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## The effects of postmenopausal hormone therapy on serum estrogen, progesterone and sex hormone binding globulin levels in healthy post-menopausal women

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### Abstract

**Objective**—Differences in disease outcomes between users and non-users of hormone therapy (HT) and between users of estrogen alone (ET) and users of estrogen plus progesterone therapy (EPT) may relate to differences in serum hormone concentrations between these populations. In this study, we examine the response of serum hormone levels in healthy post-menopausal women after one year of HT.

**Methods**—A representative sub-sample of 200 healthy adherent participants from the active and placebo groups of the Women's Health Initiative randomized, controlled clinical trials of ET (conjugated equine estrogen 0.625 mg daily) or EPT (ET plus medroxyprogesterone acetate 2.5 mg daily) were selected for determination of selected sex hormone levels at baseline and one year after randomization.

**Results**—In participants receiving active ET intervention compared to placebo, estrogenic hormone levels increased from baseline to year 1 by 3.6-fold for total estrone, 2.7-fold for total estradiol, and 1.8-fold for bioavailable and free estradiol concentrations. Serum SHBG concentrations also increased 2.5-fold. In contrast, progesterone levels decreased slightly in women taking exogenous EPT. The response of serum estrogens and SHBG did not differ substantially with the addition of progesterone. In subgroup analyses, hormone response varied by age, ethnicity, BMI, smoking, vasomotor symptoms, and baseline hormone levels.

**Conclusions**—These data provide a reference point for the serum hormone response to HT, and demonstrate that response of serum estrogens is similar for ET and EPT. The implications of the

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slight decrease in serum progesterone levels with EPT therapy are uncertain. Potential treatment interactions for estrogenic hormones were identified, which suggest a larger response to HT in women with low endogenous levels.

### Keywords

Hormone therapy; estrogen; progesterone; estradiol; hormone levels; sex hormone binding globulin; Women's Health Initiative

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### Introduction

During the menopausal transition, ovarian production of estrogen and progesterone declines. This natural endocrine transition is associated with diminished circulating levels of estrone (E1), estradiol (E2), and sex hormone binding globulin (SHBG), and an associated increase in levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH).<sup>1</sup> Use of exogenous hormones has been reported to increase the levels of circulating estrogens and SHBG.<sup>2</sup>

Prospective data on the response of serum hormone levels to hormone therapy (HT) in healthy postmenopausal women is sparse. Much of our existing knowledge about hormone changes at the menopausal transition and beyond comes from cross-sectional studies of pre- and post-menopausal women. There are relatively few longitudinal studies of levels of estrogens in women as they pass through the menopausal transition.<sup>1,3</sup> Several more recent cross-sectional studies have examined differences in post-menopausal endogenous hormones by characteristics such as race/ethnicity,<sup>4,5</sup> as well as a few studies examining the relationship between endogenous hormone levels and specific disease outcomes, such as breast cancer<sup>6,7</sup> and hip fracture.<sup>8</sup> There have been relatively few studies that have prospectively evaluated hormone levels in women on exogenous hormone therapies,<sup>9,10,11,12</sup> and these have generally been small.

Recent evidence, including that from the Women's Health Initiative (WHI), has demonstrated significant differences in disease outcomes between users and non-users of HT that differed between estrogen and progesterone and estrogen alone.<sup>13,14</sup> It is reasonable to hypothesize that these differences relate to variations in serum sex hormone concentrations between users relative to non-users of HT, and suggest that serum hormone responses may differ between users of ET and EPT. However, the physiologic implications of variations in serum hormone levels (both endogenous and as the result of exogenous hormone administration) are still being clarified. A better understanding of the response of basic serum hormone levels to HT in healthy post-menopausal women will help inform research examining specific HT regimens and disease risk.

The WHI prospective, randomized, longitudinal controlled clinical trials of hormone therapy offer an unprecedented opportunity to examine the changes in sex steroid milieu in response to HT and to determine if the response differs with the addition of a progestin. We now report the magnitude of response of estrone, estradiol, free estradiol, bioavailable estradiol, SHBG, and progesterone to HT in a representative sub-group of healthy, adherent, post-menopausal participants selected from each of the randomized HT assignments. In addition, the results will be further examined by age, BMI, oophorectomy status, race, smoking, report of vasomotor symptoms, and baseline sex hormone levels to determine whether or not serum hormone responses vary significantly according to these variables.

## Methods

### Study population

The WHI is a multi-center prospective study of post-menopausal women's health involving more than 160,000 postmenopausal women between the ages 50-79 enrolled in either an observational study (n=93,676) or one or more of four randomized controlled clinical trials. The WHI hormone trials (HT) comprised two parallel trials testing the effects of estrogen plus progestin (EPT) in 16,608 women who had an intact uterus at baseline, and the effects of estrogen alone (ET) in 10,739 women who had no uterus at baseline. Participants in the EPT arm were randomized to receive 0.625 mg of conjugated equine estrogens plus 2.5 mg of medroxyprogesterone acetate daily (Prempro, Wyeth, Philadelphia, PA) or a daily placebo pill. Participants in the ET arm were randomized to receive either 0.625 mg of conjugated equine estrogens daily (Premarin, Wyeth, Philadelphia, PA) or daily placebo pill. Participants using hormone therapies prior to study enrollment underwent a three-month washout period prior to starting study therapy or placebo. A description of the study and rationale has been reported elsewhere.<sup>15</sup> The study was approved by the investigators' institutional review boards and each participant signed written informed consent to participate in the study.

