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Differential associations of oral estradiol and conjugated equine estrogen with hemostatic biomarkers

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Abstract

Background—The risk of venous thrombosis (VT) associated with oral hormone therapy (HT) may differ by type of estrogen compound.

Objective—To compare the thrombotic profile of women using oral conjugated equine estrogens (CEE) with that of women using oral estradiol (E2).

Methods—In postmenopausal female health maintenance organization (HMO) members with no history of VT, we measured thrombin generation, levels of factor VII activity, antithrombin activity, and total protein S antigen. Mean levels of hemostasis biomarkers were cross-sectionally compared by use and type of estrogen using multiple linear regressions. The type of estrogen used was determined primarily by the HMO formulary, which changed its preferred estrogen from CEE to E2 during the study period.

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Conflicts of interest:

BM Psaty reports serving on a DSMB for a clinical trial of a device funded by the manufacturer (Zoll LifeCor).

All others authors report no conflicts of interest.

Results—The sample included 92 E2 users and 48 CEE users, with a mean age of 64.1y and mean BMI of 29.1kg/m^2. Twenty-seven percent of HT contained medroxyprogesterone acetate. Compared with E2 users, CEE users had greater thrombin generation peak values, endogenous thrombin potential, and lower total protein S (multivariate adjusted differences of 49.8 nM (95%CI: 21.0, 78.6); 175.0 nMxMin (95%CI: 54.4, 295.7); and -13.4% (95%CI: −9.8, −6.9), respectively). Factor VII and antithrombin levels were not different between E2 and CEE users. Results were similar in subgroups of users of unopposed HT, opposed HT, low-dose estrogen and standard dose estrogen.

Conclusion—The hemostatic profile of women using CEE is more prothrombotic than that of women using E2. These findings provide further evidence for a different thrombotic risk for oral CEE and oral E2.

Keywords

estrogens; estradiol; estrogens; conjugated (USP); venous thrombosis; thromboembolism; estrogen replacement therapy; hemostasis; thrombin

Introduction

Hormone therapy (HT) is commonly prescribed to treat menopausal vasomotor symptoms, even after the major downward shift in HT prescription use observed in the past decade [1,2]. In the US, oral formulations of HT are most commonly prescribed and are used by approximately 5% of insured women aged ≥50 years [1], amounting to over 2 million women. In Europe, transdermal estrogens are predominant, but oral estrogens still represent about 25% of initiated HT [3].

Oral HT can lead to cardiovascular complications. The most common is venous thrombosis (VT), with an excess of 1–2 events per 1000 treated woman-years [4,5]. Determining possible differences in VT risk between oral HT preparations is important when weighing the risks and benefits in women considering the initiation of oral HT.

We recently demonstrated that two common oral HTs, conjugated equine estrogen (CEE) and estradiol (E2), which have similar effects on climacteric symptoms, were associated with different risks of major thrombotic events [6,7]. Compared with current use of oral E2, current use of oral CEE was associated with a doubling of the risk of VT and a possible increased risk of myocardial infarction (MI). We further demonstrated an increased measure of resistance to activated protein C (APC) in CEE users compared with E2 users.

To better characterize the differential impact of HT products on hemostatic factors, we evaluated the cross-sectional associations of oral CEE and E2 with five hemostatic biomarkers. We hypothesized that oral CEE users would show a greater thrombotic propensity in global coagulation assays (thrombin generation) and concentrations of specific coagulation factors or inhibitors (factor VII, antithrombin, protein S) than oral E2 users.

Methods

Setting and Design

This cross-sectional study was conducted in Group Health Cooperative (GHC), an integrated healthcare system serving more than 500,000 residents in Washington State. The Cooperative maintains a formulary that includes preferred drugs within a class, including a preferred postmenopausal estrogen. During the study, the preferred estrogen, oral CEE, was changed in 2005 to oral E2 for primarily economic motives. We used this quasi-experimental design to characterize the hemostatic profile of the two HT products.

Population

This study is part of the Heart and Vascular Health (HVH) Study, a set of population-based, case-control studies evaluating risk factors for cardiovascular disease, including incident VT [8–11]. Eligible participants for this study were postmenopausal female HVH study controls, who were randomly-selected GHC enrollees between 2003 and 2010, matched to cases of MI on age, sex, hypertension treatment status, and year of MI. Their participation rate was 83%. Of 3692 eligible controls identified, citrated blood was drawn from 1499 women (41%) and stored for future analyses. Primary reasons for blood sample unavailability were refusal to give blood, death prior to blood draw, and inability to obtain a citrated plasma sample at blood draw. Among the 1499 women with available blood samples, after excluding women using anticoagulants and samples with processing times greater than 6 hours, our analysis included all E2 users (n=96), all CEE users (n=48) and a random subset of all non-users of oral HT (141 non-users). Most women were using estrogen-only preparations (n=103). Of the women using estrogen combined with progestogen, three users of micronized progestin were excluded to reduce heterogeneity, as all other users of estrogen preparations with progestogen used medroxyprogesterone acetate (n=38). No participant had a history of VT and none had used anticoagulants within 180 days before blood collection.

