

A Two-by-Two Factorial Trial Comparing Oral with Transdermal Estrogen Therapy and Fenretinide with Placebo on Breast Cancer Biomarkers

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ABSTRACT

Purpose: Oral conjugated equine estrogen (CEE) and medroxyprogesterone acetate (MPA) increase breast cancer risk, whereas the effect of transdermal estradiol (E2) and MPA is less known. Fenretinide may decrease second breast malignancies in premenopausal women but not in postmenopausal women, suggesting a hormone-sensitizing effect. We compared the 6 and 12-month changes in insulin-like growth factor-I (IGF-I), IGF-binding protein-3 (IGFBP-3), IGF-I:IGFBP-3 ratio, sex-hormone binding-globulin, and computerized mammographic percent density during oral CEE or transdermal E2 with sequential MPA and fenretinide or placebo.

Experimental Design: A total of 226 recent postmenopausal healthy women were randomly assigned in a two-by-two factorial design to either oral CEE 0.625 mg/day ($n = 111$) or transdermal E2, 50 μ g/day ($n = 115$) and to fenretinide 100 mg/twice a day ($n = 112$) or placebo ($n = 114$) for 12 months. Treatment effects were investigated by the Kruskal-Wallis test and analysis of covariance. P values were two-sided.

Results: After 12 months, oral CEE decreased IGF-I by 26% [95% confidence interval (CI), 22–30%] and increased sex-hormone binding-globulin by 96% (95% CI, 79–112%) relative to baseline, whereas no change occurred with transdermal E2 ($P < 0.001$ between groups). Fenretinide decreased IGFBP-3 relative to placebo ($P = 0.04$). Percentage of breast density showed an absolute increase of 3.5% (95% CI, 2.5–4.6%) during hormone therapy without differences between groups ($P = 0.39$).

Conclusions: Oral CEE has more favorable changes than transdermal E2 on circulating breast cancer risk biomarkers but gives similar effects on mammographic density. Fenretinide exerted little modulation on most biomarkers. The clinical implications of these findings require additional studies.

INTRODUCTION

Hormone replacement therapy (HRT) relieves climacteric symptoms and has been associated with reduced coronary heart disease mortality in observational studies (1). However, the use of HRT is associated with an increased risk of breast cancer, particularly with combined estrogen-progestin therapy (2, 3). A causal relationship between oral HRT and breast cancer has been demonstrated recently in the Women's Health Initiative trial, where a continuously combined regimen of oral conjugated equine estrogen (CEE) and medroxyprogesterone acetate (MPA) for a mean of 5.2 years resulted in a hazard ratio of breast cancer of 1.26 [95% confidence interval (CI), 1.00–1.59] compared with placebo (4). Breast cancers appeared to be at a higher stage, and the rate of abnormal mammograms was increased in the HRT arm (5). Moreover, a higher rate of cardiovascular disease (CVD) events (including coronary heart disease, stroke, and venous thromboembolic events) was noted in the HRT arm, with a global index of risks and benefits favoring a risk excess during HRT, notwithstanding a statistically significant reduction of osteoporotic bone fractures (6) and colorectal cancer (7).

In contrast to oral CEE, little is known of the chronic effects of transdermal 17- β estradiol (E2) on breast cancer and cardiovascular disease risk. However, the Million Women Study (3) has shown recently that compared with never users the risk of breast cancer during transdermal E2 was similar to that of oral estrogen (relative risk, 1.24; 95% CI, 1.11–1.39, compared with relative risk 1.32; 95% CI, 1.21–1.5 with oral estrogen). Moreover, unlike oral estrogen therapy, transdermal E2 does not seem to increase venous thromboembolism (8) and has a neutral effect on ultrasensitive C-reactive protein (9), an increasingly recognized risk marker of CVD (10), thus suggesting a safer profile on CVD risk.

In this two-by-two randomized trial, we compared the effects of oral CEE and sequential MPA with those of transder-

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mal E2 and sequential MPA on several biomarkers associated with breast cancer risk, including high circulating insulin-like growth factor-I (IGF-I; Refs. 11, 12), low IGF binding protein-3 (IGFBP-3; Ref. 13), high IGF-I:IGFBP-3 molar ratio (11, 13), low sex-hormone binding protein (SHBG; Ref. 14), and high computerized mammographic percent density (15, 16). Women were also randomized to either placebo or fenretinide, a synthetic amide derivative of retinoic acid, in an attempt to reduce breast cancer risk associated with HRT use. In a secondary analysis of a previous trial, fenretinide decreased second breast malignancies in premenopausal women but not in postmenopausal women, suggesting a possible sensitizing effect by sex-steroid hormones (17). Moreover, the retinoid showed a statistically significant interaction with menopausal status on the 12-month change in circulating IGF-I (18), and previous studies have shown that inhibition of breast cancer cell growth by the retinoid is partly mediated by down-regulation of the IGF system (19).

MATERIALS AND METHODS

Participants. Study participants were postmenopausal women with 6–60 month amenorrhea and follicle-stimulating hormone levels >40 units/liter who were willing to initiate HRT for menopausal relief. Women meeting any of the following criteria were excluded: prior HRT use, hysterectomy, previous malignancy, first-degree relative with breast cancer aged <50 years, endometrial proliferative disorders, alterations of metabolic, liver, renal, and cardiac function, retinoid hypersensitivity, photodermatitis, retinal diseases or glaucoma, venous thromboembolic events, active infections, severe depression, porphyry, and otosclerosis. The study was conducted at four academic institutions in Italy, including the European Institute of Oncology, Milan, and the University Clinics of Obstetrics and Gynecology of Brescia, Varese, and Trieste.

Interventions. Recent postmenopausal healthy women were randomly assigned in a two-by-two factorial design to compare oral CEE 0.625 mg/day ($n = 111$) with transdermal E2, 50 $\mu\text{g}/\text{day}$ ($n = 115$) released by a weekly patch, and fenretinide 100 mg/bid ($n = 112$) with placebo ($n = 114$) after 12 months of treatment. Assignment to the estrogen route was unblinded because the comparison in symptom relief was not a main study objective. Sequential MPA, 10 mg/day p.o. for the first 12 days of each month, was added to continuous estrogen therapy in each arm. A 3-day rest period from the retinoid capsules was prescribed monthly to increase plasma retinol levels, thus allowing sufficient uptake for normal night vision (17). At each visit women received study medications from their investigator physician to be self-administered for a 7-month period. Hormonal drugs were packaged in monthly blisters, whereas fenretinide or placebo were provided in two bottles each containing 200 capsules. Numbered tear-off drug labels were removed and attached to the case report form, which participants had to sign at each semiannual visit to confirm actual drug dispensation.

