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Factors Associated With Serum Estradiol Levels Among Postmenopausal Women Using Hormone Therapy

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Abstract

Objective—To identify factors associated with serum estradiol (E2) levels among healthy postmenopausal women using hormone therapy (HT).

Methods—This is an unplanned post hoc analysis of data from the Early versus Late Intervention Trial with Estradiol, a randomized controlled trial of 1 mg oral E2 with or without vaginal progesterone in healthy postmenopausal women. We included results from visits when women reported at least 80% compliance to HT. Mixed effects linear models identified factors associated with serum E2 levels while taking HT assessed every 6 months over a median follow-up of 4.8 years adjusted for baseline E2 level, visit and reduced E2 dose. Possible correlates evaluated included demographics, clinical characteristics, medication use and biomarkers of liver and kidney metabolic function.

Results—The analysis included 2160 E2 measurements in 275 postmenopausal women. Mean ±SD age was 55.4±3.9 vs 64.4±5.5 years and mean±SD time-since-menopause was 3.6±1.8 vs 16.0±5.6 years for early vs late postmenopausal women. Adjusted for pre-treatment E2 level, visit and reduced dose indicator, higher serum E2 levels were associated with higher body mass index

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Authors' Data Sharing Statement
Will individual participant data be available (including data dictionaries)? Yes
What data in particular will be shared? Analysis datasets used to generate tables of results in the manuscript.
What other documents will be available? Trial protocol.
When will data be available (start and end dates)? Start date: Date of publication, No end date.
By what access criteria will data be shared (including with whom, for what types of only for research purposes and only for the research approved by IRB; c) a commitment not to try to identify any individual participant; d) a commitment to securing the data using appropriate computer technology and other necessary safeguards; e) a commitment to not transfer the data to other users; and, f) a commitment to destroying the data after analyses are completed.

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(BMI), higher weight, surgical menopause, alcohol use, and antihypertensive medication use. Current and past smoking and antifungal medication use were associated with lower serum E2 levels. In the multivariable model, higher BMI and alcohol use were associated with higher serum E2 levels while current and past smoking were associated with lower serum E2 levels. These factors were similar between early and late postmenopausal women.

Conclusion—Factors associated with serum E2 levels among postmenopausal women taking HT include BMI, alcohol use and smoking. As serum E2 levels relate to HT effect, achievement of desirable E2 levels can be maximized through personalized intervention.

Clinical Trial Registration—ClinicalTrials.gov, NCT00114517.

Precis

Body mass index, alcohol use, and smoking are associated with serum estradiol levels among postmenopausal women taking hormone therapy.

Introduction

The North American Menopause Society (NAMS) in 2017 recommended that hormone therapy (HT) should be individualized to maximize benefits and minimize risks(1). Although there is no recommendation to monitor serum estradiol (E2) levels among postmenopausal women taking HT, achieved E2 levels have been shown to be associated with potential benefits on atherosclerosis as reported in Estrogen in the Prevention of Atherosclerosis Trial (EPAT) and the Early vs Late Intervention Trial with Estradiol (ELITE)(2, 3). This association may explain the reduced coronary heart disease and atherosclerosis progression only among early postmenopausal women(4, 5). The estrogen threshold hypothesis postulates that each end organ tissue varies in its sensitivity to E2(6). Some studies show that achieved E2 levels relate to biological effects of HT treatment. For instance, in the Kronos Early Estrogen Prevention Study (KEEPS), E2 level was related to intensity of hot flushes(7) and in the Ultra-Low-dose Transdermal estRogen Assessment (ULTRA) trial, E2 level was related to bone mineral density(8)

The achieved mean serum E2 levels among oral E2 compliant postmenopausal women in ELITE varied widely from 9 to 360 pg/ml. Oral E2 is metabolized in the liver through the cytochrome P450 (CYP) enzyme pathway(9, 10) and is excreted through urine and feces(11). This study aims to explore the factors associated with E2 levels to reflect this wide range among postmenopausal women taking HT. We hypothesized that demographics, clinical characteristics and factors relating to liver and kidney metabolism may be potential factors associated with E2 level in postmenopausal women taking oral HT.

