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Baseline Serum Estradiol and Fracture Reduction During Treatment With Hormone Therapy: The Women's Health Initiative Randomized Trial

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Abstract

Purpose—To test the hypothesis that the reduction in fractures with hormone therapy (HT) is greater in women with lower estradiol levels.

Methods—We conducted a nested case-control study within the Women's Health Initiative HT Trials. The sample included 231 hip fracture case-control pairs and a random sample of 519 all fracture case-control pairs. Cases and controls were matched for age, ethnicity, randomization date, fracture history and hysterectomy status. Hormones were measured prior to randomization. Incident cases of fracture identified over an average follow-up of 6.53 years.

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Conflicts of interest

Dr. Cauley has received research support from Merck & Co, Inc, Eli Lilly & Co., Pfizer Pharmaceuticals and Novartis Pharmaceuticals. She has also received consulting fees Novartis Pharmaceuticals. Dr LaCroix serves as a consultant to Procter & Gamble and receives research grant support from Pfizer and the Alliance for Better Bone Health. Dr. Wactawski-Wende was a paid consultant on a workshop for Johnson & Johnson and has served on the speaker's bureau for Merck & Co., Inc. Dr. Bauer has received research support from Novartis Pharmaceuticals, Amgen, Proctor & Gamble Pharmaceutical Co. and Merck & Co., Inc. Dr. Cummings receives research support from Amgen, Pfizer, Novartis, Eli Lilly and Co. and consulting fees or honoraria from Eli Lilly and Co., Zelos, Merck and Co., Novartis, GlaxoSmithKline, Procter & Gamble, and Aventis. Dr. Jackson has received research support from and is on the speaker's bureau for Procter & Gamble Pharmaceuticals, has received research and conference support from Novartis, and has received an honorarium as a Continuing Medical Education speaker for Aventis/Alliance for Better Bone Health.
Dr. Wallace serves on the Data Monitoring Committees for Clinical Trials sponsored by Novartis Pharmaceuticals and Merck and Co.
Dr. Chen receives research support from Eli Lilly and Co.
Drs. Robbins and Larson have no conflicts to report.

Results—There was no evidence that the effect of HT on fracture differed by baseline estradiol (E2) or sex hormone binding globulin (SHBG). Across all quartiles of E2 and SHBG, women randomized to HT had about a 50% lower risk of fracture including hip fracture, compared to placebo.

Conclusion—The effect of HT on fracture reduction is independent of estradiol and SHBG levels.

Keywords

hormone therapy; fracture; sex steroid hormones; Women's Health Initiative.

Introduction

The Women's Health Initiative (WHI) Hormone Therapy (HT) Trials tested the effects of estrogen plus progestin in postmenopausal women with an intact uterus or estrogen alone in women with hysterectomy on a number of important chronic diseases in women [1,2]. In both trials, HT was associated with a significant decrease in total fractures including hip, lower arm/wrist and clinical vertebral fractures [3,4]. Results were similar in the estrogen plus progestin trial and in the estrogen alone trial and did not differ across a variety of *clinical* risk factors. However, despite the reduction in fractures, an increased risk of stroke and dementia for both hormone regimens and increased risk of breast cancer for combined therapy have resulted in cautious recommendations about the use of HT for treatment of osteoporosis or fracture prevention [5]. Identification of women who are most likely to benefit could improve the overall risk/benefit ratio.

In a trial of estrogen plus progestin with and without calcitriol, women with the lowest circulating estradiol levels at entry experienced greater increases in hip and whole body bone mineral density (BMD) but not spine BMD [6]. More recently, the effect of ultra low dose transdermal estradiol on bone turnover was shown to differ in women by endogenous estradiol levels [7]. Compared to women in the highest quintile of free estradiol index (FEI), those in the lowest quintile had a greater reduction in markers of bone turnover and a trend toward greater improvement in hip BMD. Taken together, these results suggest that the effects of estrogen treatment may be greater in women with the low estradiol levels. Neither of these studies, however, had significant power to estimate whether effects of hormone therapy on fracture, the most serious consequence of osteoporosis, differed by baseline level of estradiol. In the current analysis, we tested the hypothesis that HT reduces fracture risk to a greater degree in women with lower circulating estrogen levels. In a secondary analysis, we also examined the association between baseline endogenous hormone levels and risk of fracture.

Methods

Overview

Details of the WHI-HT Trials design are reported elsewhere [8,9]. In brief, 16,608 postmenopausal women with an intact uterus were randomized to either conjugated equine estrogen (CEE) 0.625 mg/d plus medroxy progesterone acetate 2.5 m/d in a single tablet (n=8506) or placebo (n=8102) (E+P) [1]. In the WHI Estrogen Alone (E alone) Trial, 10,738 postmenopausal women with prior hysterectomy were randomized to either CEE 0.625 mg/d (n=5310) or matching placebo (n=5429) [2]. All of the women were 50–79 years of age at randomization and enrolled in WHI at one of 40 US clinical centers from 1993 to 1998. The E+P trial was stopped after 5.2 years and the E-alone trial after 7.1 years of follow-up [1,2]. The protocol and consent forms were approved by the institutional review boards of all institutions and all women provided written informed consent.