Study Objectives and Outcomes. The main objectives of the trial (#IEO-167) were: (a) to compare the effects of oral CEE and transdermal E2 at biologically comparable doses (20); and (b) to determine the effects of fenretinide on risk biomarkers

of breast cancer in healthy women undergoing estrogen therapy by a different route. The study biomarkers included the 6- and the 12-month levels of circulating IGF-I (the latter being the primary end point), IGFBP-3, IGF-I:IGFBP-3 molar ratio, and SHBG, and the 12-month measure of mammographic percentage of density as assessed by computerized method. Serial measurements of plasma concentrations of retinol, fenretinide, and its main metabolite 4-methoxyphenylretinamide were performed to assess relationships with drug activity and toxicity and to study pharmacokinetic interactions with the hormonal agents. This will be the subject of a separate paper.

The trial received Institutional Review Board approvals and was conducted in accordance with Good Clinical Practice procedures. All of the subjects gave their written informed consent. Women were assessed clinically at baseline, 2, 6, 12, and 18 months. Assessment included semiannual blood hematology and biochemistry examinations and baseline transvaginal ultrasounds to monitor drug safety and to exclude pretreatment endometrial abnormalities. Fasting blood samples were drawn between 8 a.m. and 10 a.m. at baseline, 6 and 12 months during the combined estrogen and MPA phase to adjust for the progestin effect. Adverse events were assessed using the National Cancer Institute Common Toxicity Criteria (21). Compliance was evaluated by a self-reported calendar and by pill count.

Whereas the study was still ongoing, the unexpected results of the Heart and Estrogen/Progestin Replacement study (22) and the initial WHI study alert became available on the Web regarding CVD risks associated with oral CEE and MPA use. The findings prompted us, for safety reasons, to study the 6- and 12-month changes in ultrasensitive C-reactive protein levels. The results of this study have been published elsewhere (9).

Assay Methods. All of the blood samples for circulating biomarkers were collected and stored at -80°C until assayed at the European Institute of Oncology. Plasma IGF-I was determined on EDTA by a chemiluminescent immunometric assay (Nichols Institute Diagnostics, San Juan, CA). The assay was performed on the automatic instrument LIAISON (Byk Santec Diagnostica, Dietzenbach, Germany). The sensitivity of the test was 0.8 nmol/liter; intra- and interassay coefficients of variation of our in-house pooled serum control sample were 5.4% and 7.8%, respectively. Serum IGFBP-3 was measured by ELISA using commercially available kits purchased from Diagnostic Systems Laboratories, Inc. (Webster, TX). The sensitivity of the assay was 0.001 nmol/liter; intra- and interassay coefficients of variation of our in-house pooled serum control sample were 4.0% and 9.9% (mean: 222 nmol/liter), respectively. All of our data were expressed in nmol concentrations to calculate the IGF-I:IGFBP-3 ratio, which is used as a more sensitive index of growth factor bioavailability (23). Moreover, IGF-I levels adjusted for IGFBP-3 were associated more strongly with breast cancer risk than IGF-I levels alone in observational studies (11). For IGF-I, to convert to ng/ml, one has to multiply by 7.633; for IGFBP-3, to convert to $\mu\text{g}/\text{ml}$, one has to divide by 24. Serum levels of SHBG were determined by a chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA) designed for the Immulite Automated analyzer. The sensitivity of the assay was 0.2 nmol/liter; intra- and interassay coefficients of variation were 2.5% and 4.5%, respectively. Pre- and post-treatment blood samples obtained from each subject

were assayed within the same run to improve analytical precision. All of the analyses were blinded to the treatment groups.

Mammographic percentage of density was measured in a randomly selected subgroup (~2 every 3 based on the randomization code) of 149 subjects who provided both baseline and 12 month radiograms and completed the study. The analysis was performed by one of us (I. M.) in Toronto under the supervision of Norman F. Boyd, using the computer-assisted method described previously (24). A calibration study was initially performed on 70 independent cases to assess the interobserver variability. Images from the craniocaudal film of the left breast of each participant were digitized and presented to the observer using a Lumisys model 85, which provides up to 12-bit density resolution and 50 μm spatial resolution. One pixel was 0.0676 mm^2 . Images from subjects at baseline and after treatment were randomly ordered, and furthermore, the pairs of baseline and post-treatment films were also randomly ordered. Reliability of computer-assisted measurements was assessed by repeated measurements of 81 images randomly distributed based on the randomization code throughout the seven reading sessions. Correlation for the percentage of density was 0.904.

Sample Size. The main outcome measure of the study was the change in IGF-I from baseline to 12 months. On the basis of small series from the literature (25, 26), we assumed a 20% relative decrease of IGF-I by CEE+placebo and sequential MPA and a 10% relative increase by E2+placebo and sequential MPA after 12 months with a correlation between baseline and 12 months of 0.8. From our previous series (18, 27), we estimated a baseline IGF-I value (mean \pm SD) of 19.7 ± 6.6 nmol/liter. Our hypothesis was that oral CEE and fenretinide would not lead to an additional reduction of IGF-I levels, whereas we anticipated a 15% relative decrease in IGF-I from baseline with transdermal E2 and fenretinide (18, 27). The number of subjects necessary to detect such an interaction with 80% power and two-tailed 5% significance level was 56/group based on two-way ANOVA model. In the absence of an interaction the power to detect the main effect of fenretinide was 98%, and the comparison of transdermal E2 with oral CEE had a power of 99%. Although the observed SDs in IGF-I at baseline and 12 months were slightly lower than assumed, at 5.3 and 6.2 nmol/liter, respectively, the correlation was also lower at 0.6. These effects cancel each other out approximately and mean that the preintervention sample size calculations remained adequate. Whereas percentage of mammographic density is an increasingly recognized risk biomarker for breast cancer (28), its changes during hormone intervention have not yet been associated with changes in breast cancer risk. Moreover, the current study had an exploratory value, because the actual sample size was not sufficient to detect significant differences between groups. However, based on its predicted endocrine effects, we anticipated a greater increase in mammographic density for those subjects on transdermal E2 than for those on oral CEE. No change with fenretinide was anticipated based on a previous pilot study in breast cancer survivors (29). The study of mammographic percentage of density could also provide additional clues on the association between baseline IGFs and SHBG levels and mammographic percentage of density, because this biomarker mainly represents breast stromal component (30), and associations between mammographic percentage of density and

IGFs in blood (31) and tissue (30), as well as with circulating SHBG (32) have been reported recently.