Methods

This was an unplanned post hoc analysis conducted among healthy postmenopausal women participating in ELITE(2, 3). ELITE was a single-center, randomized, double-blinded, placebo-controlled trial of HT in postmenopausal women, stratified by <6 years-since-menopause (early postmenopause) and 10 or more years-since-menopause (late postmenopause). ELITE was specifically designed to test the HT timing hypothesis, i.e.,

whether the effects of HT on atherosclerosis progression vary according to the timing of HT initiation in relation to menopause. Eligible women were healthy postmenopausal women with no clinical history of cardiovascular disease or diabetes. A total of 643 postmenopausal women were randomized to receive either HT or placebo according to time-since-menopause strata using a 1:1 ratio of stratified blocked randomization. The HT regimen included oral micronized 17-beta-estradiol 1 mg/day with (in women with a uterus) or without 4% vaginal micronized progesterone gel 45 mg/day for 10 days each month. After randomization, women attended study clinic visits every month for the first 6 months and every other month thereafter until trial completion. The trial was conducted from July 2005 to February 2013 with a median duration of follow-up of 4.8 (range 0.5 to 6.7) years. The primary trial results showed that HT was associated with less progression of subclinical atherosclerosis (measured as rate of change in carotid artery intima-media thickness, CIMT) compared with placebo when therapy was initiated in early, but not in late postmenopausal women(5). The ELITE trial was registered on ClinicalTrials.gov (NCT00114517), funded by the National Institute on Aging, National Institutes of Health (R01AG-024154) and was approved by the Institutional Review Board of the University of Southern California.

This analysis included ELITE visits with at least 80% compliance with active HT determined by tablet count. Compliance was calculated as the percentage of active HT tablets that should have been consumed over the inter-visit period, comparing number of pills dispensed at the prior visit to those returned at the subsequent visit.

At baseline and at every 6 months during trial follow-up, serum levels of total E2 were quantified by radioimmunoassay with preceding organic solvent extraction and Celite column partition chromatography of samples(12). Assay sensitivity was 2 pg/ml and interassay coefficients of variation were 11%, 13% and 12% at 15, 36 and 101 pg/ml, respectively.

Race–ethnicity, type of menopause (natural, surgical), multivitamin use, vitamin E use and fish oil use were determined at baseline by self-report using structured questionnaires. At baseline and each 6-month visit, we measured age, time-since-menopause, weight and body mass index (BMI). Smoking status (never, past smoker, current smoker) and alcohol use (<1 drink, 1–2 drinks, >2 drinks per day) were self-reported at each visit. Total weekly metabolic equivalent of energy expenditure (MET) calculated as weekly hours of moderate and vigorous activity were determined from a structured 7-day physical activity recall(5). Current use of any lipid-lowering medication, lipid lowering with statins in particular, antihypertensive, calcium channel blocker, antifungal, diabetes and anticonvulsant medications were determined from medications brought into each clinic visit. Creatinine, creatinine clearance, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and fasting blood glucose were measured at baseline and at annual study visits by chemistry safety laboratory measures. A reduced E2 dose indicator (yes, no) defined visits when reduced E2 dose (0.5 mg, 0.25 mg instead of 1 mg oral E2) was taken.

Baseline demographic and clinical characteristics were reported as means and standard deviations (SD) for continuous variables and frequencies and percentages for categorical variables. Baseline serum E2 levels and serum E2 levels while taking HT were reported as

mean±SD and median (IQR). The levels were compared between early and late postmenopausal women with Wilcoxon rank sum test.

Serum E2 levels were log transformed to achieve normality. As creatinine, creatinine clearance, AST, and ALT were measured annually, these measures were imputed at the 6-, 18-, 30-, 42-, and 54-month visits using the method of multiply-imputed chained equations with 10 imputations(12).

Associations between log-transformed E2 levels while taking HT and each potential correlate were first assessed using mixed effects linear models with a random intercept at the participant level, and adjusted for baseline serum E2 level, follow-up visit (as indicator variables) and reduced E2 dose indicator.