Outcomes

Reports of hip, clinical vertebral, wrist/lower arm and other fractures (excluding chest/sternum, ribs, skull/face, fingers, toes, and cervical vertebrae) were ascertained by semiannual questionnaire. All reported fractures were confirmed by review of the medical records by centrally trained local adjudicators who were blinded to treatment assignment. Hip fractures underwent a second central adjudication. The agreement between central and local adjudication for hip fracture was 94%.

Nested Case-Control Study Design

The present study is a case-control study nested within the prospective design of the WHI-HT Trials. All confirmed cases of hip fracture (n=248) and all fractures (n=2596) that occurred during the trials were selected as potential cases. Potential controls (n=24,586) were selected among those not reporting fracture for the duration of their trial participation. Potential cases and controls with less than 1.2 ml of serum available at their baseline visit were excluded (n=2153). Because the outcomes were not mutually exclusive, some participants experienced both a hip and other type of fracture.

Matching was done separately with all participants with a hip fracture matched first followed by other fractures. Cases who experienced both a hip and another fracture were matched with the hip fracture. Cases and controls were matched on age at screening (+/−1 year), ethnicity (Caucasian, Black, Hispanic, American Indian, Asian, other), hormone trial randomization date (+/−1 year), prior history of any non-vertebral fracture after age 55 (y/n) and hysterectomy status at baseline (y/n). The final analytic set included 231 hip fracture case-control pairs. Other fracture case-control pairs (n=519) were then randomly selected to reach a total of 750 fracture case control pairs.

Other Measurements

Information on baseline risk factors for fractures was assessed in a standardized manner by questionnaire, interview, and clinical examination. Race/ethnicity categorization was based on self-declaration. Weight was measured on a balance beam scale while wearing indoor clothing. Height was measured with a fixed stadiometer. Weight and height were used to calculate body mass index (BMI); weight in kilograms divided by the square of height in meters. Information on falls in the past year, personal fracture history, family history of fracture, smoking, alcohol consumption, general health status and prevalent medical conditions was obtained by questionnaire. We classified physical activity on the basis of frequency and duration of walking and mild, moderate and strenuous activities in the previous week. We calculated kilocalories of energy experienced in 1 week as a metabolic equivalent (METS) (kcal hours per week per kg). Dietary calcium intake was assessed using a modification of the Block food frequency questionnaire and expressed in milligrams per day. Information on use of calcium supplements in the previous 2 weeks was obtained by an interviewer-administered medication inventory. Total calcium intake was derived from the sum of dietary and supplemental sources.

Participants were asked to bring all medications, vitamins, and supplements to the clinic for verification of current use. Information on medication use at baseline included use of estrogen, progestin, thiazide diuretics, thyroid medications and corticosteroid use. Information on past use of HT was collected by questionnaire. Women using postmenopausal hormones at the initial screening could be enrolled after a 3-month washout period. Information was collected on use of other antiresorptive agents at baseline and follow-up years 1, 3, and 6. If a woman initiated open-label use of HT or any selective estrogen receptor modulator after randomization, she was required to discontinue study medications.

Serum Measurements of Sex Hormones

A 12-hour fasting blood sample was obtained from each participant attending the initial screening visit and stored at −80° according to strict quality control procedures [10]. Stored baseline sera for measuring sex hormone concentrations were shipped on dry ice to the Reproductive Endocrine Research Laboratory (University of Southern California, Los Angeles, CA), a WHI-designated core laboratory. Estradiol concentrations were quantified using sensitive and specific radiommunoassay (RIA) following organic solvent extraction and Celite column partition chromatography [11,12]. The sensitivity of the estradiol RIA are 3 pg/ml (11.0 pmol/L). The intraassay coefficient of variation (CV) was 7.9% at 34 pg/ml (124 pmol/L) and interassay CVs were 8.0% and 12.0% at 16 pg/ml (58.7 pmol/L) and 27 pg/ml (99.1 pmol/L), respectively. Free and bioavailable estradiol concentrations were calculated using the measured estradiol and sex hormone-binding globulin (SHBG) concentrations and an assumed constant for albumin [13]. This method has been shown to have high validity [14–16].