Randomization. Randomization was centrally performed by telephone at the European Institute of Oncology, using permuted blocks of four and stratified for the four participating centers. Women were assigned on an individual basis to one of four treatment groups. They remained on the same allocation throughout the study. A computer-generated randomization list was drawn up by the statistician and given to the data manager. Clinicians enrolling participants contacted the data manager via a centralized phone call to check eligibility. The data manager then allocated the next available number on entry into the trial, and each woman received the drugs directly from the clinician. The code was revealed to the researchers once recruitment, data collection, and laboratory analyses were complete.

Statistical Methods. The analysis of IGF-I, IGFBP-3, IGF-I:IGFBP-3, and SHBG was carried out according to the "intention to treat" approach. The "last observation carried forward" technique was adopted to deal with missing data (33). Logarithmic transformation was applied to IGF-I, IGFBP-3, their molar ratio, and SHBG. The analysis of mammographic percentage of density was performed on paired mammograms, those subjects with missing films being excluded from analysis.

Linear regression models were used to investigate the relationships between IGF-I, IGFBP-3, IGF-I:IGFBP-3, SHBG, and mammographic percentage of density and the main subject characteristics at baseline. Characteristics were age, age at menarche, parity, age at menopause, months since menopause, 5-year Gail risk (34), height, weight, body mass index (BMI), waist:hip girth ratio, current smoking status, family history of breast/ovarian cancers, or cardiovascular disease in first degree relatives. A forward regression procedure was adopted. The associations between IGF-I, IGFBP-3, IGF-I:IGFBP-3, SHBG, and mammographic percentage of density were calculated by Spearman's correlation coefficients.

Treatment effects on the study biomarkers were analyzed in two ways. First, we analyzed the percentage change in circulating biomarkers and the absolute change in mammographic density, because this is an easier way to express treatment effects on outcome measures and also because we powered the study on these end points. Differences among the treatment arms were calculated using the Kruskal-Wallis test for the median percentage change of the blood biomarkers and the ANOVA test for the absolute change in mammographic percentage of density. Bootstrap confidence intervals for the median percentage changes were based on 5000 bootstrap samples (35). Second, the logarithms of the values of IGF-I, IGFBP-3, IGF-I:IGFBP-3, and SHBG at 6 and 12 months were used as outcome measures in repeated measures analysis of covariance models, where the potential confounding effect of the general characteristics of the participants was evaluated and, whenever significant, included into the models. For mammographic percentage of density, the 1-month measure was the main end point of the analysis of covariance model, because the 6-month measure was not available. Because there was no interaction between estrogen route and fenretinide on any outcome measure, results are presented mainly as the two main contrasts, namely, oral CEE *versus*

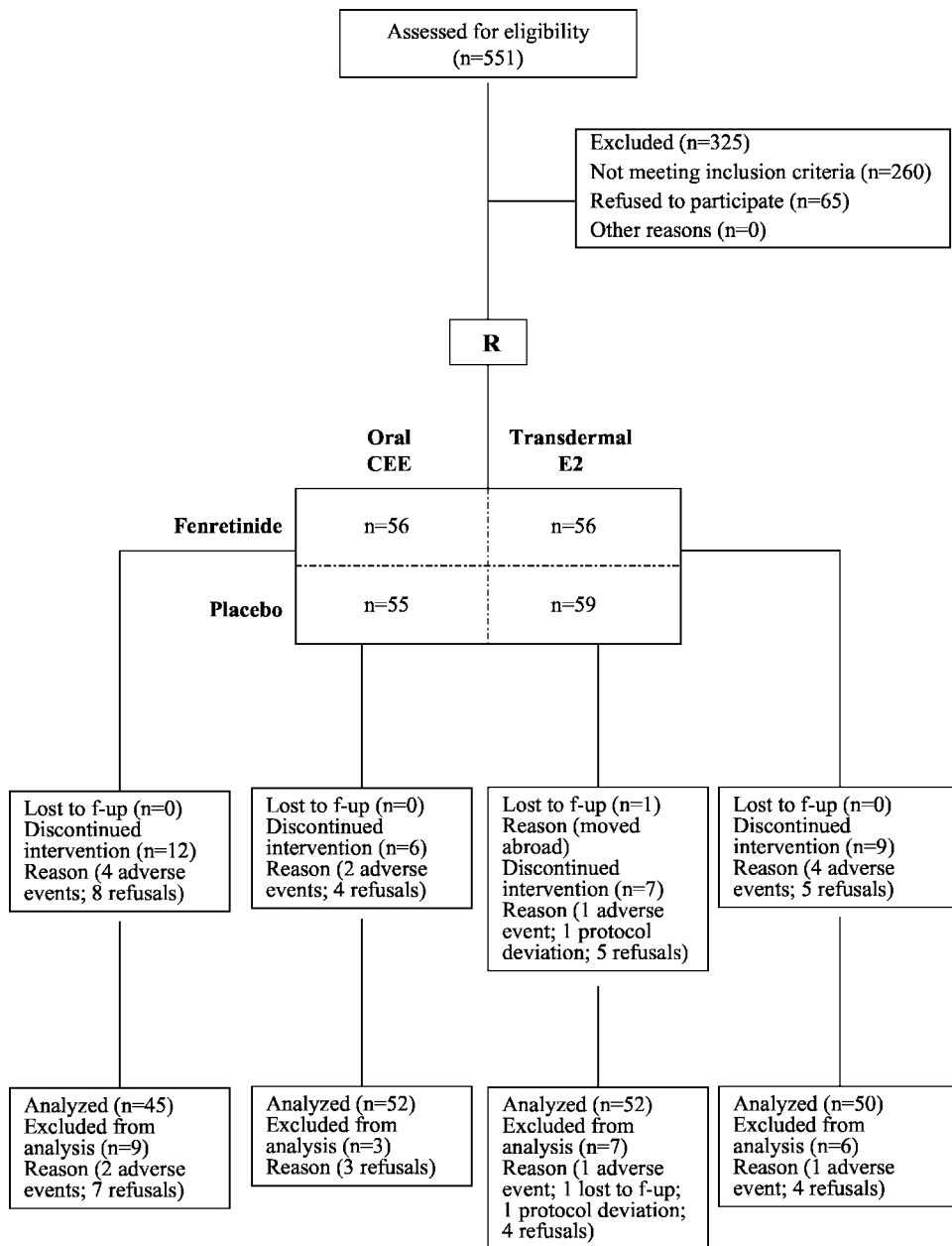


Fig. 1 Participant flow diagram. All of the subjects received sequential medroxyprogesterone acetate, 10 mg/day for 12 days each cycle.

transdermal E2 and fenretinide *versus* placebo. All of statistical analysis was carried out with SPLUS 2000 (MathSoft Inc.).

RESULTS

Subject Characteristics and Baseline Biomarkers.