Potential E2 correlates with a p-value ≤ 0.15 were included in a multivariable model. A manual backward selection approach was used to drop least significant variables from the model; significant variables with p-value less than 0.05 were retained in the final model. The final multivariable model was developed and presented among the total analysis sample and stratified by early and late postmenopause. In the total analysis sample, the interaction term of each significant factor by time-since-menopause strata was tested to determine whether the E2 association was modified by time-since-menopause.

The estimates from the model were back-transformed to show the association with E2 levels. The estimates of association with log E2 levels and model-estimated least square mean E2 levels for each variable in the multivariable model are presented in the Appendices 1–3, available online at http://links.lww.com/xxx. All statistical analyses were performed using SAS software version 9.4 (Cary, NC).

Results

Of the 643 women in the ELITE trial, 323 women were randomized to HT. Among those women, 275 women (123 early and 152 late postmenopause) had visits with at least 80% compliance to HT, contributing 2160 E2 measurements over the trial follow-up for the analysis.

The mean±SD age was 55.4±3.9 vs 64.4±5.5 years and mean time-since-menopause was 3.6±1.8 vs 16.0±5.6 years for early vs late postmenopausal women, respectively. The majority of the women were non-Hispanic white (202/275, 73.5%) and had experienced natural menopause (245/275, 89.1%). The mean±SD BMI was 27.2±5.6 kg/m^2, and mean creatinine, creatinine clearance, AST, ALT and glucose levels were within normal ranges. More than half of the women had never smoked (170/275, 61.8%) and were not currently consuming alcohol (139/275, 50.5%) (Table 1).

Among the total analysis sample, the mean±SD baseline serum E2 level was 10.8±3.3 pg/ml and the median (IQR) was 9.0 (3.0) pg/ml. Early and late postmenopausal women had similar mean±SD (10.6±2.9 vs 10.9±3.5 pg/ml) and median (IQR) (9.0 (2.0) vs 9.0 (3.0) pg/ml) baseline serum E2 (Wilcoxon rank sum p=0.57). (Data not presented in a table)

The average serum E2 level while taking HT in the total analysis sample was 55.6±47.5 pg/ml and the median (IQR) was 45 (40) pg/ml. Early and late postmenopausal women had similar mean (57.8±56.7 vs 53.6±36.9 pg/ml) and median (46 (40) vs 45 (39) pg/ml) serum E2 level while taking HT (Wilcoxon rank sum p=0.39). (Data not presented in a table)

Adjusted for baseline serum E2 level, visit and a reduced E2 dose indicator variable, higher serum E2 levels were significantly associated with higher BMI (p=0.002), surgical menopause (p=0.04), consumption of > 2 alcoholic beverages per day (p<0.001), and antihypertensive medication use (p=0.02). Lower serum E2 levels were associated with smoking (p<0.001) and antifungal medication use (p=0.02) (Table 2 and 3 with back-transformed estimates with E2 levels and Appendix 1 [http://links.lww.com/xxx] with estimates from model with log E2 levels). Alcohol use of >2 drinks per day was significantly associated with higher serum E2 level (p<0.001).

Compared to non-smokers, past smokers had significantly lower serum E2 levels (p=0.005) and current smokers had the lowest serum E2 levels (p<0.001. Higher intake of alcohol was significantly associated with higher serum E2 levels (p-value for trend=0.003).

Age, race–ethnicity, time-since-menopause, creatinine, creatinine clearance, AST, ALT, fasting blood glucose, total weekly MET hours, hours of weekly moderate and vigorous activity, use of lipid-lowering medication, calcium channel blocker, diabetic medication, anticonvulsant medication, multivitamins, vitamin E and fish oil supplements were not significantly associated with serum E2 levels.

