.04 and 2.25 $\mu\text{g/L}$, respectively ($P<0.05$ versus both placebo and raloxifene). From 6 months on, CEEs decreased the mean PAI-1 level by 30.6 to 48.6 $\mu\text{g/L}$ ($P<0.02$ versus both placebo and raloxifene) as well as the mean tPA-PAI-1 ratio. The increase in the tPA-PAI-1 ratio was significant versus placebo at 6 and 12 months ($P<0.002$). The overall change in PAI-1 from baseline in the CEE group differed significantly from the overall change from baseline in the other groups ($P<0.001$, the Figure). PAP as well as D-dimer showed wide scatter and did not change significantly in any of the groups. There was, however, at all time points an inverse correlation between PAI-1 and PAP ($r=-0.38$ to -0.62 ; all $P<0.005$) and a positive correlation between the tPA-PAI-1 ratio and PAP ($r=0.48$ to 0.68 ; $P<0.001$) in the entire group. Furthermore, any change versus baseline in PAI-1 was negatively associated with any change versus baseline in PAP ($r=-0.42$ to -0.62 ;

TABLE 3. Change From Baseline: Active Treatment Versus Placebo—Coagulation Markers

	Months	Ral 60 mg/d	P	Ral 150 mg/d	P	CEEs 0.625 mg/d	P
Fibrinogen, g/L	6	-0.41 (-0.88, 0.06)		-0.22 (-0.71, 0.27)		-0.20 (-0.68, 0.28)	
	12	-0.70 (-1.29, -0.12)		-0.63 (-1.24, -0.03)		-0.57 (-1.15, 0.02)	
	24	-0.86 (-1.46, -0.26)	†	-0.71 (-1.33, -0.08)	*	-0.77 (-1.37, -0.16)	*
Factor VII antigen, %	6	-2.1 (-9.9, 5.6)		-3.2 (-11.1, 4.7)		3.2 (-4.5, 11.0)	
	12	2.4 (-6.3, 11.1)		3.0 (-5.8, 11.8)		4.6 (-4.0, 13.1)	
	24	3.2 (-7.4, 13.7)		6.6 (-4.2, 17.4)		8.6 (-1.8, 19.0)	
F1+2, nmol/L	6	0.07 (-0.25, 0.40)		0.21 (-0.12, 0.55)		0.35 (0.02, 0.67)	
	12	0.04 (-0.38, 0.47)	‡	0.36 (-0.08, 0.80)		0.79 (0.36, 1.22)	‡
	24	-0.10 (-0.54, 0.33)		0.05 (-0.40, 0.50)		0.05 (-0.38, 0.48)	
TAT, $\mu\text{g/L}$	6	-2.0 (-6.7, 2.7)		-1.7 (-6.5, 3.2)		-3.1 (-7.9, 1.7)	
	12	-2.0 (-7.8, 3.8)		5.8 (-0.2, 11.9)		-0.1 (-5.9, 5.7)	
	24	-4.6 (-10.1, 0.9)		-3.6 (-9.3, 2.0)		-4.2 (-9.8, 1.3)	

See the footnote to Table 2 for an explanation of symbols and abbreviations. †P=0.06; ‡P=0.07; *P<0.05; **P<0.01; ***P<0.001.

TABLE 4. Change From Baseline: Active Treatment Versus Placebo—Fibrinolysis Markers

	Months	Ral 60 mg/d	<i>P</i>	Ral 150 mg/d	<i>P</i>	CEEs 0.625 mg/d	<i>P</i>
tPA, μg/L	6	-0.70 (-1.73, 0.32)	§	-0.47 (-1.53, 0.59)	¶	-2.04 (-3.09, -1.00)	‡
	12	0.42 (-1.02, 1.87)		-0.38 (-1.88, 1.11)		-1.30 (-2.74, 0.15)	
	24	-0.55 (-2.20, 1.09)	§	0.15 (-1.56, 1.85)	¶	-2.25 (-3.90, -0.61)	†
tPA-PAI-1 ratio	6	0.04 (-0.01, 0.09)		0.03 (-0.02, 0.08)	§	0.09 (0.04, 0.14)	†
	12	0.05 (0.00, 0.11)	§	0.04 (-0.02, 0.10)	§	0.11 (0.06, 0.17)	‡
	24	0.03 (-0.02, 0.09)		0.02 (-0.03, 0.09)		0.08 (0.02, 0.13)	
PAI-1, μg/L	6	-14.1 (-47.6, 19.4)	§	-0.3 (-34.9, 34.4)	¶	-48.6 (-82.6, -14.6)	‡
	12	-5.4 (-49.7, 38.9)	¶	2.8 (-43.0, 48.7)	¶	-48.4 (-92.7, -4.1)	‡
	24	-6.3 (-34.0, 21.4)	§	15.8 (-12.9, 44.4)	¶	-30.6 (-58.3, -2.9)	‡
PAP, μg/L	6	-3.8 (-89.3, 81.8)		-15.1 (-103.6, 73.5)		50.0 (-37.0, 136.9)	
	12	-53.8 (-146.3, 38.7)		-29.4 (-125.1, 66.4)		15.2 (-77.3, 107.7)	
	24	-83.2 (-192.3, 25.8)		-79.9 (-192.8, 33.1)		-52.5 (-161.7, 56.7)	
D-dimer, μg/L	6	13 (-36, 61)		1 (-49, 51)		-11 (-60, 38)	
	12	18 (-35, 71)		19 (-35, 74)		-14 (-67, 39)	
	24	8 (-45, 62)		-1 (-57, 55)		-20 (-73, 34)	

See the footnote to Table 2 for an explanation of symbols and abbreviations.