From September 1, 1998 to October 1, 2000, 551 subjects were screened and 226 subjects were randomized into the trial. A description of the participant flow diagram is summarized in Fig. 1. As of April 30, 2002, when the trial ended, 34 subjects had dropped out due to adverse events ($n = 11$), voluntary withdrawals ($n = 22$), or protocol deviation (1 subject allocated to transdermal E2 received oral CEE). The 11 cases of adverse events were the following: 1 abdominal pain, 2 weight gain

>10%, 3 dermatological (1 skin rash, 1 allergy to the patch, and 1 cheilitis), 1 ocular edema, 1 blurred-vision and night blindness, 1 phlebitis, 1 edema of the lower arm, and 1 hypertriglyceridemia.

The distribution of the main subject characteristics and the baseline levels of circulating biomarkers according to the treatment groups are shown in Table 1. All of the variables were evenly distributed among groups. There were negative associations between IGF-I and age ($P = 0.01$), IGFBP-3, and the time since last menstrual period ($P = 0.03$), and IGF-I:IGFBP-3 and age ($P = 0.02$) and BMI ($P = 0.05$). Also, SHBG was negatively associated with BMI ($P < 0.001$) and age at menopause ($P = 0.04$). Mammographic percentage of density was nega-

Table 1 Characteristics of study participants (mean \pm SD) and main outcome measures (mean and 95% CI^a) at baseline by treatment group^b

	Treatment group			
	CEE+placebo (n = 55)	CEE+fenretinide (n = 56)	E2+placebo (n = 59)	E2+fenretinide (n = 56)
Age (years)	51.9 \pm 3.3	52.6 \pm 3.3	52.5 \pm 3.1	52.9 \pm 3.5
Age at menarche	12.8 \pm 1.5	12.8 \pm 1.7	12.7 \pm 1.4	12.5 \pm 1.5
Age at menopause	49.8 \pm 3.2	50.6 \pm 3.0	50.4 \pm 2.9	50.7 \pm 3.0
Months since menopause	22.0 \pm 16.5	20.4 \pm 14.6	22.3 \pm 14.3	23.5 \pm 16.0
5-year Gail risk (%)	1.22 \pm 0.34	1.22 \pm 0.37	1.21 \pm 0.33	1.28 \pm 0.53
BMI (kg/m ²)	24.5 \pm 3.5	23.9 \pm 3.6	25.4 \pm 4.7	25.0 \pm 3.6
Waist:hip ratio	0.81 \pm 0.06	0.82 \pm 0.07	0.82 \pm 0.08	0.83 \pm 0.10
Current smoker (%)	18	27	20	39
IGF-I (nmol/liter)	17.8 (16.4–19.3)	16.2 (14.8–17.8)	16.2 (14.8–17.8)	16.6 (15.3–18.0)
IGFBP-3 (nmol/liter)	81.5 (76.9–86.5)	81.4 (76.7–86.3)	78.4 (72.7–84.6)	85.7 (80.9–90.7)
IGF-I:IGFBP-3	0.22 (0.20–0.23)	0.20 (0.18–0.22)	0.21 (0.19–0.22)	0.19 (0.18–0.21)
SHBG (nmol/liter)	53.2 (46.8–60.4)	56.5 (50.1–63.9)	50.7 (45.2–57.0)	51.6 (46.0–57.9)

^a CI, confidence interval; CEE, conjugated equine estrogen; E2, estradiol; BMI, body mass index; IGF-I, insulin-like growth factor-I; IGFBP, insulin-like growth factor-binding protein-3; SHBG, sex-hormone binding protein; MPA, medroxyprogesterone acetate.

^b All subjects received sequential MPA, 10 mg/day for 12 days each cycle.

tively associated with BMI ($P < 0.001$), which explained 23% of its variation, and with IGFBP-3 ($P = 0.03$), but not with IGF-I and IGF-I:IGFBP-3. Finally, SHBG was negatively associated with IGF-I and IGFBP-3 ($P = 0.01$ and $P < 0.001$, respectively) and was positively associated with mammographic percentage of density ($P < 0.001$).

Percentage Changes in Circulating Biomarkers during Treatment. The median percentage changes in the circulating biomarker levels from baseline to 6 months and 12 months in the four treatment groups is shown in Table 2. Most effects were already evident after 6 months of treatment. There was a statistically significant difference in IGF-I ($P < 0.001$), IGF-I:IGFBP-3 molar ratio ($P < 0.001$), and SHBG ($P < 0.001$) between oral CEE and transdermal E2 (Table 2). Specifically, oral CEE and sequential MPA decreased the median IGF-I by 26% (95% CI, 22–30%) and increased the median SHBG by

96% (95% CI, 79–112%) relative to baseline, whereas no change occurred with transdermal E2 and sequential MPA. A slight difference in the median percentage change in IGFBP-3 was found as a result of a mild decrease by fenretinide relative to placebo ($P = 0.04$).

Repeated Measures Analysis of Circulating Biomarkers during Treatment. Women on transdermal E2 had higher IGF-I levels at 6 and 12 months compared with oral CEE ($P < 0.001$), whereas fenretinide had no effect compared with placebo ($P = 0.39$) nor was there any interaction between fenretinide and estrogen route ($P = 0.11$). For a woman with median IGF-I level (17 nmol/liter) and median age (52 years), the model predicted a 28% relative decrease (95% CI, 25–32%) in IGF-I at 12 months on oral CEE and a 6% relative decrease (95% CI, 0–9%) on transdermal E2 (Fig. 2).

Levels of IGFBP-3 under transdermal E2 were not statis-

Table 2 Median percentage change and 95% CI^a in IGF-I, IGFBP-3, IGF-I:IGFBP-3, and SHBG from baseline to 6 and 12 months by treatment group^b

		6 months			12 months		
		Median	(95% CI)	<i>P</i>	Median	(95% CI)	<i>P</i>
IGF-I (nmol/liter)	CEE	-28.7	(-31.8 to -25.2)	<0.001	-25.6	(-30.3 to -21.7)	<0.001
	E2	-0.5	(-5.6 to 0.0)		-0.4	(-2.8 to 0.0)	
	Fenretinide	-11.5	(-18.8 to -2.8)	0.51	-10.8	(-17.7 to -3.3)	0.32
	Placebo	-17.8	(-24.1 to -8.6)		-17.5	(-23.4 to -8.7)	
IGFBP-3 (nmol/liter)	CEE	-4.8	(-8.1 to 0.0)	0.24	-3.1	(-8.6 to 0.0)	0.09
	E2	-7.1	(-9.4 to -3.0)		-7.6	(-11.8 to -4.5)	
	Fenretinide	-8.3	(-11.9 to -5.3)	0.04	-8.3	(-11.9 to -3.2)	0.04
	Placebo	-3.7	(-7.4 to 0.0)		-3.1	(-8.4 to 0.0)	
IGF-I:IGFBP-3	CEE	-22.9	(-29.2 to -17.6)	<0.001	-23.9	(-28.1 to -19.0)	<0.001
	E2	2.6	(0.0–6.8)		3.9	(0.0–9.6)	
	Fenretinide	-2.9	(-11.8 to 0.0)	0.17	-2.4	(-13.4 to 0.0)	0.08
	Placebo	-10.0	(-16.4 to -4.0)		-9.7	(-16.2 to -3.4)	
SHBG (nmol/liter)	CEE	85.9	(71.3–107.5)	<0.001	95.6	(78.9–111.6)	<0.001
	E2	-2.0	(-5.9 to 0.0)		-3.8	(-8.1 to 0.0)	
	Fenretinide	2.7	(0.0–18.1)	0.36	4.9	(0.0–20.9)	0.53
	Placebo	10.9	(0.0–25.3)		12.8	(0.0–30.7)	