In a multivariable mixed effects model in the total analysis sample (275 women, 2160 visits), higher serum E2 levels were significantly associated with higher BMI and consumption of >2 alcohol beverages per day (p<0.001). Lower serum E2 levels were significantly associated with current and past smoking (p<0.001) (Table 4 with back-transformed estimates with E2 levels and Appendix 2 [http://links.lww.com/xxx] with estimates from model with log E2 levels). Alcohol use of >2 drinks per day was significantly associated with higher serum E2 levels (p<0.001). The beta coefficients showed a trend in increasing serum E2 level with the amount of alcohol use (p for trend=0.002).

The factors associated with serum E2 levels were similar between early (123 women, 1037 visits) and late (152 women, 1123 visits) postmenopausal women based on similar beta estimates and non-significant interaction by time-since-menopause. While both current and past smoking was associated with serum E2 level among early postmenopausal women, only current smoking was associated with serum E2 level among late postmenopausal women. The association of alcohol use on serum E2 level was statistically significant among early but not late postmenopausal women (alcohol use by time-since-menopause interaction = 0.06). Model-estimated mean serum E2 levels for each factor are presented in the Appendix 3 (http://links.lww.com/xxx).

Discussion

Our study identified BMI, alcohol use, and smoking as statistically significant correlates of serum E2 levels among postmenopausal women taking oral E2 therapy. When considering

each alcohol use category, we found that alcohol use of > 2 drinks per day was significantly associated with higher serum E2 levels. The beta coefficients showed a significant trend in increasing serum E2 levels with the increasing alcohol use. The factors associated with serum E2 level were similar between early and late postmenopausal women. Though the association of alcohol use and E2 levels appeared to be stronger in early compared to late postmenopausal women, the interaction by time-since-menopause was not significant (p=0.06). The biological explanation for BMI, alcohol use and smoking on E2 levels involves the complex pathway of E2 metabolism(10) as discussed below.

The positive association of E2 levels with BMI is consistent with several prior reports(13, 14). The EPAT showed that overweight and obese postmenopausal women using E2 therapy attained significantly greater concentrations of both total E2 and free E2 adjusted for age (p=0.01)(15). In postmenopausal women, the aromatization from androstenedione to E1 and E2 occurs mainly in adipose tissue(16); hence elevated BMI which indicates increased fat mass could explain the increased E2 levels(17).

The association between alcohol use and serum E2 levels found in this study confirmed the findings from prior smaller studies. Acute alcohol consumption increased E2 levels among 12 postmenopausal women taking HT to a level of 300% higher than the targeted level in a randomized, double-blind, placebo-controlled crossover study(18). A meta-analysis of 13 prospective cohort studies reported that higher levels of alcohol consumption were significantly associated with increased E2 levels compared to non-drinkers (p trend=0.002) (19). A cross-sectional analysis in the Women's Health Initiative observational study reported a positive association between alcohol consumption and E2, E2 metabolites and E1 among HT users. The association was stronger with increasing dose of alcohol (p trend=0.02)(20). An alcohol effect on E2 metabolism was hypothesized to occur through increased aromatase activity in the conversion of androgens to E2 as alcohol drinkers had lower testosterone and higher E2 to testosterone ratio compared to non-drinkers(21). Alcohol was also reported to promote adrenal gland cell signaling of dehydroepiandrosterone sulfate production, a precursor of E2 production(22, 23). Alcohol consumption also increases the reduced nicotinamide-adenine dinucleotide (NADH) to NAD^+ ratio in the liver(24) and leads to decreased catabolism of steroid hormones through oxidation and inhibition of E2 conversion to E1(18).