SHBG was quantified by a solid-phase, two-site chemiluminescent immunoassay using the Immulite Analyzer (Diagnostic Products Corp, Los Angeles, CA). The solid phase is a polystyrene bead with a monoclonal antibody specific for SHBG. The intraassay CVs ranged from 4.1% to 7.7% and the interassay CVs ranged from 5.8% to 13%. The sensitivity of the SHBG assay was 0.2 nmol/L (0.005 μg/dL). (To convert pg/ml to pmol/L, multiply by 3.67; to convert μg/dl to nmol/L, multiply by 34.67). Laboratory personnel were blinded to case-control status, and samples were analyzed in random order.

Statistical Analyses

We compared the characteristics of the women by randomized group using chi-square tests for categorical variables and t-tests for continuous variables (Table 1). Characteristics of interest included those previously identified as independent risk factors for hip fracture in WHI [17]. We examined the mean biomarker by case-control status in all fracture case-control pairs and separately in hip fracture case-control pairs (Table 2 and Table 3). To evaluate the significance of the relationship between the biomarkers and hormone use status, a linear model was run for each marker, modeling the biomarker as a function of hormone use. The resulting p-value from the F-test for hormone use is presented.

To evaluate whether the effects of estradiol and SHBG are independent from the effects of hormone use on fracture, we examined the interaction between the randomization arms of the hormone trials and the biomarkers (Table 4 and Table 5). Each model contained an indicator for hormone use (E,E+P vs. Placebo), the biomarker of interest, their interaction, and was adjusted for the matching variables. Odds ratios for hormone use are presented for each level of the four biomarkers; p-values are taken from the interaction term. Models were run in the combined hormone trials with both hip fractures and total fractures (Table 4), and then in the individual hormone trials for the total fracture outcome (Table 5).

To evaluate the effect of the biomarkers on fracture outcomes, a series of unconditional logistic regression models were run for both total fractures and specifically hip fractures (Table 6 and Table 7). Sex hormones were divided into quartiles based on the distribution within the controls. First, we examined the main effect of each biomarker on fracture in a base model, adjusting for the matching factors of age, ethnicity, randomization date, fracture history, and hysterectomy status. A second set of models was then run with the addition of BMI. The correlation between BMI and estradiol in this study population was r=0.14. A third and final model was run with the additional factors of treated diabetes and self-reported health status, both of which were related to hip fracture in WHI [17]. Tests for trend were carried out using a logistic regressing model with quartiles of biomarkers coded as a continuous variable, 1 to

4. To address collinearity, a correlation matrix of the various adjustment factors as well as hormone use was examined and no strong associations that could have influenced the results were found.

In a secondary analyses, we performed a sensitivity analyses excluding any participant who reported less than 80% adherence at the time of her fracture event (for cases) or at the time of her corresponding case's fracture event (for controls). In doing this for our adherence analysis, we ended up excluding 268 controls and 317 cases. All analyses were conducted using SAS Version 9.0 (SAS Institute, Cary, NC).

Results

In this nested case-control subset, women were on average 65 years of age, 90% were Caucasian and their mean BMI was in the overweight range, Table 1. About 10% of women reported current smoking and 20% reported past history of fracture. Alcohol intake was modest with one third reporting not drinking alcohol and 10–12%, averaging 1or more drinks per day. Most of the women reported some leisure time physical activity. A history of 2 or more falls in the year before randomization was reported by about 15% of the women. The mean calcium intake was over 1000 mg/day in both groups of women. About 45% of women had a prior hysterectomy; 90% reported good or excellent health and about 5% reported treated diabetes. Few of the women reported corticosteroid use.

In this subset, women randomized to E+P or E alone had a higher body weight, and BMI, and were more likely to report past use of HT, Table 1. There was no difference in age, race, smoking status, personal or parental fracture history, alcohol consumption, physical activity, hysterectomy status or use of thiazide diuretics. There was a tendency for women in the hormone group to report two or more falls and history of diabetes and slightly lower calcium intake than women in the placebo group. Similar findings were observed when we limited the analyses to hip fracture case-control pairs (data not shown).

Sex Hormones in Cases and Controls

We initially compared sex steroid hormones within the cases and controls by randomization group, Table 2a and Table 2b. There were no differences in sex steroid hormones by randomization group in either the cases or controls. Comparing hip fracture cases and matched pairs, hip fracture cases had significantly lower free and bioavailable estradiol and higher SHBG than controls. Fracture cases had higher levels of SHBG than controls.