### Selection of Women from Active Arms of HT trials and Controls

Out of the 27,237 participants in the HT, 9810 met the eligibility criteria. These criteria were designed to identify a group of adherent participants representing a range of age and weight categories, while ensuring prudent use of stored serum samples within the WHI. Participants were selected who had excellent adherence to the assigned treatment arm during the first year of the study (defined as taking  $\geq 90\%$  of assigned study pills during the first year on study and no use of hormone therapy for those randomized to placebo), at least 4 mL available fasting serum for baseline and year one visits, and no missing values for body mass index (BMI). Women were excluded if they had a diagnosis of cardiovascular disease, cancer, fracture, or death during an average (standard deviation) of 8.1(1.5) years of follow-up.

From the sub-group of eligible women, 200 women were randomly selected. These included 50 from each active and placebo arm, with a specified number of women selected in age and body mass index categories. Specifically, 3, 4 and 3 women with screening ages in 50-59, 60-69, and 70-79 years, respectively, were selected in each of the body mass index (kilograms/meters squared) categories of <25.0 (underweight/normal), overweight I (25.0-29.9), obesity I (30.0-34.9), obesity II (35.0-39.9) and extreme obesity ( $\geq 40$ ), in each of the four active and placebo groups.

### Blood Samples and Measurement of Sex Hormones and SHBG

Blood was collected from each study participant at the baseline visit and after 1 year of randomization following at least a 12-hour fast, and then was stored at  $-70^{\circ}\text{C}$ . On average (standard deviation), participants had their baseline blood draw 75(37) days prior to initiating study pills, and 367(22) days elapsed between the initiation of study pills and the year-one follow-up blood draw. Samples were shipped on dry ice to the Reproductive Endocrine Research Laboratory (University of Southern California, Los Angeles CA). Laboratory personnel were blinded to treatment status and specimens were randomly ordered and sent to the laboratory in batches that included blind duplicate and pooled quality control samples. Estrone, estradiol and progesterone (the last in EPT participants only) were quantified by radioimmunoassay (RIA) following a hexane:ethyl acetate extraction. Intraassay CVs ranged from 4.1-7.7% for the assays, with interassay CVs ranging from 5.8-13%. Sex hormone binding globulin was assayed via a solid-phase, two-site chemiluminescent immunoassay using the Immulite analyzer. Free estradiol (non-albumin or SHBG bound) and bioavailable estradiol (non-SHBG bound) values were calculated based upon a validated algorithm using measured

values for estradiol and SHBG, and an average value for albumin. The sensitivity of the estradiol assay was 3 pg/ml (11.0 pmol/L), and concentrations below these values were deemed undetectable.

### Statistical analyses

We compared log transformed sex hormone levels at Year 1 in relation to HT randomization assignment using linear regression models adjusted for age, BMI, smoking, and log-transformed baseline sex hormone levels. In addition, we calculated ratios of year 1 to baseline levels for each woman to estimate the relative change.

To examine subgroup effects for each sex hormone we tested whether or not the main effects of EPT and ET differed. If no significant differences were found, we pooled the two active arms (CEE active and CEE + MPA active) and the two placebo arms and fit linear regression models, testing one at a time, to determine whether the HT effect was modified by age, BMI, race, smoking, baseline sex hormone levels, self-report of vasomotor symptoms at baseline, and oophorectomy. For age, BMI and baseline sex hormone levels, we used a one-degree-of-freedom test for trend.

Comparisons of baseline characteristics by randomization assignment were made by Fisher's exact test of association and two sample t-tests. Geometric means are presented for biomarkers. All analyses were done in SAS version 9.1.3, based on the intention-to-treat principle and all statistical tests were two sided. All p-values are nominal. Given the number of subgroup analyses performed, we can expect to have approximately two false positives at the  $p = 0.05$  level.

### Results

Baseline characteristics of all trial participants in the WHI EPT and ET trials are available.<sup>15</sup> Among the 200 women included in this study, the active study populations were similar to their corresponding placebo groups within the ET and EPT cohorts, with the exception of slightly more current smokers in the active EPT group relative to EPT placebo ( $p=0.05$ ) and a trend towards more past smokers in the active ET group relative to the ET placebo ( $p=0.06$ ) (Table 1). Two women in the EPT trial had baseline progesterone values near 1000 pg/ml (one in the active group and one in the placebo group), however excluding these points did not change the overall pattern of response to exogenous progesterone and so these two participants were retained in the analysis.

Participants in this study had a mean age of 64.1 ( $\pm 7.6$ ) years. Because women were selected by BMI status, the cohort in this study had a higher average BMI ( $33.0 \pm 8.3 \text{ kg/m}^2$ ) and weight ( $85.8 \pm 21.4 \text{ kg}$ ) than in the overall WHI HT cohort ( $29.1 \pm 6.0 \text{ kg/m}^2$ ,  $76.2 \pm 17.0 \text{ kg}$ ). At baseline, ET and EPT groups were similar in age, weight, BMI, ethnicity, report of vasomotor symptoms, and smoking history, however (as anticipated) women in the ET group were further from menopause and were substantially more likely to have had bilateral oophorectomy (38% of ET women relative to 0% of EPT women). The ET group also included significantly more women who reported ever using HT, and significantly more reporting "current" HT use prior to the three month washout period.