A pharmacokinetic study of oral E2 among postmenopausal women reported that smoking enhances the hepatic metabolism of oral E2, resulting in lower E2 levels(25). In a randomized cross-over study of oral and transdermal E2 therapy, E2 levels were 40–70% lower among smokers compared with non-smokers; a statistically significant difference in E2 level was seen among postmenopausal women taking oral, but not transdermal E2 therapy(26). Smoking also reduces or completely cancels the efficacy of oral E2 therapy among postmenopausal women on E2-related effects such as alleviation of hot flushes and urogenital symptoms, beneficial effects on lipid metabolism, osteoporosis and cardiovascular disease(27, 28). As the effect of smoking on E2 levels has been demonstrated only with oral E2, the mechanism could involve the elevated hepatic clearance of E2 as smoking increases 2-hydroxylation of E2 and leads to decreased bioavailability of E2(29). Additional mechanisms of a smoking effect on E2 include a smoking-related reduction of

aromatase activity in granulosa cell and fatty tissue, a reduction in steroid production from cholesterol through smoking-related inhibition of C-20, 22 desmolase, an increase in A-ring metabolism of the CYP450 enzyme system, increased sex-hormone binding globulin (SHBG) capacity, stimulation of adrenal function and increased hepatic and renal clearance(28).

We further explored the association between past smokers and E2 levels by conducting sensitivity analysis removing women who had recently stopped smoking and women with secondhand smoking exposure. The magnitude and statistical significance of the association of past smoking with E2 level did not change. One possible explanation could be that the induction of liver enzymatic activity by smoking may be permanent or may take many decades to return to baseline levels, similar to the decreasing risk over decades of lung cancer seen in individuals who stop smoking. However, this is conjecture and will require further study to untangle this interesting but complex finding.

We found that antifungal medication use was not statistically associated with E2 level in the final model. Other studies have reported no effect of co-administration of oral E2 and antifungal medication on E2 levels(30–32). CYP3A4 inhibitors such as ketoconazole(32) and grapefruit juice(30) have been reported to increase E1 (but not E2) levels among women taking oral E2. We evaluated other CYP3A4-related medications which could result in a drug interaction with oral E2 through liver metabolism including antiepileptic, antihypertensive, lipid-lowering and antidiabetic medications (33–35) and commonly used supplements (multivitamin, vitamin E and fish oil). None of these medications were associated with serum E2 level.

A post hoc analysis from the EPAT trial showed that exercise and physical activity were associated with lower levels of E2 over 2 years of intervention with oral E2 1 mg/day(36); we did not find this association in the ELITE sample. As EPAT participants were older, had higher BMI and higher baseline E2 levels and E2 levels while taking HT than participants in ELITE, the difference may be due to different population characteristics.

Endogenous serum E2 levels among premenopausal and perimenopausal women significantly differed across race–ethnicity at menopausal transition in the Study of Women Across the Nation (SWAN), with highest serum E2 levels in Hispanic women and lowest levels in Asian women. The difference in serum E2 level disappeared after adjustment for BMI(37). In ELITE participants who were postmenopausal women taking HT, race–ethnicity were not related to achieved serum E2 levels while BMI was significantly associated with serum E2 level. These findings may suggest that metabolism of both endogenous and exogenous E2 is related to BMI rather than race–ethnicity.

The strength of this study is the randomized clinical trial data providing prospective repeated measurements of E2 levels and possible correlates that decreased variability of measurements and allowed determination of active factors associated with E2 levels among postmenopausal women taking oral E2 therapy with good compliance. Despite the overall significant trend in serum E2 levels by level of daily alcohol use, the estimate of association with alcohol intake of more than 2 drinks per day was based on a small sample size, thus

needs further exploration with a larger sample size. The analysis was also limited to total serum E2 levels and did not account for other estrogen components including free E2, E1 and SHBG. Only 11% of women were surgically menopausal and 18 women used antifungal medications during the study, limiting analyses of these specific associations.