Sex Hormones and Intervention Effect

There was no evidence that the effect of HT on any fracture reduction or specifically hip fracture reduction differed by baseline level of estradiol, Table 3. Across all quartiles of estradiol, women randomized to HT had about a 50% lower risk of experiencing a non-spine fracture or hip fracture. This association was significant across all quartiles of estradiol for non-spine fractures but for hip fracture, only for the first quartile. Nevertheless, there was no evidence of an interaction for all fractures or for hip fractures. We further analyzed all fractures with an additional cutpoint at the 10^{th} percentile of estradiol, giving new groupings of ≤5, >5- 6, >6–10, >10–14 and >14 pg/ml. Women with the lowest estradiol levels (<10^{th} percentile, ≤5 pg/ml) did not show any evidence of a greater benefit (OR=0.55; 95% CI 0.34 to 0.91) for HT. This compares to an OR of 0.40 (95% CI 0.19–0.85) for the women with estradiol levels in the 10^{th} to 25^{th} percentile (> 5–6 pg/ml). There was also no interaction between SHBG and the hormone intervention on overall fracture reduction or hip fracture reduction. Across all quartiles of SHBG, women randomized to HT had a reduced risk of fracture, although it was only statistically significant in the highest quartile of SHBG for hip fracture.

We examined the two trials separately and tested whether the effect of E+P versus E alone on fracture reduction differed by sex steroid hormone level. These analyses are limited to total fractures because of the smaller number of hip fractures. As shown in Table 4, both the E+P and E alone trials showed similar reductions in total fracture, irrespective of baseline estradiol levels. There was a significant interaction between E+P therapy and SHBG level whereby fracture reduction was greatest in women with the highest SHBG, but there was no clear linear pattern. There was no interaction between E alone treatment and SHBG on fracture reduction. Finally, a sensitivity analysis including only adherent women revealed similar results (data not shown).

Main Effects of Sex Hormones on Fracture

We found no association between circulating estradiol and total fractures, Table 5a. However, women with the highest SHBG, (Quartile IV, > 1.67μg/dl), had a 50% increased risk of total fracture compared to women with the lowest SHBG. Adjustment for BMI attenuated the associations slightly (p trend=0.067), but in the full multivariate model women with the highest SHBG had a significant 53% increased risk of fracture.

Women with the highest free and bioavailable estradiol had about a 50% lower risk of hip fracture (Table 5b) compared to women with lowest levels. However, further adjustment for BMI attenuated these associations and they were no longer statistically significant. On the other hand, there was a stepwise increase in the risk of hip fracture across quartiles of SHBG. Women with the highest SHBG had over a four-fold increased risk of hip fracture. Additional multivariate adjustment for BMI, health status and diabetes had only a modest effect on these associations. The correlation between SHBG and bioavailable estradiol was r=−0.35 but, addition of bioavailable estradiol to the SHBG models had little effect on the association. For example, the addition of bioavailable estradiol to the multivariate models had little effect on the association between SHBG quartiles and hip fracture risk: The OR (95% CI) for hip fracture in the second (OR=2.10; 1.06, 4.20), third (OR=2.64; 1.32, 5.29) and fourth (OR=3.84; 1.84, 8.01) quartile (p trend=0.0004) in comparison to the lowest quartile.

Conclusion

The WHI-HT Trials were the largest trials of HT in postmenopausal women and were the first to show that treatment with either estrogen plus progestin or estrogen alone results in a significant reduction in hip and other fractures [3,4]. The women enrolled in WHI were generally not osteoporotic, but there was little evidence that the reduction of fractures differed across a summary fracture risk score [3,4]. In previous analyses, we tested whether the effect of HT on fracture reduction differed across individual clinical risk factors and found no evidence of a differential effect of HT on fracture across levels of risk factors [3,4]. Our current findings extend these previous results by testing whether the effect of HT on fracture differed across levels of baseline estradiol or SHBG, measured prior to randomization. Contrary to our hypothesis, there was no evidence that HT reduced fractures to a greater extent in women with the lowest estradiol levels or highest SHBG levels at entry to the study. Thus, measurement of sex steroids before treatment is unlikely to identify women who may be most likely to benefit from HT, at least at the doses and regimen that were given as part of WHI.

To our knowledge, only two previous studies have assessed whether the response to treatment with estrogen on skeletal outcomes is influenced by endogenous sex steroid levels. A total of 489 women aged 65–77 were randomized to placebo, HT (E+P or E alone depending on uterine status) or HT plus calcitriol and followed for three years [6]. Of these, 260 women were included in the longitudinal analyses. Results showed that the increase in BMD was 4–6% higher in women with serum bioavailable estradiol in the lowest tertile (<8.8 pg/ml) at study entry compared to women with the highest bioavailable estradiol (≥14.3 pg/ml). The authors

concluded that women with serum estradiol <9 pg/ml are optimal candidates for hormonal therapy for osteoporosis prevention.

In the second study, the Ultra Low Dose Transdermal Estrogen 2 year Trial, Huang et al. randomized 382 women (mean age 66 yrs) to 0.014 mg/d transdermal estradiol patch or placebo [7]. The free estradiol index (FEI) was calculated as the ratio of total estradiol to SHBG. Compared to women with the highest FEI, those in the lowest quartile had a 15–26% greater reduction in bone turnover markers in response to estrogen treatment. There was some suggestion of a greater response for hip BMD (not spine BMD) in those with lower versus higher FEI levels, but the results were not significant.