Baseline values for all of the measured hormones (SHBG, estrone, estradiol, bioavailable estradiol, free estradiol, and progesterone) were comparable between active and placebo groups at baseline. In contrast, there was a consistent trend towards higher estrogenic hormone values and slightly lower SHBG values in the ET group relative to the EPT group. For all participants, (geometric) mean baseline levels of estradiol ( $13.8 \pm 1.8 \text{ pg/mL}$  vs  $11.1 \pm 1.7 \text{ pg/mL}$ ,  $p=0.007$ ), bioavailable estradiol ( $9.4 \pm 1.9 \text{ pg/mL}$  vs  $7.5 \pm 1.9 \text{ pg/mL}$ ,  $p=0.01$ ), and free

estradiol (0.37 $\pm$  1.90 pg/mL vs 0.28 $\pm$  1.86 pg/mL,  $p=0.003$ ) were significantly higher in ET trial participants relative to EPT trial participants at baseline. The trends for estrone (41.8  $\pm$  1.6 ng/dL vs 39.7 $\pm$  1.5 ng/dL,  $p=0.43$ ) and SHBG (35.5 $\pm$  1.6 Nmol/L vs 39.0 $\pm$  1.6 Nmol/L,  $p=0.16$ ) did not reach statistical significance.

Mean unadjusted hormone values for each treatment group are presented in table 2, with the ratio of active year 1 to baseline values presented in the right-hand column for each study arm (these are adjusted for log<sub>2</sub> baseline hormone values, age, BMI, and smoking history). The mean baseline SHBG concentration ranged from 35.1 Nmol/L (ET placebo) to 40.8 Nmol/L (EPT active). Following one year on placebo, SHBG concentrations remained essentially unchanged. Women on active hormone, however, saw a roughly 2.5 fold increase in serum SHBG concentration from baseline, to 102.7 Nmol/L in EPT participants and 90.7 Nmol/L in ET. Values for the measured total estrogens (estrone and estradiol) followed a similar pattern (see table 2), with a roughly 4 fold increase in estrone and a roughly 3 fold increase in total estradiol concentrations from baseline to year 1 in active hormone recipients. As expected, the increase in serum bioavailable and free estradiol concentrations was of a slightly smaller magnitude (approximately 2-fold increase relative to baseline).

Progesterone concentrations were only assayed for those women in the EPT trial. For women taking placebo, progesterone concentrations increased from 69.9 pg/mL to 75.1 pg/mL. In contrast, measured serum progesterone concentrations for women taking active EPT decreased from 72.3 pg/mL to 62.0 pg/mL. The corresponding ratio of change in the active group relative to the placebo group was 0.8 (CI 0.7-0.9).

The effects of EPT did not differ from ET for the estrogenic hormones or SHBG (all  $p$ -values  $> 0.4$ ), so for the subgroup analysis the two active arms (ET and EPT) were combined for comparison to the pooled placebo arms. In multiple regression analysis, serum estrogenic hormone responses varied significantly by BMI, smoking status, age, black vs white race, report of vasomotor symptoms, and baseline hormone values (Table 3). Heavier women had a smaller magnitude of serum estrogenic hormone response to exogenous hormone administration than lighter-weight women. For example, compared to placebo, total estrone levels increased with HT use 5.7 times for normal weight women and 3.2 times for obese women ( $p$ -int  $< 0.001$ ). Similarly, bioavailable estradiol levels increased 2.5 times for normal weight women, but only 1.8 times for obese women ( $p$ -int = 0.02).

Smoking (never, past, current) showed a graded relationship to an increase in estrogenic hormones (estrone, total estradiol and bioavailable estradiol); compared to placebo, the increase in estrone for non-smokers was 4.3 times, for past smokers 3.4 times, and for current smokers 2.1 times ( $p$ -int = 0.004). Similar differences were estimated for total estradiol and bioavailable estradiol.

Women with lower baseline hormone values had a larger relative response to exogenous hormones for all four estrogenic hormone assays evaluated. Adjusted mean total bioavailable estradiol concentrations increased 2.3-fold relative to baseline for women whose baseline bioavailable estradiol concentrations were in the lowest tertile, relative to 1.5-fold for women whose baseline concentrations were in the highest tertile ( $p=0.003$ ).

Older age was associated with a significantly greater response for estrone (total) and total estradiol only; women 70-79 exhibited a 3.7-fold increase in total estradiol compared with a 2.4-fold increase in women 50-59 ( $p$ -int=0.006). There was a suggestion that black women had a smaller magnitude of response of serum estrone, total estradiol, bioavailable estradiol, and free estradiol. However, this trend only reached statistical significance for total estradiol, which increased 1.8 times in black women and 3.0 times in white women ( $p$ -int = 0.01). Women reporting vasomotor symptoms had a significantly smaller magnitude of response for all of the

estrogenic hormone values tested and for SHBG. For example, the bioavailable estradiol level increased 2.1-fold among women with no vasomotor symptoms compared with 1.8-fold for women with mild and 1.4-fold among women reporting moderate to severe symptoms. In separate cross-tabulations, women with vasomotor symptoms were found to be younger than those without, however the pattern of decreasing response to hormone therapy among women with vasomotor symptoms held within each age tertile (data not presented).