Although titration of HT to specified serum E2 levels is not recommended by the American College of Obstetricians and Gynecologists (ACOG), American Society for Reproductive Medicine (ASRM) or NAMS, this study has important public health implications for postmenopausal women taking HT, as achieved E2 levels relate to the biological response of HT(6–8). In particular, we have previously reported that the effect of HT on atherosclerosis progression among postmenopausal women is related to achieved serum E2 levels(2, 3). Higher E2 levels were associated with reduced atherosclerosis progression when initiated in early postmenopause (<6 years since menopause), however, higher E2 levels were associated with greater atherosclerosis progression when initiated in late postmenopause (≥10 years since menopause)(3). Many significant factors associated with E2 levels in this study such as weight, alcohol and smoking are modifiable. Postmenopausal women should control their weight and refrain from smoking and limit alcohol use with a goal of obtaining the lowest effective dose of HT. Postmenopausal women who are obese, drink more than 2 alcoholic beverages per day and are 10 years since menopause may be at higher risk for atherosclerosis due to higher E2 levels when taking HT. Postmenopausal women who continue to smoke may need higher E2 dosages to maintain benefits in clinical outcomes. Health care professionals prescribing HT need to consider these modifiable life-style factors since these factors may require adjustment for the most appropriate treatment dose for each individual woman to acquire the desired outcome with minimal side effects and maximum compliance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline demographic and clinical characteristics Continuous variables are presented as mean and standard deviation; categorical variables are presented as frequency, percent.

Variable	Mean/Frequency	Standard deviation/Percent
Age (years)		
Total analysis sample	60.7	6.8
Early postmenopause	55.4	3.9
Late postmenopause	64.4	5.5
Time since menopause (years)		
Total analysis sample	10.2	7.6
Early postmenopause	3.6	1.8
Late postmenopause	16	5.6
Race/Ethnicity		
White, non-Hispanic	202	73.5%
African American, non-Hispanic	21	7.6%
Hispanic	30	10.9%
Asian, non-Hispanic	22	8.0%
Menopause type		
Natural	245	89.1%
Surgical	30	10.9%
Body mass index (kg/m2)	27.2	5.6
Creatinine (mg/dL)	0.85	0.15
Creatinine clearance (mL/min)	80.7	22.0
Aspartate aminotransferase (U/L)	21.3	6.8
Alanine aminotransferase (U/L)	20.5	9.0
Fasting blood glucose (mg/dL)	96.3	11.0
Smoking status		
Never	170	61.8%
Past smoker	94	34.2%
Current smoker	11	4.0%
Alcohol use		
None	139	50.5%
<1 drink per day	97	35.3%
1–2 drinks per day	30	10.9%
>2 drinks per day	9	3.3%
Total weekly metabolic equivalent hours	247	23.3
Medications and supplements use		
Lipid-lowering	55	20%
Statin	51	18.5%
Antihypertensive medication	58	21.1%
Calcium channel blocker	14	5.1%
Antifungal	2	0.7%

Variable	Mean/Frequency	Standard deviation/Percent
Anticonvulsant	8	2.9%
Multivitamin	163	59.3%
Vitamin E	46	16.7%
Fish oil	83	30.3%

Table 2

Association of serum estradiol levels while taking hormone therapy with demographic and clinical characteristics and median estradiol level by categorical variable Beta estimates and p-values are from mixed effects linear model, adjusted for baseline serum estradiol level, follow-up visit and reduced estradiol dose indicator; Beta estimates and 95% confidence interval are back-transformed from the model with log estradiol level.; Back transformed betas represent a fold change in means from reference category for categorical variable or per increasing unit of continuous variable.; p-trend for alcohol use p=0.003