P<0.002), and any change in the tPA-PAI-1 ratio was positively associated with any change in PAP (*r*=0.51 to 0.55; *P*<0.001). No consistent association existed between the level of PAI-1 or the tPA-PAI-1 ratio and D-dimer.

CRP and Carbohydrate Metabolism

Compared with placebo, raloxifene did not change CRP levels; at 6 months of treatment, however, CEE induced a transient increase (*P*=0.006, Table 5). The overall increase from baseline in the CEE group differed marginally from the overall change from baseline in both the placebo and raloxifene treatment groups (*P*=0.07; the Figure). Compared with placebo, neither raloxifene nor CEEs showed a significant effect on fasting levels of glucose and/or insulin.

Additional Analyses

Adjustment for smoking habits by ANCOVA did not materially affect the results (data not shown).

Discussion

There were 2 main findings. First, both raloxifene and CEEs lowered LDL-C and fibrinogen levels. Second, CEEs, but not raloxifene, decreased PAI-1 and increased HDL-C, TG, F1+2, and CRP levels. These results suggest that both CEEs

and raloxifene are associated with a sustained improvement of the cardiovascular risk profile but that there are important differences between these agents.

Comparable Effects of Raloxifene and CEEs

The sustained decrease in LDL-C and fibrinogen levels after both CEE and raloxifene treatment may be considered beneficial from the cardiovascular point of view.^{19,20} LDL-C may promote cardiovascular disease by the induction of endothelial dysfunction²¹ and fibrinogen by its effects on hemostasis, blood rheology, platelet aggregation, and endothelial function.²⁰ The effects of CEEs^{22,23} and raloxifene^{13,14} on LDL-C levels that we found are in line with earlier studies. Similarly, previous studies have shown that estrogen replacement was associated with a diminished increase or a slight decrease in fibrinogen levels over time^{23,24} and that treatment with tamoxifen²⁵ and raloxifene¹⁴ decreased fibrinogen levels. Our study confirms and extends these data¹⁴ by showing that the effects of raloxifene increase with time. It is thought that estrogens and, presumably, raloxifene reduce LDL-C levels by enhancing LDL clearance as a result of hepatic LDL receptor induction.^{26,27} How estrogens and selective estro-

TABLE 5. Changes From Baseline: Active Treatment Versus Placebo—CRP and Carbohydrate Metabolism

	Months	Ral 60 mg/d	<i>P</i>	Ral 150 mg/d	<i>P</i>	CEEs 0.625 mg/d	<i>P</i>
CRP, mg/L	6	-1.5 (-4.1, 1.1)	§	0.8 (-1.9, 3.6)		1.0 (-1.6, 3.7)	†
	12	-3.0 (-6.5, 0.5)		-1.5 (-5.1, 2.1)		0.4 (-3.0, 3.9)	
	24	-3.1 (-6.2, 0.04)		-1.4 (-4.6, 1.9)		-1.3 (-4.4, 1.9)	
Glucose, mmol/L	6	-0.01 (-0.3, 0.3)		-0.1 (-0.4, 0.2)		-0.2 (-0.5, 0.1)	
	12	-0.1 (-0.4, 0.2)		-0.3 (-0.6, 0.0)		-0.1 (-0.5, 0.2)	
	24	-0.1 (-0.4, 0.2)		-0.1 (-0.4, 0.2)		-0.2 (-0.5, 0.1)	
Insulin, pmol/L	6	-9 (-24, 6)		-5 (-20, 10)		-7 (-22, 7)	
	12	2 (-14, 18)		1 (-16, 18)		-4 (-20, 12)	
	24	-1 (-20, 19)		7 (-14, 27)		-3 (-23, 17)	

See the footnote to Table 2 for an explanation of symbols and abbreviations.

gen receptor modulators influence fibrinogen levels is not known.

Different Effects of Raloxifene and CEEs

In contrast to CEEs, raloxifene did not affect the levels of HDL-C, TG, PAI-1, tPA, F1+2, and/or CRP. Observational studies have shown that cardiovascular prognosis is adversely affected by a decrease in HDL-C^{28,29} and by an increase in TG,^{28,30} PAI-1,^{31–33} tPA,^{33,34} and CRP.^{35–37}

It is therefore reasonable to assume that the increase in HDL-C observed with CEEs, which is thought to be mediated at least partly by an effect on hepatic lipase,^{38,39} represents a favorable change. The CEE-associated increase in TG may be innocuous, as this likely reflects enhanced synthesis of large, nonatherogenic, VLDL particles.²² Our results are in agreement with earlier reports on the effects of estrogen^{22,23} and raloxifene^{13,14} on HDL-C and TG. The mechanistic basis of the difference between CEEs and raloxifene, however, is not known.