Sex hormone binding globulin concentrations increased a total of 2.5-fold and varied significantly by participant race only, with black women exhibiting a lower overall magnitude of response to hormone therapy of 1.7-fold relative to 2.6-fold for white women. Progesterone concentrations decreased 0.8-fold relative to baseline following adjustment in recipients of EPT, and did not vary significantly by any of the examined characteristics in the regression model. History of bilateral oophorectomy did not significantly impact the magnitude of response of serum hormone levels to exogenous hormone administration for any of the hormones tested.

For a given covariate (such as age) and a specific hormone (such as total estradiol), the relative magnitude of response to hormone therapy could vary because a) the absolute values for that hormone varied according to that covariate at baseline, b) because that hormone varied according to that covariate at year 1, or c) because that hormone varied according to that covariate at both baseline and year 1. All three patterns were observed. For example, the different BMI groups demonstrated variation in total estradiol concentrations at baseline. Baseline total estradiol concentrations in the active hormone groups (ET and EPT combined) were 9.1 pg/mL, 11.1 pg/mL, and 13.6 pg/mL at baseline for women with a BMI <25, 25-39.9, or  $\geq 30$  respectively. By year one, however, hormone values were more comparable across those BMI groups (30.2 pg/mL, 30.9 pg/mL, and 35.3 pg/mL respectively). On the other hand, baseline concentrations of total estradiol were 12.4 pg/mL, 12.2 pg/mL, and 11.5 pg/mL among the age tertiles 50-59, 60-69, and 70-79 (relatively similar); by year one these values were 28.6 pg/mL, 34.2 pg/mL, and 37.7 pg/mL. Lastly, in the case of smoking, current smokers had both lower baseline hormone values (9.9 pg/mL relative to 12.5 pg/mL and 11.9 pg/mL among never and past smokers, respectively) and a diminished response to HT (16.3 pg/mL relative to 39.8 pg/mL and 32.6 pg/mL following one year of therapy). Given that lower baseline hormone concentrations were associated with a greater magnitude of response to hormone therapy and that a number of the variables were associated with lower hormone levels at baseline (i.e. lighter weight women had lower baseline estrogenic hormone concentrations), these analyses were repeated with correction for baseline hormone values. However, this correction did not substantially alter the findings.

## Discussion

Serum hormone responses in healthy, postmenopausal women taking exogenous hormones were similar among those taking combined estrogen plus progestin and estrogen alone (ET). We observed a roughly 4 fold increase in total estrone and a roughly 3 fold increase in total estradiol concentrations from baseline to year 1 in active hormone recipients, with an increase in serum bioavailable and free estradiol concentrations of approximately 2-fold relative to baseline. The smaller magnitude increase in bioavailable and free serum estradiol concentrations is expected, as a proportion of the increased value of the total serum hormone measurements reflects that proportion that is bound to SHBG, which is known to increase in the setting of hormone therapy<sup>1</sup> and which is not considered to be bioavailable. We observed a roughly 2.5 fold increase in serum SHBG concentration relative to baseline for women taking active hormone therapy.

We did not observe significant changes in serum hormone concentrations from baseline to year 1 in women on placebo, and in fact, the ratios of SHBG and the various estrogens from baseline to year 1 are nearly identical to those from year 1 active vs placebo. In the case of serum progesterone in women with a uterus, however, concentrations did tend to increase slightly from baseline to year 1 in participants on placebo. The serum SHBG response was relatively comparable following one year of hormone therapy across all demographic characteristics (roughly 2.5 fold), and only varied significantly by race, with black women having a smaller SHBG and estrogenic hormone response than white women. The relatively small sub-sample of women evaluated in this study did not allow for comparisons across other racial groups, and further study will also be necessary to evaluate the relative SHBG and estrogenic hormone response in other racial and ethnic groups.

Serum estrogenic hormone level response to exogenous hormone therapy also varied significantly by participant age, BMI, smoking status, report of vasomotor symptoms, and baseline hormone concentration. A larger response to HT was observed for women with lower BMI and in older women for all four estrogenic compounds assayed, with the greatest variability in the total hormone measurements. It might be expected that women with lower baseline hormone values would experience a larger increase in serum hormone concentrations, even if the absolute serum hormone concentrations were similar across these groups following therapy. This does appear to be the case, with a significantly greater magnitude of response of the estrogenic hormones observed for women in the lowest tertile of baseline hormone values relative to the highest tertile ( $p < 0.01$ , see table 3). Similarly, lighter weight women and older women might be anticipated to have lower baseline hormone values, and therefore a greater response to HT might be anticipated in these populations. Importantly, however, controlling for baseline hormone values did not significantly alter our findings, and the observed variations in serum hormone response to HT between subpopulations does not simply represent variations in hormone concentrations in these populations at baseline.