An important limitation to both of these reports is their focus on intermediate outcomes of BMD or bone turnover, neither of which can fully explain the fracture reduction observed with anti-resorptive agents [18–20]. Fractures, especially hip fractures, are the most serious consequence of osteoporosis and have been associated with a significant increase in morbidity, disability, institutionalization and mortality [21]. We found no evidence that the benefit of HT on fracture reduction depends on circulating estradiol or SHBG levels before treatment. Our results are consistent with the effect of raloxifene on vertebral fractures [22] whereby the reduction in vertebral fractures with raloxifene was similar across all levels of estradiol. In contrast, women with higher levels of endogenous estradiol experienced greater reduction in breast cancer risk with raloxifene treatment compared to women with lower levels.

Women with the highest SHBG concentration (>1.7 μg/dl) had a four-fold increased risk of hip fracture that was independent of BMI, other risk factors and estradiol in our study. These *hip* fracture results are consistent with an earlier report based on the WHI-Observational Cohort (OS) [23]. Our results, however, extend these earlier findings to include an association with all fractures: Women with the highest SHBG were also 50% more likely to experience *any* fracture. These results are consistent with an independent effect of SHBG on fracture risk. The underlying mechanism for this effect is not known. Others have suggested that SHBG may play a larger role as a mediator of multiple signaling pathways in sex hormone responsive cells [24–27]. Administration of CEE has been shown to increase SHBG [28], but the reduction in fractures with HT occurs despite the increase in SHBG. In the Tromso Osteoporosis Study women with the highest SHBG had an increased risk of fracture but this was largely explained by BMD, suggesting that effects of SHBG on fracture risk are mediated by BMD [29].

Women with higher bioavailable or free estradiol appeared to have a lower risk of hip fracture but this was attenuated in models adjusting for BMI. We found no association between estradiol and total fractures. An increased risk of hip and vertebral fractures was observed with lower estradiol levels in some [30–34] but not all studies [23,29]. In an earlier study based on the WHI-OS, the association between serum estradiol and hip fracture was not independent of SHBG levels [23].

This study has several strengths. It is the largest study testing whether the skeletal benefits of hormone therapy differs by levels of estradiol or SHBG. We focused on fractures, including hip fractures, the most important clinical consequence of osteoporosis. Our research endocrine laboratory used sensitive extraction based radioimmunoassay for measurement of estradiol. We also controlled for other important risk factors for fracture. There are however, several limitations. Only one type of estrogen formulation was tested, although it was the most commonly prescribed postmenopausal hormone therapy regimen in the US at the time the study was designed. The large pharmacologic dose of estrogen in the trial may have overwhelmed any small difference in baseline estradiol. The WHI-HT primarily enrolled Caucasian women but there was little evidence of a differential treatment effect by race/ethnicity [3,4]. BMD was

measured in only a subset of WHI women and we could not test whether the effects of HT on BMD differed across sex steroid hormone levels.

In conclusion, the reduction in fracture, including hip fracture, observed with hormone therapy is independent of circulating estradiol and SHBG levels. Measurement of sex hormone levels do not appear to be useful for identifying optimal candidates for hormone therapy as prescribed in WHI for fracture reduction.

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Table 1

Descriptive characteristics by randomization assignment: biomarker nested case-control subset.

Characteristic	E+P, E[a]	PBO[a]	P-value
Caucasian, n (%)	655 (90.8)	689 (88.4)	0.128
Age (y), mean (SD)	65.5 (7.7)	65.8 (7.3)	0.350
Menopause (y) mean (SD)	17.3 (9.6)	18.2 (9.6)	0.080
Weight (kg), mean (SD)	75.5 (17.3)	73.6 (16.0)	0.027
Height (cm), mean (SD)	161.7 (6.7)	161.7 (6.2)	0.931
BMI (kg/m^2), mean (SD)	28.8 (6.2)	28.1 (5.5)	0.011
Smoking Status, n (%)			0.236
Never	352 (48.8)	405 (52.0)	
Past	291 (40.4)	294 (37.7)	
Current	72 (10.0)	67 (8.6)	
Past hormone therapy (yes) n (%)	245 (34.0)	313 (40.2)	0.028
Positive history of fracture, n (%)	157 (21.8)	161 (20.7)	0.600
Alcohol use, n (%)			0.792
Non-drinker	232 (32.2)	237 (30.4)	
<1 drink per day	407 (56.4)	442 (56.7)	
1+ drinks per day	78 (10.8)	95 (12.2)	
Physical activity (METS), n (%)			0.365
0, inactive	114 (15.8)	133 (17.1)	
<5	152 (21.1)	171 (22.0)	
5–<12	146 (20.2)	167 (21.4)	
≥12	229 (31.8)	245 (31.5)	
Fall history (2 or more falls at baseline), n (%)	111 (15.4)	108 (13.9)	0.052
Calcium intake (mg/day), mean (SD)	1067.2 (609.4)	1130.2 (727.8)	0.070
Baseline thiazide diuretic use, n (%)	40 (5.5)	39 (5.0)	0.639
Parental history of fracture, n (%)	254 (35.2)	289 (37.1)	0.702
Hysterectomy, n (%)	316 (43.8)	366 (47.0)	0.220
Self-reported health (≥ good), n (%)	651 (90.3)	708 (90.9)	0.734
Corticosteroid use, n (%)	1 (0.1)	1 (0.1)	0.956
Treated diabetes (pills or shots), n (%)	44 (6.1)	29 (3.7)	0.064