It is less clear how the effects of CEEs on the markers of hemostasis, PAI-1, tPA, and F1+2, should be interpreted. The CEE-induced decrease in both PAI-1 and tPA could be regarded as beneficial, because PAI-1 is an essential antagonist of fibrinolysis and because the antigen levels of tPA primarily reflect the levels of circulating tPA–PAI-1 complexes. Although the level of PAP, a marker of fibrinolysis activation, and of D-dimer, a degradation product of cross-linked fibrin, did not increase significantly, for the whole group any decrease in PAI-1 was significantly associated with an increase in PAP ($r = -0.42$ to -0.62 ; $P < 0.002$), and any increase in the tPA–PAI ratio was significantly associated with an increase in PAP ($r = 0.51$ to 0.55 ; $P < 0.001$). Our data concerning the effects of CEEs on fibrinolysis are consistent with previous experience.^{40–45} None of these previous studies, however, had a duration of >1 year, nor did they include a placebo group. Oral estrogens may decrease both PAI-1 and tPA levels by affecting adipose tissue metabolism⁴⁶ or by the upregulation of the hepatic clearance of tPA–PAI-1 complexes.^{47,48} Our study suggests no important influence of raloxifene on fibrinolysis.

CEEs, but not raloxifene, transiently increased levels of F1+2, a marker of activated coagulation, which is in accordance with previous experience in short-term trials.^{14,43,45,49} Raloxifene may thus have some advantage over CEEs in this respect. The temporary character of the F1+2 increase is reminiscent of the results of the HERS study, which, in the first year of hormone use, showed more coronary events in the hormone group than in the placebo group.⁷

It must be noted that part of the changes induced by CEEs may result from the hepatic first-pass effect of oral estrogen. With respect to some cardiovascular parameters, transdermal estrogen administration may have effects that are similar to those of oral raloxifene. For example, transdermal estrogen does not affect circulating levels of TG,²² HDL-C,⁵⁰ PAI-1,^{40,42,45} and F1+2.⁴⁵ This may have implications for the choice of prescription: transdermal estrogen or raloxifene, for example, may be preferred in subjects with hypertriglyceridemia. So far, however, the implications with regard to clinical events of the differential effects of oral versus transdermal estrogen are unclear.

In contrast to raloxifene, CEEs transiently increased levels of CRP. There appear to be no previous published data on this issue. Even slightly increased CRP levels are a strong predictor of an adverse cardiovascular prognosis in both men^{35,36} and women.³⁷ The interpretation of these findings is unclear and includes at least 2 concepts: that elevated CRP levels reflect the hepatic response to inflammation in the vascular wall,³⁶ an important component of atherosclerosis, and that CRP itself activates processes that lead to vascular damage.⁵¹ The contrasting effects of CEEs on fibrinogen (decrease) and CRP (increase) argue somewhat against a CEE-associated increase in inflammatory activity but do not exclude it. This issue requires further investigation.

No Effect of Raloxifene and CEEs

Finally, we assessed the effects of CEEs and raloxifene on factor VII antigen, glucose, and insulin levels and on blood pressure and found no differences compared with placebo. This conclusion is limited, however, by the fact that we used relatively insensitive methods to assess these variables.

Study Limitations and Conclusions

We included only hysterectomized women, which enabled us to study the effects of conjugated estrogen without the addition of a progestogen and to compare the effects of raloxifene with estrogen-only therapy. This study had some limitations. First, it was relatively small, and we therefore cannot exclude relatively small treatment effects. Second, besides the effects described in this article, estrogen replacement and potentially raloxifene may have important direct (ie, LDL-C-independent) effects on endothelium-dependent and -independent vascular function.^{52–55}

In conclusion, this study, which comprised hysterectomized but otherwise healthy postmenopausal women aged 45 to 60 years, clearly shows that with respect to cardiovascular risk factors, raloxifene behaves as a partial estrogen agonist. Like CEEs, raloxifene showed a favorable influence on 2 generally recognized risk markers of cardiovascular disease, ie, LDL-C and fibrinogen levels. Unlike CEEs, however, it neither increased HDL-C nor decreased PAI-1 levels. Both raloxifene and CEEs express beneficial effects on bone mineral density; in contrast to CEEs, raloxifene seems not to influence the endometrium and breast tissue. In healthy postmenopausal women, raloxifene may thus be an attractive alternative for estrogen replacement in the prevention of cardiovascular disease and osteoporosis. Further prospective, randomized trials with clinical end points, however, are necessary to prove that CEEs and raloxifene improve cardiovascular prognosis in postmenopausal women.

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