It is somewhat surprising that women reporting vasomotor symptoms at baseline had a smaller magnitude of response to hormone therapy than women who did not report such symptoms. Previous work has shown that vasomotor symptoms correlate with lower estrogenic hormone levels and with higher FSH levels, with some evidence suggesting it is the higher FSH itself that may be the mediator of these symptoms.<sup>16</sup> We might have anticipated that women with vasomotor symptoms would have lower baseline hormone levels. We would therefore have anticipated a greater relative response to HT, however this does not appear to be the case. It is possible that we are not fully correcting for a common factor underlying both increased self-report of vasomotor symptoms at baseline and decreased magnitude of response to exogenous hormones in our model. As relief of vasomotor symptoms is one of the common indicators for initiating HT, further investigation into hormone levels before and after hormone therapy and their relationship to report of and relief of vasomotor symptoms is warranted.

Smoking status was a strong and consistent effect modifier of all measured serum estrogenic hormone levels. Current smokers had a significantly smaller response to hormone supplementation than never smokers, with former smokers having intermediate levels. No effect of smoking was observed for either SHBG or for progesterone. The effects of smoking on hormone metabolism are known to be complex and at least partially mediated by hepatic P-450 enzyme system, and the physiologic effects of HT are known to be diminished in smokers.<sup>17</sup>

In post-menopausal women, the ovaries remain an important source of endogenous hormone production. In these postmenopausal women, history of bilateral oophorectomy did not impact serum hormone response to HT. It is impossible to evaluate the effects of hysterectomy separately from hormone preparation on response to supplementation in the current study, since

hysterectomized women receive a different hormone preparation than women with an intact uterus. Baseline levels of estrogenic hormones were higher in the ET participants relative to the EPT participants, which may be related to the absence of a uterus in these women. On the other hand, women who have had a hysterectomy are known to differ in a number of demographic characteristics (higher rates of obesity, lower average socioeconomic status, etc) relative to those who have not, and these factors may contribute to the observed baseline hormone concentrations. Overall, serum hormone responses to therapy were similar among ET and EPT participants, suggesting that neither prior hysterectomy nor the addition of progesterone have an important impact on circulating estrogen levels in postmenopausal women using exogenous hormones.

Further, the similar hormone responses among ET and EPT participants suggests that differences in serum hormone levels alone do not account, or do not fully account, for the differences in disease risk between women taking ET vs. EPT that have been observed in the WHI and in observational cohort studies. While evaluation of serum hormone response relative to specific disease risk is beyond the scope of the present study, such studies are ongoing utilizing WHI data and stored serum samples. Available evidence from other recent studies,<sup>18,19</sup> however, suggests that there is a modest increase in breast cancer risk among women with a high response of serum estradiol to HT, at least among some patient subgroups. The relatively similar hormone responses observed among ET and EPT women suggests that other factors may account for outcomes differences between ET and EPT participants. For example, rather comprehensive analyses of serum proteomic changes between baseline and 1-year in the ET trial have recently been reported,<sup>20</sup> and analyses comparing ET and EPT proteomic changes are currently under journal review.

The greatest strengths of this study relate to the study design, including parallel randomized trials of ET and EPT and the ability to control for variables including age, BMI, smoking status, and race. The availability of pre- and post-treatment serum hormone values allows for a longitudinal examination of hormone levels in treated and in placebo control women. In addition, only adherent women were included in this study, which limits the potential for dilution of the treatment effect.

On the other hand, a relatively small sub-sample of women was selected for measurement of serum hormone values, and serum hormone assays were limited to estrogens and SHBG, with progesterone assayed only in women in the EPT trial (women with a uterus, both active and placebo groups), limiting interpretation of the progesterone findings. In addition, the timing of hormone dosing relative to blood collection at year one was not collected. However, the available literature<sup>12</sup> suggests limited fluctuations of plasma estrogen concentrations at steady state associated with hormone dosing, and any such fluctuation would not be expected to significantly impact either the mean hormone concentrations for active hormone participants at year one or the differential responses among patient subgroups. Measured serum progesterone concentrations one year following initiation of hormone therapy with EPT decreased modestly, while measured serum progesterone concentrations increased modestly in those on placebo. The corrected ratio of progesterone response to hormone therapy relative to placebo at one year was 0.8 (CI 0.7-0.9), suggesting a slight but significant decrease in measured serum progesterone following one year of EPT therapy.

This slight decrease in measured progesterone on CEE+MPA therapy is unexpected and its significance is unclear. The concentration of serum progesterone as identified by the clinical or research laboratory may not accurately reflect the biological implications of exogenous progestin administration. Specifically, MPA is not detected by most progesterone assays, including the one used here. The undetected, exogenous progestin may suppress endogenous progesterone production, resulting in the decrease in detected progesterone. Alternatively,



estrogen and/or progesterone administration could result in a decreased serum progesterone measurement via an alternate mechanism, such as decreased hepatic production of corticosteroid binding globulin. Unfortunately, we do not know if similar decreases in progesterone levels are seen in women taking exogenous estrogen alone (as progesterone was only assayed in EPT trial participants). There is evidence that the biological effects of progesterone and MPA are similar, with similar (but not identical) effects on breast cancer risk.<sup>21</sup> Further study will be needed to clarify the effects of exogenous hormone administration on serum progestin levels, and may indicate a need to assay both progesterone and the exogenous progestin (such as MPA) in future studies.