Hormone Use

Hormone use (estrogen and progestogen) was based on electronic pharmacy records that capture information on drug, dose, and the number of pills dispensed to GHC members. Use of HT at the time of blood draw was determined assuming a compliance of 80% with prescribing instructions. Pharmacies served by the electronic pharmacy records are used by more than 95% of postmenopausal GHC participants for HT preparations [12]. The time of the blood draw primarily determined the estrogen type. Most users of CEE had a blood collection date prior to 5/2005 (36/48, 75%) and most users of E2 had a blood collection thereafter (87/93, 94%). These users (36 CEE and 87 E2) were defined as preferred estrogen users.

Covariates

Demographic characteristics, body mass index (BMI), and medical history, including diabetes and cancer, were recorded by review of the complete GHC medical records by trained abstractors. Race/ethnicity, self-reported health status, and smoking status were determined by a standardized telephone interview for 98% of the women. The factor V

Leiden polymorphism (FVL, rs6025, MAF 0.02) was measured or imputed using DNA from the blood draw (see below). Statin use was assessed from electronic pharmacy records, using a similar method as for HT.

Blood collection

Venous blood from the antecubital vein was collected into tubes of 3.2% sodium citrate. All samples were centrifuged at 4°C for 10 minutes at 1300G and stored at -70°C within 6 hours of collection. They were shipped on dry ice from Seattle, Washington, USA to Leiden University Medical Center (LUMC), Leiden, The Netherlands, where the laboratory assays were conducted. The mean storage time was 4.9 years (SD 2.0), and was longer for CEE users (6.7y) than estradiol users (4.4y).

Laboratory measurements

Four hemostatic measurements were performed: [1] thrombin generation (TG) assay; [2] factor VII activity (FVIIc); [3] antithrombin activity (ATc); and [4] total protein S antigen. Four different parameters were measured in the TG assay: the lag-time, the time to peak, the peak value of thrombin concentration, and the area under the curve, which represents an individual's endogenous thrombin potential (ETP).

Thrombin generation was measured directly using a fluorogenic assay (Diagnostica Stago, Asnières, France), with a coefficient of variation (CV) of 19.8% (for ETP) from the normal pooled plasma values. After addition of 10µl Tris-NaCl buffer to the wells of a pre-heated micro-titre plate (37°C), 70µl plasma sample was added. Additionally, a thrombin calibrator was added to a second plasma sample (20µl). After addition of 20µl of platelet poor reagent, the micro-titre plate was placed in the thrombinoscope. Measurement was started after automated addition of 20 µl of fluorescent substrate to all samples. Analysis of the factor VII (CV 9.2%) and antithrombin activity (CV 3.0%) were performed according to the instructions of the manufacturer of the STA-R analyser (Diagnostica Stago, Asnières, France). For the measurement of factor VII levels, 50µl factor VII was added to 50µl of plasma in a 1/20 dilution with diluent buffer. After incubation for 240 seconds, 100 µl Neoplastin was added and the measurement started. For AT, 100 µl AT was added to 100 µl plasma in a 1/20 dilution with Diluent buffer. After incubation for 60 seconds, 100 µl AT substrate was added and measurement started. All steps were automatically performed by the STA analyser. Total protein S antigen levels (CV using commercial quality control: 4.2%) were measured by an enzyme-linked immunosorbent assay (Diagnostica Stago, Asnières, France). (Diagnostica Stago, Asnières, France).

Laboratory technicians were blinded to any characteristic of the individuals of the samples. Samples were thawed once previously for the measurement of TG, FVIIc, ATc, and twice previously for the measurement of protein S.

Statistical analyses

The primary analysis compared CEE users with E2 users. We used multiple linear regressions with robust standard errors to estimate adjusted differences in mean biomarker concentrations between these groups (CEE-E2). All regression models were adjusted for

confounding by including the following variables selected prior to analyses: age (linear), BMI (linear), cancer (diagnosed within 5 years before the date of blood collection), FVL, diabetes, current statin use, progestogen use/dose, and estrogen dose (bio-equivalence of 0.625mg of CEE and 1mg of E2 [13]). Data were complete for all measures except FVL, which was missing for 65 participants (23%). We assumed that participants with missing FVL status were non-carriers.