[a]Estrogen progestin, Estrogen alone or Placebo.

Table 2

Mean +/− SD baseline biomarkers by case-control status and by randomized group: all fracture cases vs. controls.

	Cases					Controls					
	N	E+P, E Mean (SD)	N	PBO[a] Mean (SD)	P-value[b]	N	E+P, E Mean (SD)	N	PBO[a] Mean (SD)	P-value[b]	P-value[c]
Total Estradiol (pg/ml)	282	12.62 (10.40)	428	12.31 (17.36)	0.79	398	13.94 (26.14)	314	12.64 (16.46)	0.44	0.35
Free Estradiol (pg/ml)	282	0.32 (0.28)	428	0.31 (0.45)	0.65	398	0.35 (0.55)	314	0.32 (0.39)	0.45	0.32
Bioavailable E2 (pg/ml)	282	8.26 (7.21)	428	7.93 (11.30)	0.66	398	9.08 (14.64)	314	8.35 (10.17)	0.45	0.25
SHBG (μg/dl)	304	1.40 (0.71)	446	1.48 (0.75)	0.18	417	1.36 (0.68)	333	1.34 (0.69)	0.79	0.01

[a] Placebo.

[b] p-value from a linear model modeling the biomarker of interest as a function of HT status.

[c] p-value from a linear model modeling the biomarker of interest as a function of cases-control status.

Table 3

Mean +/− SD baseline biomarkers by case-control status and by randomized group: hip fracture cases vs. controls.

	Cases					Controls					
	E+P, E		PBO[a]			E+P, E		PBO[a]			
	N	Mean (SD)	N	Mean (SD)	P-value[b]	N	Mean (SD)	N	Mean (SD)	P-value[b]	P-value[c]
Total Estradiol (pg/ml)	88	11.69 (8.47)	131	10.19 (8.12)	0.19	127	12.32 (8.49)	90	13.24 (25.21)	0.70	0.15
Free Estradiol (pg/ml)	88	0.29 (0.21)	131	0.25 (0.22)	0.17	127	0.32 (0.25)	90	0.32 (0.52)	0.94	0.05
Bioavailable E2 (pg/ml)	88	7.23 (5.32)	131	6.25 (5.61)	0.20	127	8.23 (6.40)	90	8.41 (13.79)	0.90	0.03
SHBG (μg/dl)	95	1.56 (0.72)	136	1.73 (0.81)	0.09	134	1.37 (0.68)	97	1.36 (0.61)	0.84	<.0001

*[a] Placebo.

*[b] p-value from a linear model modeling the biomarker of interest as a function of HT status.

*[c] p-value from a linear model modeling the biomarker of interest as a function of cases-control status.

Table 4

Odds ratio (95% CI) of fracture among women randomized to hormone therapy compared to placebo according to levels of sex steroid hormones at baseline.