Variable	Women	Visits	Median estradiol level (pg/ml)	Beta	(95%Confidence interval)	P-value	Overall P-value
Age * (years)	275	2160		0.9978	(0.9899 – 1.0059)	0.60	
Race/Ethnicity	275	2160					
White, non-Hispanic	202	1615	45.0		Reference		0.99
African American, non-Hispanic	21	141	43.0	0.9807	(0.7985 – 1.2046)	0.85	
Hispanic	30	225	54.0	1.0164	(0.8529 – 1.2114)	0.86	
Asian, non-Hispanic	22	179	41.0	1.0047	(0.8224 – 1.2276)	0.96	
Body mass index * (kg/m^2)	275	2160		1.0139	(1.0052 – 1.0227)	0.002	
Weight * (kg)	275	2147		1.0020	(1.0007 – 1.0034)	0.006	
Time since menopause (years)	259	2046		0.9977	(0.9903 – 1.0052)	0.55	
Time since menopause *(years)	259	2046		0.9976	(0.9903 – 1.0051)	0.52	
Time since menopause	275	2160					
Early postmenopause	123	1037	46.0		Reference		
Late postmenopause	152	1123	45.0	0.9615	(0.8634 – 1.0707)	0.47	
Menopause type	275	2160					
Natural	245	1916	43.0		Reference		
Surgical	30	244	64.0	1.1982	(1.0096 – 1.4221)	0.04	
Menopause type	275	2160					
Natural, early	117	981	44.0		Reference		0.12
Natural, late	128	935	41.0	0.9458	(0.8449 – 1.0589)	0.33	
Surgical, early	6	56	61.5	1.3209	(0.9115 – 1.9143)	0.14	
Surgical, late	24	188	64.5	1.1303	(0.9283 – 1.3765)	0.22	
Creatinine * (mg/dL)	258	2143		1.1149	(0.8359 – 1.4873)	0.46	
Creatinine clearance * (ml/min)	258	2130		1.0011	(0.9994 – 1.0029)	0.25	
Aspartate aminotransferase * (U/L)	258	2143		0.9996	(0.994 – 1.0053)	0.89	
Alanine aminotransferase * (U/L)	258	2143		0.9989	(0.9943 – 1.0037)	0.64	
Fasting blood glucose * (mg/dl)	256	1042		1.0020	(0.9975 – 1.0066)	0.37	

* Time varying variable, assessed at each visit

Table 3

Association of serum estradiol levels while taking hormone therapy with demographic and clinical characteristics and median estradiol level by categorical variable Beta estimates and p-values are from mixed effects linear model, adjusted for baseline serum estradiol level, follow-up visit and reduced estradiol dose indicator; Beta estimates and 95% confidence interval are back-transformed from the model with log estradiol level.; Back transformed betas represent a fold change in means from reference category for categorical variable or per increasing unit of continuous variable.; p-trend for alcohol use p=0.003

Variable	Women	Visits	Median estradiol level (pg/ml)	Beta	(95%Confidence interval)	P-value	Overll P-value
Smoking status *	275	2160					
Never	170	1338	50.0		Reference		<0.001
Past smoker	101	741	40.0	0.8535	(0.7647 – 0.9528)	0.005	
Current smoker	14	81	26.0	0.6694	(0.5516 – 0.8125)	<0.001	
Alcohol use *	275	2160					
None	167	1059	46.0		Reference		<0.001
<1 drink per day	165	828	42.0	1.0359	(0.9667 – 1.1102)	0.32	
1–2 drinks per day	56	231	51.0	1.0941	(0.9798 – 1.2218)	0.11	
>2 drinks per day	11	42	94.5	1.6780	(1.3386 – 2.1035)	<0.001	
Total weekly MET hours *	275	2155		0.9997	(0.9988 – 1.0007)	0.63	
Moderate + vigorous activity weekly hours *, tertiles	275	2156					
0 – 2.60	193	719	47.0		Reference		0.35
2.62 – 6.75	222	727	44.0	0.9624	(0.569 – 1.6281)	0.15	
6.80+	197	710	44.0	0.9707	(0.9143 – 1.0308)	0.33	
Medications and supplements							
Lipid lowering medication *	275	2160					
No	224	1632	44.0		Reference		
Yes	86	528	49.0	1.0166	(0.9299 – 1.1115)	0.72	
Statin use *	275	2160					
No	228	1684	45.0		Reference		
Yes	79	476	46.0	1.0232	(0.9324 – 1.1228)	0.63	
Antihypertensive medication *	275	2160					
No	223	1677	45.0		Reference		
Yes	82	483	46.0	1.1155	(1.0166 – 1.2241)	0.02	
Calcium channel blocker *	275	2160					
No	263	2065	45.0		Reference		
Yes	20	95	54.0	1.0592	(0.8841 – 1.269)	0.53	
Diabetes medication *	275	2160					
No	273	2136	45.0		Reference		
Yes	3	24	43.0	0.9157	(0.6014 – 1.3942)	0.68	
Antifungal medication *	275	2160					