^a CI, confidence interval; CEE, conjugated equine estrogen; E2, estradiol; IGF-I, insulin-like growth factor-I; IGFBP, insulin-like growth factor-binding protein-3; SHBG, sex-hormone binding protein; MPA, medroxyprogesterone acetate.

^b All subjects received sequential MPA, 10 mg/day for 12 days each cycle.

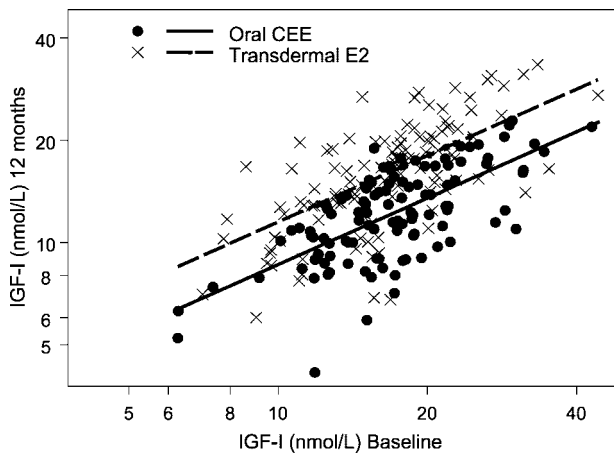


Fig. 2 Relationship between baseline and post-treatment (12 months) insulin-like growth factor-I (IGF-I) levels. The solid black line corresponds to the regression line of the predicted values of IGF-I on oral conjugated equine estrogen (CEE; ●); the dotted line corresponds to the regression line of the predicted values of IGF-I on transdermal E2 (×). All of the subjects received sequential medroxyprogesterone acetate, 10 mg/day for 12 days each cycle. Predicted IGF-I values were obtained from analysis of covariance for post-treatment IGF-I values for a woman with median age of 52 years. Note the log scale on both axes.

tically significantly different from oral CEE at 6 months ($P = 0.21$), but there was a trend toward lower IGFBP-3 levels at 12 months relative to oral CEE ($P = 0.06$). There was no statistically significant effect of fenretinide relative to placebo using this model ($P = 0.10$), although the results do not differ substantially from the percentage of change analysis. For a woman with median IGFBP-3 (84 nmol/liter), the model predicted a 6% relative decrease (95% CI, 4–9%) in IGFBP-3 at 12 months on oral CEE and a 10% relative decrease (95% CI, 8–13%) on transdermal E2 (data not shown).

Women on transdermal E2 exhibited higher values of IGF-I:IGFBP-3 at 6 and 12 months compared with those on oral CEE ($P < 0.001$). Fenretinide did not change IGF-I:IGFBP-3 levels at 6 and 12 months compared with placebo ($P = 0.10$), and its effect was not different depending on estrogen route ($P = 0.09$). For a woman with median IGF-I:IGFBP-3 (0.21) and median age (52 years), the model predicted a 24% relative decrease (95% CI, 20–28%) in IGF-I:IGFBP-3 at 12 months on oral CEE and a 6% relative increase (95% CI, 1–11%) on transdermal E2 (data not shown).

Treatment with oral CEE increased SHBG levels at 6 and 12 months compared with transdermal E2 ($P < 0.001$), whereas fenretinide showed no difference compared with placebo ($P = 0.17$) nor did it affect SHBG depending on HRT route ($P = 0.35$). For a woman with median SHBG (56 nmol/liter) and median BMI (24 kg/m²), the model predicted a 85% relative increase (95% CI, 75–95%) in SHBG at 12 months on oral CEE and a 8% relative decrease (95% CI, 3–13%) on transdermal E2 (Fig. 3).

Change of Mammographic Percentage of Density during Treatment. The mean levels of mammographic percentage of density at baseline and 12 months and their absolute change after 12 months of treatment are reported in Table 3. In

total, there was a mean absolute increment of 3.5% (95% CI, 2.5–4.6%; $P < 0.001$) after 12 months of HRT but no statistically significant difference among the four groups ($P = 0.39$). Likewise, there was no difference between oral CEE and transdermal E2 ($P = 0.33$) or between fenretinide and placebo ($P = 0.29$). The analysis of covariance gave similar results.

Treatment Compliance and Adverse Events. Assessment of compliance by pill count showed >80% compliance in 194 subjects (86%), whereas 10 subjects (4.4%) had 50–80% compliance, 5 subjects (2.2%) had <50% compliance, and 17 subjects (7.6%) were not assessable because they did not return study medications.

The number of subjects reporting at least one adverse event was 27, 34, 27, and 36 in the CEE+placebo, CEE+fenretinide, E2+placebo, and E2+fenretinide groups, respectively ($P = 0.14$ among groups; $P = 0.03$ between fenretinide and placebo), whereas the total number of adverse events was 62, 81, 51, and 84, respectively. Two subjects experienced breast cancer (1 on E2+placebo and the other on E2+fenretinide), whereas the vast majority of the remaining adverse events were grade 1 or 2, those occurring in >10% of subjects being weight gain (12 on CEE and 7 on E2), dry skin, skin rash, and desquamation (30 on fenretinide and 11 on placebo), nausea or dyspepsia (22 on fenretinide and 11 on placebo), ocular/visual symptoms (19 on fenretinide and 9 on placebo), and pain (headache/backache, 14 on CEE and 12 on E2).