	All Fractures				Hip Fractures			
	E, E+P Cases	**PBO Cases**	**OR (95% CI) for fracture**	**Interaction p-value**	**E, E+P Cases**	**PBO Cases**	**OR (95% CI) for fracture**	**Interaction p-value**
Total Estradiol (pg/ml)				0.981				0.772
≤6	78	131	0.49 (0.33, 0.74)		25	48	0.36.(0.17, 0.79)	
>6 – 10	72	112	0.50 (0.33, 0.75)		25	32	0.62 (0.30, 1.30)	
>10 – 14	59	88	0.52 (0.32, 0.83)		16	29	0.44 (0.18, 1.06)	
>14	71	92	0.55 (0.36, 0.85)		21	22	0.55 (0.23, 1.33)	
Free Estradiol (pg/ml)				0.881				0.752
≤0.16	76	142	0.48 (0.32, 0.73)		24	54	0.34 (0.16 0.74)	
>0.16 – 0.24	61	91	0.46 (0.29, 0.72)		22	28	0.55 (0.25, 1.20)	
>0.24 – 0.39	70	100	0.57 (0.37, 0.88)		23	33	0.58 (0.26, 1.29)	
>0.39	73	90	0.56 (0.36, 0.86)		18	16	0.58 (0.23, 1.51)	
Bioavailable E2 (pg/ml)				0.937				0.854
≤4.0	84	146	0.50 (0.34, 0.74)		26	56	0.41 (0.20, 0.83)	
>4.0 – 6.1	51	80	0.46 (0.28, 0.74)		19	26	0.47 (0.20, 1.06)	
>6.1 – 9.9	73	107	0.55 (0.36, 0.84)		24	33	0.74 (0.34, 1.57)	
>9.9	72	90	0.55 (0.35, 0.85)		18	16	0.50 (0.20, 1.28)	
SHBG (μg/dl)				0.139				0.157
<0.87	62	93	0.54 (0.35, 0.84)		8	14	0.35 (0.12, 1.03)	
>0.87–1.21	94	101	0.78 (0.52, 1.17)		28	24	0.98 (0.45, 2.15)	
>1.21–1.67	70	97	0.47 (0.31, 0.72)		30	36	0.60 (0.29, 1.23)	
>1.67	76	150	0.41 (0.27, 0.61)		28	62	0.31 (0.15, 0.64)	

Adjusted for age, ethnicity, randomization date, fracture history and hysterectomy status.

Table 5

Odds radio (95% CI) of fracture among women randomized to hormone therapy compared to placebo according to levels of sex steroid hormone at baseline: E+P vs E Alone Trial.

	E+P Trial				E Alone Trial			
	E+P Cases	**PBO Cases**	**OR (95% CI) for fracture**	**Interaction p-value**	**E Cases**	**PBO Cases**	**OR (95% CI) for fracture**	**Interaction p-value**
Total Estradiol				0.630				0.453
≤6	47	68	0.61 (0.35, 1.04)		31	63	0.38 (0.20, 0.71)	
>6 – 10	39	62	0.37 (0.21, 0.65)		33	50	0.64 (0.35, 1.15)	
>10 – 14	34	55	0.49 (0.26, 0.91)		25	33	0.56 (0.27, 1.18)	
>14	37	51	0.42 (0.23, 0.76)		34	41	0.78 (0.41, 1.51)	
Free Estradiol				0.704				0.495
≤0.16	42	76	0.57 (0.33, 0.97)		34	66	0.39 (0.21, 0.73)	
>0.16 – 0.24	36	48	0.36 (0.19, 0.67)		25	43	0.58 (0.30, 1.12)	
>0.24 – 0.39	41	62	0.53 (0.29, 0.97)		29	38	0.64 (0.33, 1.22)	
>0.39	38	50	0.43 (0.24, 0.78)		35	40	0.78 (0.40, 1.52)	
Bioavailable E2				0.545				0.467
≤4.0	47	76	0.62 (0.37, 1.05)		37	70	0.39 (0.22, 0.70)	
>4.0 – 6.1	30	43	0.33 (0.17, 0.65)		21	37	0.63 (0.30, 1.29)	
>6.1 – 9.9	42	66	0.49 (0.27, 0.87)		31	41	0.65 (0.34, 1.23)	
>9.9	38	51	0.43 (0.24, 0.77)		34	39	0.76 (0.39, 1.47)	
SHBG (μg/dl)				0.006				0.986
< 0.87	33	46	0.46 (0.25, 0.87)		29	47	0.61 (0.33, 1.12)	
> 0.87–1.21	54	54	1.01 (0.58, 1.75)		40	47	0.57 (0.31, 1.06)	
> 1.21–1.67	39	54	0.34 (0.19, 0.62)		31	43	0.65 (0.35, 1.22)	
> 1.67	38	88	0.27 (0.16, 0.47)		38	62	0.67 (0.36, 1.25)	

Adjusted for age, ethnicity, randomization date, fracture history and hysterectomy status.

Table 6

Odds ratio (95% CI) of fracture according to levels of sex steroid hormone at baseline: all fractures main effects.