Variable	Women	Visits	Median estradiol level (pg/ml)	Beta	(95%Confidence interval)	P-value	Overll P-value
No	275	2115	45.0		Reference		
Yes	18	45	46.0	0.8049	(0.6839 – 0.9476)	0.02	
Anticonvulsant medication *	275	2160					
No	271	2079	45.0		Reference		
Yes	17	81	46.0	0.9026	(0.7685 – 1.0602)	0.21	
Multivitamin use	275	2160					0.70
Never	51	396	48.0		Reference		
Took regularly in the past	61	463	47.0	0.9743	(0.8229 – 1.1537)	0.76	
Take regularly now	163	1301	44.0	0.9457	(0.8202 – 1.0906)	0.44	
Vitamin E use	275	2160					0.27
Never	148	1178	43.0		Reference		
Took regularly in the past	81	613	50.0	1.1035	(0.9756 – 1.2484)	0.12	
Take regularly now	46	369	48.0	1.0733	(0.9246 – 1.2459)	0.35	
Fish oil use	274	2156					0.53
Never	162	1309	43.0		Reference		
Took regularly in the past	29	243	48.0	1.0949	(0.9161 – 1.3088)	0.32	
Take regularly now	83	604	48.0	1.0466	(0.9267 – 1.1821)	0.46	

* Time varying variable, assessed at each visit

Table 4

Multivariable association of estradiol levels while taking hormone therapy with demographic and clinical characteristics among total analysis sample and by postmenopausal strata Beta estimates and p-values are from mixed effects linear model, adjusted for baseline serum estradiol level, follow-up visit and reduced estradiol dose indicator; Beta estimates and 95% confidence interval are back-transformed from the model with log estradiol level.; Back transformed betas represent a fold change in means from reference category for categorical variable or per increasing unit of continuous variable.; p-trend for alcohol use for total analysis sample p=0.002, early postmenopause p=0.005 and late postmenopause p=0.15; Interaction of alcohol use by time-since-menopause p=0.06

	Total analysis sample					Early postmenopause					Late postmenopause				
Variable	**Women**	**Visits**	**Beta**	**(95%Confidence interval)**	**P-value**	**Women**	**Visits**	**Beta**	**(95%Confidence interval)**	**P-value**	**Women**	**Visits**	**Beta**	**(95%Confidence interval)**	**P-value**
n	275	2160				123	1037				152	1123			
Body mass index (kg/m2) *	275	2160	1.015	(1.0067 – 1.0235)	<0.001	123	1037	1.0109	(0.9991 – 1.0229)	0.07	152	1123	1.0181	(1.0064 – 1.03)	0.002
Smoking status *					<0.001					<0.001					0.04
Never	170	1338		Reference		80	690		Reference		90	648		Reference	
Past smoker	101	741	0.8516	(0.7651 – 0.9481)	0.003	43	301	0.7806	(0.664 – 0.9178)	0.003	58	440	0.9261	(0.8022 – 1.0692)	0.30
Current smoker	14	81	0.6709	(0.5545 – 0.8119)	<0.001	8	46	0.6515	(0.5109 – 0.8309)	<0.001	6	35	0.6576	(0.4742 – 0.9122)	0.01
Alcohol use *					<0.001					<0.001					0.42
None	167	1059		Reference		69	467		Reference		98	592		Reference	
<1 drink per day	165	828	1.0414	(0.9722 – 1.1157)	0.25	76	420	1.0163	(0.915 – 1.129)	0.76	89	408	1.0556	(0.9631 – 1.157)	0.25
1–2 drinks per day	56	231	1.0999	(0.9862 – 1.2268)	0.09	28	128	1.1190	(0.9561 – 1.3097)	0.16	28	103	1.0661	(0.9132 – 1.2447)	0.42
>2 drinks per day	11	42	1.6979	(1.3572 – 2.1243)	<0.001	6	22	2.2371	(1.6276 – 3.0751)	<0.001	5	20	1.2607	(0.9189 – 1.7299)	0.15

* Time varying variable