DISCUSSION

In contrast to oral CEE, the risk of breast cancer and CVD associated with E2 administered by transdermal route is less known. However, the Million Women Study has suggested recently that the risk of breast cancer associated with use of

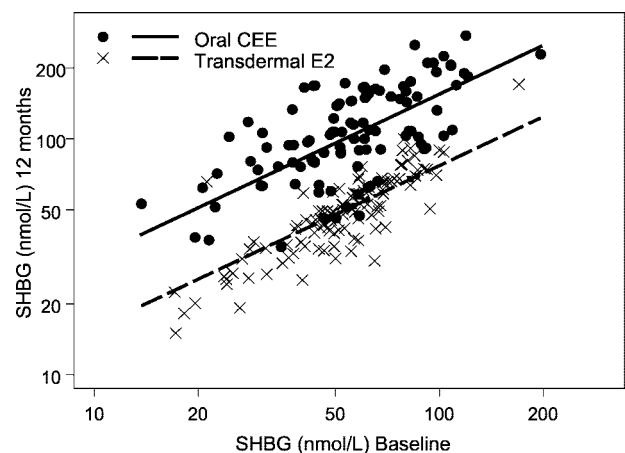


Fig. 3 Relationship between baseline and post-treatment (12 months) sex-hormone binding protein (SHBG) levels. The solid black line corresponds to the regression line of the predicted values of SHBG on oral conjugated equine estrogen (CEE; ●); the dotted line corresponds to the regression line of the predicted values of SHBG on transdermal estradiol (E2; ×). All of the subjects received sequential medroxyprogesterone acetate, 10 mg/day for 12 days each cycle. Predicted SHBG values were obtained from analysis of covariance for post-treatment SHBG values for a woman with median body mass index of 24 kg/m². Note the log scale on both axes.

Table 3 Mean mammographic percent density and 95% CI^a at baseline and 12 months and absolute change by treatment group^b

	n	Percentage breast density					
		Baseline		12 months		Absolute difference	
		Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)
CEE	73	30.3	(26.8–33.8)	33.3	(29.8–36.7)	3.0	(1.5–4.4)
E2	76	28.4	(24.9–31.9)	32.4	(28.9–35.9)	4.0	(2.5–5.5)
Fenretinide	71	28.3	(24.8–31.8)	31.2	(27.8–34.6)	2.9	(1.5–4.3)
Placebo	78	30.3	(26.7–33.8)	34.3	(30.9–37.8)	4.1	(2.5–5.6)

^a CI, confidence interval; CEE, conjugated equine estrogen; E2, estradiol; MPA, medroxyprogesterone acetate.

^b All subjects received sequential MPA, 10 mg/day for 12 days each cycle.

transdermal route is not higher than with oral estrogen (3). Moreover, unlike oral CEE and sequential MPA, transdermal E2 and sequential MPA do not increase ultrasensitive C-reactive protein (9), a risk biomarker of CVD, which may directly enhance vascular inflammation (36, 37). Because transdermal E2 does not seem to be associated with an increase in venous thromboembolism relative to oral estrogen therapy (8), its use might have a safer profile on CVD risk in women receiving HRT for menopausal symptoms or osteoporosis.

To provide additional insight into the effects of transdermal E2 on breast cancer risk, we have compared the changes induced by transdermal E2 and oral CEE administered in a continuous sequential regimen on risk biomarkers of breast cancer, including high levels of IGF-I (11, 12), low levels of IGFBP-3 (13), high IGF-I:IGFBP-3 (11, 13), low levels of SHBG (14), and high percentage mammographic density (15, 16). Secondly, we assessed the effect of fenretinide during HRT use given its potentially favorable clinical effect on second breast malignancy in premenopausal women but not in postmenopausal women (17), in line with the notion that sex hormones sensitize breast cancer cells to the retinoid-induced growth inhibition (38, 39).

Our results indicate that oral CEE and transdermal E2 given with sequential MPA have different effects on circulating IGF-I and SHBG. In contrast, no differences were observed on mammographic percentage of density, nor were changes of IGFBP-3 found with either estrogen route, whereas a reduction of IGFBP-3 was observed during fenretinide, which otherwise had no effect on the remaining circulating biomarkers. Baseline IGF-I and IGFBP-3 levels were negatively associated with SHBG levels, and IGFBP-3 and SHBG were negatively and positively associated with mammographic percentage of density, respectively. These results are in line with previous literature data (31, 32, 40), thus providing internal consistency to our results.

The difference between oral CEE and transdermal E2 on IGF-I and SHBG supports the notion that transdermal E2 and oral CEE have different hormonal and metabolic effects, presumably as a consequence of the elevated concentration of oral estrogens at first-pass hepatic level (41, 42). In contrast, no substantial difference in IGFBP-3 was noted between oral CEE and transdermal E2, in agreement with the notion that circulating IGFBP-3 is not under strict hormonal control (43). Because high IGF-I and low SHBG levels are associated with increased breast cancer risk (11, 13, 14), our results may suggest that transdermal E2 confers a greater risk of breast cancer than oral

CEE. Studies have shown that a 30% difference in IGF-I levels between top and bottom tertiles is associated with a 2-fold risk of breast cancer only in premenopausal women (11, 12), but an association between high IGF-I levels and breast cancer risk was observed only in postmenopausal women who were on HRT in a recent study (44). Thus, higher IGF-I levels seem to be associated with breast cancer risk in the presence of adequate levels of sex-steroid hormones, consistent with the notion that IGF-I interacts with the estrogen signal to increase breast cell proliferation (45, 46). Regarding SHBG, a recent meta-analysis of nine prospective studies (14) has shown an association between low SHBG levels and increased breast cancer risk, but the all-studies relative risk estimate associated with a doubling in SHBG was not statistically significant after adjustment for estradiol level (0.91; 95% CI, 0.80–1.06). Also, use of HRT substantially attenuated the difference in relative risk for breast cancer (14). Hence, the doubling of SHBG observed in the present trial with oral CEE relative to transdermal E2 may be associated with a marginal risk reduction of breast cancer.

In contrast to the observed differences in circulating IGF-I and SHBG, mammographic percentage of density, a risk biomarker more directly related to the target organ, was not affected differently by oral CEE and transdermal E2. Importantly, the value of mammographic percentage of density analysis is undermined by the lack of a HRT untreated control group. Also, the mean increases by 3.0% (95% CI, 1.5–4.4%) after 12 months of oral CEE and by 4.0% (95% CI, 2.5–5.5%) after transdermal E2 and sequential MPA may be underestimated given the recent menopausal status of most subjects, a time period associated with a greater decline in mammographic percentage of density (24). However, our results are similar to those reported in the Postmenopausal Estrogen/Progestin Intervention trial using a comparable computerized method (47) where the mean absolute changes in mammographic percentage of density after 12 months ranged from –0.07% (95% CI, –1.5 to 1.4%) on placebo up to 4.8% (95% CI, 3.3–6.2%) on oral CEE and sequential MPA.