Sensitivity analyses tested the assumption of compliance for HT use (by defining current use with 100% compliance instead of 80%), the assumption for missing FVL status (by defining participants with missing FVL as FVL carriers instead of non-carriers), and whether results were similar among only preferred estrogen users.

In secondary analyses we determined if concomitant progestogen use and estrogen dose modified the primary associations of interest. To do so, we evaluated differences between CEE users and E2 users in subgroups defined by the presence of progestogen (unopposed HT = estrogen only and opposed HT = estrogen + MPA) and by estrogen doses (low: CEE 0.3125 mg/day or E2 0.5 mg/day; modal: CEE 0.625mg/d or E2 1mg/day ; users of higher-doses excluded because of the low number (n=15)).

Finally, we also compared levels of hemostatic markers between HT users (CEE or E2) and non-HT users, to assess the impact of HT use on thrombin generation and the other hemostatic biomarkers.

In the main analysis, we present nominal 95% confidence intervals. Bonferroni statistical correction for testing seven hemostasis phenotypes in the primary analysis would yield a two-sided alpha threshold less than 0.007. Possible outliers were assessed graphically and through the estimation of *dfbetas*, with no meaningful influence on the results. Analyses were conducted with Stata 11 (StataCorp LP, College Station, Tx).

Results

Characteristics of women

The study comprised 282 mostly White women: 48 oral CEE users, 93 oral E2 users, and 141 non-users of any HT. Characteristics of the three groups were generally similar with a mean age and BMI of 64.1 (SD 10.0) years and 29.1 (SD 6.8) kg/m^2, respectively (Table 1). Among estrogen users, modal daily estrogen doses were 0.625 mg of CEE and 1 mg of E2. The mean duration of oral HT from enrollment in GHC until blood draw was 16 years in both CEE and E2 users.

Comparison of CEE and E2 users

We observed differences in hemostatic levels between CEE and E2 users (Table 2). In multiple regression models, compared with E2 users, CEE users had greater TG peak values (+49.8 nM; 95% CI: 21.0, 78.6; p-value 0.001) and ETP (+175.0 nMxMin; 95% CI: 54.4, 295.7; p-value 0.005), and lower total protein S concentrations (−3.4%; 95% CI: −9.8, −.9; p-value <0.001). Levels of the two other TG measures, lag time and time-to-peak, and FVIIc and ATc measures were similar in E2 and CEE users.

In sensitivity analyses, results were not materially altered when defining current use of HT with a prescription compliance of 100%, when we assumed that all women with missing FVL status (23%) were FVL carriers (instead of non-carriers) or when restricting to preferred estrogen users; differences in hemostatic measurements between groups persisted (data not shown).

In secondary analyses we did not find strong evidence for the presence of effect modification by the use of progestogen or dose of estrogen: all subgroups showed elements of a greater thrombogenicity for CEE use than for E2 use (Table 3) and interaction p-values were >0.05 except for lag-time (interacting with estrogen dose, p=0.014). Differences for TG peak values and ETP were found among users of unopposed HT, users of opposed HT and users of modal-dose estrogen but not among users of low-dose estrogen. However, users of low-dose CEE had lower levels of protein S and shorter TG lag-time and time-to-peak than users of low-dose E2.

Comparison of HT users with non-users

Compared with non-HT users, CEE users displayed a more prothrombotic profile with greater TG peak value and ETP, shorter TG lag-time and time-to-peak measures, and lower total protein S concentrations (Table 4). In contrast, this difference with non-users was much less pronounced for E2 users: only TG lag-time and time–to-peak measures were shorter in E2 users.

Furthermore, levels of factor VIIc tended to be higher, and levels of ATc tended to be lower in HT users (either CEE or E2) than non-users; however these differences were not statistically significant.

Discussion

In this cross-sectional study, we found a more thrombotic profile in women using oral CEE than in women using oral E2. CEE use was associated with higher TG peak values and ETP and with lower total protein S concentrations than E2 use. This differential association of estrogen subtypes on hemostatic biomarkers provides further evidence and possible mechanisms for the previously reported finding of an increased VT risk associated with the use of CEE compared with the use of E2 [4,5,7]. Our findings were similar in users of unopposed HT and users of opposed HT, and differences between CEE and E2 use were observed in subgroups of users of low-dose estrogen and modal-dose estrogen. These latter groups are of particular interest, as current recommendations advise the use of the lowest effective dose consistent with treatment goals [6,7,14].