The primary objective of this study was to determine the magnitude of response of selected serum sex hormones to exogenous oral hormone treatment. The differential hormone responses observed for personal characteristics such as age, BMI, race, vasomotor symptoms, and smoking status were relatively unexpected, and the sample size in this study is not adequate to fully address all of the issues raised by these observations. A full understanding of how individual variables influence response to serum hormone therapy and its influence on health will require further study. The generally similar responses to serum hormone concentrations between ET and EPT may suggest that differences in outcomes between these two populations are the result of factors not directly mediated by serum hormone levels, however further detailed analysis of serum hormone concentrations relative to specific disease outcomes will be necessary.

## Conclusions

In postmenopausal women, circulating levels of estrogens and SHBG are elevated by two- to four-fold with use of either estrogen alone or combined estrogen plus progesterone. Circulating progesterone levels exhibit a modest but statistically significant reduction with use of combined hormone therapy. Age, BMI, smoking status, report of vasomotor symptoms, and baseline hormone levels are significant modifiers of the effects of hormone therapy on serum estrogens. These data provide a reference point for the serum hormone response to HT, and demonstrate that this response is similar for EPT and ET therapy and suggest a larger response to HT in women with low endogenous levels.

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Table 1

Baseline Characteristics vs. Randomization Assignment

	CEE Trial				CEE+MPA Trial				P-value <sup>†</sup>
	Active	Placebo	Active	Placebo	Active	Placebo	Active	Placebo	
	N	%	N	%	N	%	N	%	
<b>Age at screening, years</b>									1.00
50-59	15	30.0	15	30.0	15	30.0	15	30.0	1.00
60-69	20	40.0	20	40.0	20	40.0	20	40.0	
70-79	15	30.0	15	30.0	15	30.0	15	30.0	
<b>Years from menopause</b>									0.99
<5 years	4	8.9	3	8.3	6	12.8	4	9.8	0.68
5-10 years	4	8.9	2	5.6	10	21.3	9	22.0	
10-15 years	13	28.9	7	19.4	12	25.5	10	24.4	
≥15 years	24	53.3	24	66.7	19	40.4	18	43.9	
<b>Ethnicity</b>									0.69
White	41	82.0	42	84.0	46	92.0	44	88.0	1.00
Black	7	14.0	6	12.0	1	2.0	4	8.0	
Hispanic	0	0.0	1	2.0	1	2.0	1	2.0	
Asian/Pacific Islander	1	2.0	0	0.0	2	4.0	1	2.0	
Unknown	1	2.0	1	2.0	2	4.0	1	2.0	
<b>BMI</b>									1.00
Normal (18.5 - 24.9)	10	20.0	10	20.0	10	20.0	10	20.0	1.00
Overweight (25.0 - 29.9)	10	20.0	10	20.0	10	20.0	10	20.0	
Obesity I (30.0 - 34.9)	10	20.0	10	20.0	10	20.0	10	20.0	
Obesity II (35.0 - 39.9)	10	20.0	10	20.0	10	20.0	10	20.0	
Extreme Obesity III (≥= 40)	10	20.0	10	20.0	10	20.0	10	20.0	
<b>Smoking</b>									0.06
Never smoked	24	49.0	34	69.4	28	57.1	25	50.0	0.05
Past smoker	20	40.8	9	18.4	15	30.6	24	48.0	
Current smoker	5	10.2	6	12.2	6	12.2	1	2.0	

	CEE Trial				CEE+MPA Trial				P-value	
	Active		Placebo		Active		Placebo			
	N	%	N	%	N	%	N	%		
<b>Prior bilateral oophorectomy</b>									0.83	N/A
Yes	19	39.6	18	36.7	0	0	0	0	0	0
No	29	60.4	31	63.3	50	100.0	50	100.0	50	100.0
<b>Vasomotor symptoms</b>									0.16	0.49
None	24	49.0	32	65.3	33	66.0	29	59.2		
Mild	17	34.7	9	18.4	11	22.0	16	32.7		
Moderate/severe	8	16.3	8	16.3	6	12.0	4	8.2		
<b>HT Usage Status</b>									0.11	1.00
Never used	23	46.0	30	60.0	41	82.0	41	82.0		
Past user	20	40.0	10	20.0	8	16.0	7	14.0		
Current user	7	14.0	10	20.0	1	2.0	2	4.0		
<b>Time Since Quitting HT Categorized</b>									0.14	0.55
Non-User	23	46.0	30	60.0	41	82.0	41	82.0		
Past, < 5 years	6	12.0	5	10.0	3	6.0	3	6.0		
Past, 5-10 years	1	2.0	1	2.0	3	6.0	0	0.0		
Past, >10 years	13	26.0	4	8.0	2	4.0	4	8.0		
Current	7	14.0	10	20.0	1	2.0	2	4.0		
	<b>Mean</b>	<b>STD</b>	<b>Mean</b>	<b>STD</b>	<b>Mean</b>	<b>STD</b>	<b>Mean</b>	<b>STD</b>	<b>Mean</b>	<b>STD</b>
<b>Age at screening, years</b>	64.0	7.6	64.2	7.6	63.9	7.8	64.3	7.6	64.3	7.6
<b>Weight, kg</b>	88.2	24.2	86.4	23.1	84.0	19.9	84.5	18.1	84.5	18.1
<b>BMI, kg/m<sup>2</sup></b>	33.2	8.6	33.4	9.2	32.8	7.1	32.6	8.3	32.6	8.3
<b>Total expend from phys act (MET hrs/wk)</b>	8.7	13.0	7.8	15.0	9.2	9.2	8.9	7.7	8.9	7.7