According to previous studies by Boyd *et al.* (15), an increase of 1% in computerized percentage of density corresponds to a 2% increase in the relative risk of breast cancer. Our results indicate that oral CEE or transdermal E2 plus sequential MPA administered for 12 months increase mammographic percentage of density by 3–4%. Because changes in mammographic percentage of density occur during the first year of HRT and tend to plateau thereafter (48), our data seem to be consist-

ent with the 9% increased risk of breast cancer observed under oral CEE and MPA in the WHI trial in previously untreated women (5). Large randomized clinical trials are warranted to validate computerized mammographic percentage of density as a surrogate end point biomarker for breast cancer prevention.

Our results show that fenretinide exerted little modulation on most breast cancer biomarkers. Whereas the decline of IGFBP-3 is in line with our recent data in premenopausal women with prior breast cancer (13), we did not observe a similar decline of IGF-I. The lack of modulation of IGF-I by fenretinide among women on HRT may be explained assuming that the pharmacological effects of HRT have interfered with the ability of fenretinide to inhibit IGF-I synthesis, in contrast with the physiological interaction with endogenous estrogen levels observed in premenopausal women (17, 19). Moreover, extrapolations from recent *in vitro* studies indicate that a daily dose of 200 mg may not achieve growth-inhibitory (apoptotic) concentrations at the target tissue, *i.e.*, $>5 \mu\text{M}$ (49, 50), which are about five times those attained in the blood with 200 mg/day (51). Treatment with fenretinide was well tolerated, and compliance to all of the treatments was elevated. The retinoid showed a statistically significant excess of mild adverse events that were expected from previous reports (52).

The clinical implications of our findings still remain unsettled. Our results may in the first place suggest that transdermal E2 and sequential MPA is associated with a higher risk of breast cancer compared with oral CEE and sequential MPA because of the different effects on IGF-I and SHBG levels. However, the role of SHBG and IGF-I as risk biomarkers in HRT users is unclear (14, 44), and no difference in mammographic percentage of density was noted between oral CEE and transdermal E2 with sequential MPA, consistent with the similar risk of breast cancer noted in the Million Women Study (3). Notably, our study was conducted in women who received HRT for menopausal symptoms who may have lower endogenous hormone levels and, therefore, be at lower risk of breast cancer compared with asymptomatic women. Indeed, in the WHI trial the women allocated to placebo who had used HRT before the study developed fewer breast cancers than those who never used HRT (5).

In the context of the global effects of HRT on women's health, which includes an excess of CVD with oral CEE and MPA (4) and the neutral effect of transdermal E2 and MPA on ultrasensitive C-reactive protein (9) and venous thrombosis (8), our results might favor the use of transdermal E2 over oral CEE for the period necessary to relieve menopausal symptoms, particularly in women at increased risk for CVD. Additional studies are necessary to support our contention. Our results also suggest that fenretinide is not an active antidote for reducing breast cancer risk promoted by HRT use. By contrast, tamoxifen has shown a statistically significant reduction of breast cancer relative to placebo in women on estrogen therapy in a primary prevention trial (53), and a phase III trial addressing this *post-hoc* finding is currently taking place (54).

In conclusion, our study indicates that transdermal E2 exerts different effects than oral CEE on circulating IGF-I and SHBG but is associated with a similar increase on mammographic percentage of density. The addition of fenretinide provided little modulation on these biomarkers. Because transder-

mal E2 may be safer than oral CEE at cardiovascular level, additional clinical studies are warranted to assess the effects of transdermal E2 on breast cancer risk.

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REFERENCES

- Grodstein F, Stampfer MJ, Colditz GA, et al. Postmenopausal hormone therapy and mortality. *N Engl J Med* 1997;336:1769–75.
- Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. *Lancet* 1997;350:1047–59.
- Beral V, Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 2003;362:419–27.
- Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *J Am Med Assoc* 2002;288:321–33.
- Chlebowski RT, Hendrix SL, Langer RD, et al. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. *J Am Med Assoc* 2003;289:3243–53.
- Cauley JA, Robbins J, Chen Z, et al. Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. *J Am Med Assoc* 2003;290:1729–38.
- Chlebowski RT, Wactawski-Wende J, Ritenbaugh C, et al. Estrogen plus progestin and colorectal cancer in postmenopausal women. *N Engl J Med* 2004;350:991–1004.
- Scarabin PY, Oger E, Plu-Bureau G. Differential association of oral and transdermal oestrogen-replacement therapy with venous thromboembolism risk. *Lancet* 2003;362:428–32.
- Decensi A, Omodei U, Robertson C, et al. Effect of transdermal estradiol and oral conjugated estrogen on C-reactive protein in retinoid-placebo trial in healthy women. *Circulation* 2002;106:1224–8.
- Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med* 2002;347:1557–65.
- Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet* 1998;351:1393–6.
- Toniolo P, Bruening PF, Akhmedkhanov A, et al. Serum insulin-like growth factor-I and breast cancer. *Int J Cancer* 2000;88:828–32.
- Decensi A, Veronesi U, Miceli R, et al. Relationships between plasma IGF-I and IGFBP-3 and second breast cancer risk in a prevention trial of fenretinide. *Clin Cancer Res* 2003;9:4722–9.
- Key T, Appleby P, Barnes I, et al. The Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst* 2002;94:606–16.
- Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. *J Natl Cancer Inst* 1995;87:670–5.
- Byrne C, Schairer C, Wolfe J, et al. Mammographic features and breast cancer risk: effects with time, age, and menopause status. *J Natl Cancer Inst* 1995;87:1622–9.