All Fracture	Cases	Controls	Model 1 Base Model[a] OR (95% CI) for fracture	p trend	Model 2 Additional Adjustment for BMI OR (95% CI) for fracture	p trend	Model 3 Full MV Model[b] OR (95% CI) for fracture	p trend
Total Estradiol (pg/ml)				0.170		0.256		0.213
≤6	209	179	1.00		1.00		1.00	
>6 – 10	184	213	0.73 (0.55, 0.97)		0.76 (0.57, 1.01)		0.74 (0.56, 0.99)	
>10 – 14	147	140	0.90 (0.66, 1.22)		0.94 (0.69, 1.29)		0.93 (0.68, 1.28)	
>14	163	172	0.80 (0.60, 1.08)		0.87 (0.63, 1.18)		0.88 (0.64, 1.20)	
Free Estradiol (pg/ml)				0.163		0.300		
≤0.16	218	185	1.00		1.00		1.00	0.296
>0.16 – 0.24	152	176	0.73 (0.54, 0.98)		0.75 (0.56, 1.04)		0.75 (0.55, 1.01)	
>0.24 – 0.39	170	167	0.86 (0.65, 1.15)		0.91 (0.67, 1.22)		0.89 (0.66, 1.20)	
>0.39	163	176	0.78 (0.58, 1.04)		0.85 (0.62, 1.17)		0.86 (0.63, 1.19)	
Bioavailable E2 (pg/ml)				0.243		0.384		0.389
≤4.0	230	202	1.00		1.00		1.00	
>4.0+ – 6.1	131	153	0.75 (0.55, 1.01)		0.77 (0.57, 1.04)		0.77 (0.56, 1.04)	
>6.1 – 9.9	180	175	0.90 (0.68, 1.20)		0.95 (0.71, 1.27)		0.93 (0.70, 1.25)	
>9.9	162	174	0.81 (0.60, 1.08)		0.89 (0.65, 1.22)		0.91 (0.66, 1.24)	
SHBG (μg/dl)				0.026		0.067		0.048
≤0.87	155	188	1.00		1.00		1.00	
>0.87–1.21	195	187	1.28 (0.95, 1.71)		1.26 (0.93, 1.70)		1.29 (0.95, 1.74)	
>1.21–1.67	167	185	1.11 (0.82, 1.50)		1.08 (0.79, 1.48)		1.12 (0.82, 1.53)	
>1.67	226	182	1.53 (1.14, 2.06)		1.48 (1.07, 2.04)		1.53 (1.11, 2.12)	

[a] Adjusted for age, ethnicity, randomization date, fracture history and hysterectomy status.

[b] Additional adjustment for BMI, treated diabetes, self-reported health status.

Table 7

Odds ratio (95% CI) of fracture according to levels of sex steroid hormone at baseline: hip fractures main effects.

Hip Fracture	Cases	Controls	Model 1 Base Model[a] OR (95% CI) for fracture	p trend	Model 2 Additional Adjustment for BM OR (95% CI) For fracture	p trend	Model 3 Full MV Model[b] OR (95% CI) for fracture	p trend
Total Estradiol (pg/ml)				0.137		0.354		0.325
≤6	73	50	1.00		1.00		1.00	
>6 – 10	57	69	0.57 (0.34, 0.94)		0.63 (0.38, 1.05)		0.63 (0.37, 1.06)	
>10 – 14	45	46	0.67 (0.39, 1.16)		0.75 (0.43, 1.31)		0.74 (0.42, 1.31)	
>14	43	47	0.62 (0.36, 1.08)		0.83 (0.46, 1.48)		0.89 (0.49, 1.61)	
Free Estradiol (pg/ml)				0.027		0.188		0.271
≤ 0.16	78	49	1.00		1.00		1.00	
>0.16 – 0.24	50	63	0.50 (0.30, 0.84)		0.55 (0.33, 0.94)		0.58 (0.34, 1.00)	
>0.24 – 0.39	56	56	0.63 (0.37, 1.05)		0.77 (0.45, 1.31)		0.75 (0.43, 1.30)	
> 0.39	34	44	0.48 (0.27, 0.86)		0.72 (0.38, 1.35)		0.77 (0.40, 1.47)	
Bioavailable E2 (pg/ml)				0.056		0.404		0.496
≤ 4.0	82	55	1.00		1.00		1.00	
>4.0+ – 6.1	45	54	0.56 (0.33, 0.94)		0.63 (0.37, 1.08)		0.65 (0.38, 1.13)	
>6.1+ – 9.9	57	58	0.66 (0.40, 1.08)		0.80 (0.47, 1.35)		0.78 (0.46, 1.33)	
> 9.9	34	45	0.50 (0.28, 0.89)		0.75 (0.40, 1.39)		0.80 (0.42, 1.50)	
SHBG (μg/dl)				<0.001		0.004		0.002
≤ 0.87	22	52	1.00		1.00		1.00	
> 0.87–1.21	52	60	2.14 (1.14, 4.03)		1.94 (1.02, 3.70)		2.11 (1.08, 4.14)	
> 1.21–1.67	66	61	2.64 (1.43, 4.89)		2.28 (1.20, 4.34)		2.69 (1.37, 5.29)	
> 1.67	90	53	4.23 (2.28, 7.85)		3.42 (1.74, 6.71)		4.01 (1.98, 8.15)	

[a] Adjusted for age, ethnicity, randomization date, fracture history and hysterectomy status.

[b] Additional adjustment for BMI, treated diabetes, self-reported health status.