<sup>1</sup> Tests of association based on Fisher's exact test (categorical variables) or t-tests (continuous variables).  
 Note: CEE = conjugated equine estrogen; CEE+MPA = conjugated equine estrogen plus medroxyprogesterone acetate; BMI = body mass index

Table 2

Hormone Levels at Baseline, Year 1, and Multivariable Adjusted<sup>1</sup> Hormone Ratios<sup>2</sup> (Active/Placebo) at Year 1 in Participants on Active Hormone Therapy and Placebo

Variable	CEE			CEE+MPA			P-het <sup>3</sup>		
	Active	Placebo	Year 1 Ratio (Active/Placebo)	Active	Placebo	Year 1 Ratio (Active/Placebo)			
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	
<b>SHBG (Nmol/L)</b>									
Baseline	36.0	(30.9, 42.0)	35.1	(31.3, 39.4)	40.8	(35.7, 46.7)	37.2	(33.0, 42.0)	0.63
Year 1	90.7	(77.2, 106.6)	36.1	(32.4, 40.1)	2.6	(2.3, 2.9)	102.7	(89.6, 117.8)	2.5
Ratio (Year1/baseline)	2.5	(2.2, 2.9)	1.0	(1.0, 1.1)	2.5	(2.3, 2.8)	1.1	(1.0, 1.1)	
<b>Estrone (ng/dL)</b>									
Baseline	41.4	(36.1, 47.4)	42.1	(36.9, 48.1)	41.4	(36.6, 46.8)	38.1	(33.7, 43.0)	0.65
Year 1	149.9	(121.3, 185.2)	40.5	(35.2, 46.5)	4.0	(3.3, 4.9)	138.6	(119.7, 160.4)	3.8
Ratio (Year1/baseline)	3.6	(2.9, 4.5)	1.0	(0.9, 1.0)	3.3	(2.8, 4.0)	1.0	(0.9, 1.1)	
<b>Estradiol (PG/mL)</b>									
Baseline	13.3	(11.3, 15.6)	14.4	(12.2, 17.1)	10.9	(9.4, 12.8)	11.3	(9.7, 13.3)	0.54
Year 1	35.5	(29.5, 42.7)	14.0	(11.7, 16.6)	2.8	(2.4, 3.4)	31.3	(27.1, 36.1)	3.0
Ratio (Year1/baseline)	2.7	(2.3, 3.2)	1.0	(0.9, 1.1)	2.9	(2.5, 3.3)	1.0	(0.9, 1.1)	
<b>Bioavailable estradiol (PG/mL)</b>									
Baseline	8.9	(7.4, 10.7)	9.9	(8.2, 11.9)	7.2	(6.0, 8.6)	7.7	(6.5, 9.2)	0.70
Year 1	16.1	(13.8, 18.8)	9.5	(7.8, 11.5)	1.9	(1.6, 2.2)	13.8	(11.8, 16.1)	2.0
Ratio (Year1/baseline)	1.8	(1.6, 2.1)	1.0	(0.9, 1.1)	1.9	(1.7, 2.2)	1.0	(0.9, 1.1)	
<b>Free estradiol (PG/mL)</b>									
Baseline	0.4	(0.3, 0.4)	0.4	(0.3, 0.5)	0.3	(0.2, 0.3)	0.3	(0.2, 0.3)	0.46
Year 1	0.6	(0.5, 0.7)	0.4	(0.3, 0.5)	1.9	(1.6, 2.2)	0.5	(0.4, 0.6)	2.0
Ratio (Year1/baseline)	1.8	(1.6, 2.1)	1.0	(0.9, 1.1)	1.9	(1.7, 2.2)	1.0	(0.9, 1.0)	
<b>Progesterone<sup>4</sup> (PG/mL)</b>									
Baseline					72.3	(60.7, 86.1)	69.9	(59.2, 82.4)	
Year 1					62.0	(54.7, 70.3)	75.1	(64.9, 86.9)	0.8



**Table 3**  
Multivariable Adjusted<sup>1</sup> Hormone Ratios<sup>2</sup> (Active/Placebo) at Year 1 by Selected Participant Characteristics.