17. Veronesi U, De Palo G, Marubini E, et al. Randomized trial of fenretinide to prevent second breast malignancy in women with early breast cancer. *J Natl Cancer Inst* 1999;91:1847–56.
18. Torrisi R, Pensa F, Orengo MA, et al. The synthetic retinoid fenretinide lowers plasma insulin-like growth factor I levels in breast cancer patients. *Cancer Res* 1993;53:4769–71.
19. Favoni RE, de Cupis A, Bruno S, et al. Modulation of the insulin-like growth factor-I system by N-(4-hydroxyphenyl)-retinamide in human breast cancer cell lines. *Br J Cancer* 1998;77:2138–47.
20. Hillard TC, Whitcroft SJ, Marsh MS, et al. Long-term effects of transdermal and oral hormone replacement therapy on postmenopausal bone loss. *Osteoporos Int* 1994;4:341–8.
21. National Cancer Institute. *Common Toxicity Criteria*. Ed. 2 ed. Bethesda, MD: National Cancer Institute, 1999.
22. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *J Am Med Assoc* 1998;280:605–13.
23. Juul A, Main K, Blum WF, Lindholm J, Ranke MB, Skakkebaek NE. The ratio between serum levels of insulin-like growth factor (IGF)-I and the IGF binding proteins (IGFBP-1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic patients. *Clin Endocrinol (Oxf)* 1994;41:85–93.
24. Boyd N, Martin L, Stone J, Little L, Minkin S, Yaffe M. A longitudinal study of the effects of menopause on mammographic features. *Cancer Epidemiol Biomark Prev* 2002;11:1048–53.
25. Weissberger AJ, Ho KK, Lazarus L. Contrasting effects of oral and transdermal routes of estrogen replacement therapy on 24-hour growth hormone (GH) secretion, insulin-like growth factor I, and GH-binding protein in postmenopausal women. *J Clin Endocrinol Metab* 1991;72:374–81.
26. Helle SI, Omsjo IH, Hughes SC, et al. Effects of oral and transdermal oestrogen replacement therapy on plasma levels of insulin-like growth factors and IGF binding proteins 1 and 3: a cross-over study. *Clin Endocrinol (Oxf)* 1996;45:727–32.
27. Torrisi R, Parodi S, Fontana V, et al. Effect of fenretinide on plasma IGF-I and IGFBP-3 in early breast cancer patients. *Int J Cancer* 1998;76:787–90.
28. Chlebowski RT, McTiernan A. Biological significance of interventions that change breast density. *J Natl Cancer Inst* 2003;95:4–5.
29. Cassano E, Coopmans dY, Ferranti C, et al. Mammographic patterns in breast cancer chemoprevention with fenretinide (4-HPR). *Eur J Cancer* 1993;29A:2161–3.
30. Guo YP, Martin LJ, Hanna W, et al. Growth factors and stromal matrix proteins associated with mammographic densities. *Cancer Epidemiol Biomark Prev* 2001;10:243–248.
31. Byrne C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankinson SE. Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. *Cancer Res* 2000;60:3744–8.
32. Boyd NF, Stone J, Martin LJ, et al. The association of breast mitogens with mammographic densities. *Br J Cancer* 2002;87:876–82.
33. Piantadosi S. Clinical trials: a methodologic perspective. In: V. Barnett, R.A. Bradley, N. I. Fisher, J. S. Hunter, J. B., Kadane, D. G., Kendall, D. W., Scott, A. F. M., Smith, J. L., Teugels, G. S. Watson (eds). New York: Wiley-Interscience Publication, 1997.
34. Gail MH, Costantino JP, Bryant J, et al. Weighing the risks and benefits of tamoxifen treatment for preventing breast cancer. *J Natl Cancer Inst* 1999;91:1829–46.
35. Davison AC, Hinkley DV. Bootstrap methods and their application. In: R. Gill, B. D. Ripley, S. Ross, M. Stein, and D. Williams (eds.). Cambridge, United Kingdom: Cambridge University Press, 1997.
36. Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, Zeiher AM. Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. *Circulation* 2000;102:1000–6.
37. Yeh ET, Anderson HV, Pasceri V, Willerson JT. C-reactive protein: linking inflammation to cardiovascular complications. *Circulation* 2001;104:974–5.
38. Rubin M, Fenig E, Rosenauer A, et al. 9-Cis retinoic acid inhibits growth of breast cancer cells and down-regulates estrogen receptor RNA and protein. *Cancer Res* 1994;54:6549–56.
39. Roman SD, Ormandy CJ, Manning DL, et al. Estradiol induction of retinoic acid receptors in human breast cancer cells. *Cancer Res* 1993;53:5940–5.
40. Pfeilschifter J, Scheidt-Nave C, Leidig-Bruckner G, et al. Relationship between circulating insulin-like growth factor components and sex hormones in a population-based sample of 50- to 80-year-old men and women. *J Clin Endocrinol Metab* 1996;81:2534–40.
41. Nachtigall LE. Emerging delivery systems for estrogen replacement: aspects of transdermal and oral delivery. *Am J Obstet Gynecol* 1995;173:993–7.
42. Vehkavaara S, Silveira A, Hakala AP, et al. Effects of oral and transdermal estrogen replacement therapy on markers of coagulation, fibrinolysis, inflammation and serum lipids and lipoproteins in postmenopausal women. *Thromb Haemost* 2001;85:619–25.
43. Bonanni B, Johansson H, Gandini S, et al. Effect of low dose tamoxifen on the insulin-like growth factor system in healthy women. *Breast Cancer Res Treat* 2001;69:21–7.
44. Kaaks R, Lundin E, Rinaldi S, et al. Prospective study of IGF-I, IGF-binding proteins, and breast cancer risk, in northern and southern Sweden. *Cancer Causes Control* 2002;13:307–16.
45. Clarke RB, Howell A, Anderson E. Type I insulin-like growth factor receptor gene expression in normal human breast tissue treated with oestrogen and progesterone. *Br J Cancer* 1997;75:251–7.
46. Lai A, Sarcevic B, Prall OW, Sutherland RL. Insulin/insulin-like growth factor-I and estrogen cooperate to stimulate cyclin E-Cdk2 activation and cell cycle progression in MCF-7 breast cancer cells through differential regulation of cyclin E and p21(WAF1/Cip1). *J Biol Chem* 2001;276:25823–33.
47. Greendale GA, Reboussin BA, Slone S, Wasilauskas C, Pike MC, Ursin G. Postmenopausal hormone therapy and change in mammographic density. *J Natl Cancer Inst* 2003;95:30–7.
48. Greendale GA, Reboussin BA, Sie A, et al. Effects of estrogen and estrogen-progestin on mammographic parenchymal density. Postmenopausal Estrogen/Progestin Interventions (PEPI) Investigators. *Ann Intern Med* 1999;130:262–9.
49. Lotan R. Retinoids and apoptosis: implications for cancer chemoprevention and therapy. *J Natl Cancer Inst* 1995;87:1655–7.
50. Erdreich-Epstein A, Tran LB, Bowman NN, et al. Ceramide signaling in fenretinide-induced endothelial cell apoptosis. *J Biol Chem* 2002;277:49531–7.
51. Formelli F, Clerici M, Campa T, et al. Five-year administration of fenretinide: pharmacokinetics and effects on plasma retinol concentrations. *J Clin Oncol* 1993;11:2036–42.
52. Camerini T, Mariani L, De Palo G, et al. Safety of the synthetic retinoid fenretinide: long-term results from a controlled clinical trial for the prevention of contralateral breast cancer. *J Clin Oncol* 2001;19:1664–70.
53. Veronesi U, Maisonneuve P, Sacchini V, Rotmensz N, Boyle P. Tamoxifen for breast cancer among hysterectomised women. *Lancet* 2002;359:1122–4.
54. Guerrieri-Gonzaga A, Galli A, Rotmensz N, Decensi A. The Italian breast cancer prevention trial with tamoxifen: findings and new perspectives. *Ann N Y Acad Sci* 2001;949:113–22.