	SHBG			Estrone (total)			Estradiol (bioavailable)			Estradiol (total)			Progesterone (total) <sup>3</sup>		
	Ratio	95%CI	p <sup>4</sup>	Ratio	95%CI	p	Ratio	95%CI	p	Ratio	95%CI	p	Ratio	95%CI	p
<b>Overall</b>	2.5	(2.3, 2.7)	<0.001	3.7	(3.3, 4.2)	<0.001	1.9	(1.7, 2.1)	<0.001	2.9	(2.6, 3.2)	<0.001	0.8	(0.7, 0.9)	0.01
<b>Age at screening, years</b>															
50-59	2.2	(1.9, 2.5)	0.13	3.3	(2.6, 4.1)	0.04	1.8	(1.5, 2.2)	0.08	2.4	(2.0, 3.0)	0.006	1.0	(0.7, 1.3)	0.12
60-69	2.7	(2.4, 3.0)		3.5	(2.8, 4.2)		1.8	(1.5, 2.1)		2.7	(2.3, 3.2)		0.8	(0.6, 1.0)	
70-79	2.5	(2.2, 2.9)		4.7	(3.7, 5.9)		2.3	(1.9, 2.8)		3.7	(3.0, 4.5)		0.7	(0.5, 0.9)	
<b>Ethnicity<sup>5</sup></b>															
White	2.6	(2.4, 2.8)	0.006	3.8	(3.3, 4.3)	0.13	2.0	(1.8, 2.2)	0.06	3.0	(2.7, 3.3)	0.01	0.8	(0.7, 0.9)	>0.99
Black	1.7	(1.3, 2.3)		2.7	(1.7, 4.1)		1.4	(1.0, 1.9)		1.8	(1.2, 2.6)		0.8	(0.3, 2.0)	
<b>BMI</b>															
Normal (18.5 - 24.9)	2.3	(2.0, 2.8)	0.47	5.7	(4.3, 7.6)	<0.001	2.5	(2.0, 3.1)	0.02	3.8	(3.0, 4.9)	0.006	0.8	(0.5, 1.1)	0.61
Overweight (25.0 - 29.9)	2.6	(2.2, 3.1)		3.9	(3.0, 5.2)		1.9	(1.5, 2.4)		3.0	(2.4, 3.9)		0.8	(0.5, 1.1)	
Obesity I-III (>=30.0)	2.5	(2.3, 2.8)		3.2	(2.7, 3.8)		1.8	(1.5, 2.0)		2.6	(2.2, 2.9)		0.8	(0.7, 1.0)	
<b>Smoking</b>															
Never smoked	2.6	(2.3, 2.9)	0.16	4.3	(3.7, 5.1)	0.004	2.2	(1.9, 2.5)	<0.001	3.3	(2.9, 3.8)	<0.001	0.8	(0.6, 1.0)	0.40
Past smoker	2.5	(2.2, 2.8)		3.4	(2.8, 4.2)		1.7	(1.5, 2.1)		2.6	(2.2, 3.1)		0.8	(0.6, 1.0)	
Current smoker	2.0	(1.5, 2.6)		2.1	(1.4, 3.2)		1.1	(0.8, 1.5)		1.5	(1.1, 2.2)		1.4	(0.6, 3.5)	
<b>Vasomotor symptoms</b>															
None	2.7	(2.4, 2.9)	0.03	4.1	(3.5, 4.8)	0.03	2.1	(1.9, 2.4)	0.003	3.3	(2.9, 3.8)	<0.001	0.9	(0.7, 1.1)	0.07
Mild	2.4	(2.0, 2.7)		3.7	(2.9, 4.7)		1.8	(1.4, 2.2)		2.6	(2.1, 3.2)		0.7	(0.5, 1.0)	
Moderate/severe	2.1	(1.7, 2.6)		2.6	(1.8, 3.7)		1.4	(1.0, 1.8)		1.9	(1.4, 2.5)		0.6	(0.3, 0.9)	
<b>Prior bilateral oophorectomy</b>															
No	2.5	(2.3, 2.7)	0.65	3.7	(3.2, 4.3)	0.80	1.9	(1.7, 2.2)	0.51	2.7	(2.1, 3.5)		NA		
Yes	2.6	(2.2, 3.1)		3.6	(2.7, 4.8)		1.8	(1.4, 2.2)							
<b>Baseline hormone</b>															

	SHBG		Estrone (total)			Estradiol (bioavailable)			Estradiol (total)			Progesterone (total) <sup>3</sup>			
	Ratio	95%CI	p <sup>4</sup>	Ratio	95%CI	p	Ratio	95%CI	p	Ratio	95%CI	p	Ratio	95%CI	p
Lowest tertile	2.8	(2.4, 3.2)	0.10	4.5	(3.6, 5.6)	0.02	2.3	(1.9, 2.9)	0.003	3.4	(2.7, 4.3)	0.01	0.8	(0.6, 1.1)	0.25
Middle tertile	2.4	(2.1, 2.8)		3.8	(3.1, 4.7)		2.0	(1.6, 2.5)		3.0	(2.4, 3.8)		0.9	(0.7, 1.2)	
Highest tertile	2.3	(2.0, 2.7)		3.1	(2.5, 3.9)		1.5	(1.2, 1.9)		2.3	(1.8, 2.8)		0.6	(0.5, 0.9)	

<sup>1</sup> Adjusted for age, BMI, smoking and baseline biomarker.

<sup>2</sup> From a multivariable adjusted linear regression model where biomarkers are fit on log<sub>2</sub> scale. Ratios (95% Confidence Intervals) are back-transformed and presented on the original scale.

<sup>3</sup> Includes only participants in the CEE+MPA trial.

<sup>4</sup> P-value for interaction.

<sup>5</sup> Model includes only Whites and Blacks.

Note: CEE = conjugated equine estrogen; CEE+MPA = conjugated equine estrogen plus medroxyprogesterone acetate; SHBG = sex hormone binding globulin; CI = confidence interval; BMI = body mass index