Although no previously-published study has directly compared hemostatic measures between users of CEE and E2, one prospective study compared levels of sex hormone binding globulin (SHBG), the main binding protein for circulating E2, which appears to be a useful marker of VT risk among users of oral contraceptives [18]. Unadjusted levels of SHBG were greater in users of oral CEE (n=37) than oral E2 (n=25) [17], but whether this difference in SHBG levels is a predictor of VT risk is unknown.

Thrombin generation is a global hemostatic assay measuring the initiation, propagation, and neutralization phase of TG triggered by tissue factor, and is likely determined by a multitude of coagulation factors (fibrinogen, FV, FIX) or inhibitors (AT, protein S, TFPI) [8–11,15]. It is useful in assessing bleeding and thrombotic risk; in particular its measurements of peak thrombin value or ETP can identify groups with different risks of incident [16,17] or recurrent VT [18,19]. In our study, both peak thrombin values and ETP were greater in CEE users than E2 users, suggesting an increased global hemostatic activation with CEE use.

Within this same sample, we recently observed a 70% increased APC resistance in analyses comparing CEE users with E2 users [7]. Increased APC resistance may be an important pathway explaining the increased thrombotic risk associated with exogenous estrogens both for HT and oral contraceptives [20–23]. It has been associated with an increased risk of VT in non-carriers of FVL and thus represents a valid intermediate coagulation endpoint for hemostatic studies of these drugs [24,25]. The difference in APC resistance may be partly explained by the observed differences in protein S concentrations. Protein C and tissue factor pathway inhibitor concentrations may also play a role but were not measured in our study [15,22].

Our secondary analysis showed differences in TG biomarkers and protein S in CEE users compared with non-HT users. For E2 and non-HT user comparisons, E2 users had shorter TG lag-time and time-to-peak but similar TG peak value and ETP and similar protein S levels. These findings contrast with previous literature on oral E2: two previous clinic-based studies showed greater TG peak thrombin and ETP in users of oral estrogen, including users of E2 [26,27], and prospective trials have demonstrated a decrease of 5–20% of protein S levels after 3–12 months of treatment with oral E2 [28–30]. Several explanations for these differences may exist. First, the precision of our estimates does not permit the rejection of meaningful differences between E2 and non-HT users. Second, the long duration of treatment of the users in our study may have led to the loss of more vulnerable individuals, who may have displayed greater changes in thrombin generation or protein S. While this is possible, it is unlikely to account for the differences observed between users of E2 and users of CEE, who had on average the same duration of treatment. Third, doses of oral E2 may be important. There was a much larger proportion of high doses of E2 (≥2mg) in studies showing differences in TG ETP and peak thrombin between E2 users and non-HT users than in ours [26,27]. The decrease in protein S levels induced by E2 may also be less important with lower drug doses [28–30], and this may be compatible with our results.

The observed hemostatic differences between CEE and E2 may be due to the differences in the estrogen content of CEE and E2. The thrombogenicity of oral estrogens is thought to be the consequence of the liver's exposure during the first-pass metabolism after gut absorption [31,32]. CEE is a mixture of multiple estrogens derived from the urine of pregnant mares, of which estrone and equilin sulfates are the most abundant. In contrast, E2 only contains 17β-estradiol, which is chemically identical to the predominant estrogen in circulation before menopause and which is converted to estrone in the liver and other tissues [13,32]. These differences in estrogen composition result in different circulating concentrations of E2, estrone and their ratio [33], with possibly different influences on the production of hemostatic factors [27] and on the clinical risk of VT.

Our findings may help guide medication use decisions made by health care providers and their postmenopausal patients. As the effectiveness of oral CEE and oral E2 does not differ significantly, E2 may be preferred if the oral route is chosen, while remembering that the differential influence of these drugs on other safety outcomes, such as cancer, is not well characterized.

We acknowledge limitations to our non-randomized study design. Even though the likelihood of confounding was reduced because the choice of estrogen was mainly driven by the formulary preference, residual confounding associated with possible changes in prescription patterns over the study period cannot be excluded. Although information on FVL or other prothrombotic genetic variants was incomplete or missing, we do not expect thrombophilia status to confound and bias the results: it was unknown to clinicians and did not guide prescribing decisions, and the sensitivity analysis on FVL status did not modify the results. Whether the long storage of frozen citrated samples (longer for CEE users than E2 users) may have influenced our results is unknown, as studies have shown a good stability of most hemostatic biomarkers for up to 3 years, but data for longer period are lacking [2,34] . Finally, the duration of use of HT was long and could have led to the loss of vulnerable individuals. This duration was however similar for CEE and E2 users.

The strengths of our study include the quasi-experimental comparison of CEE and E2 users, secondary to the HMO formulary change, the choice of validated intermediate biomarkers and the population-based design without restrictions for health conditions, providing good generalizability of our findings.

In conclusion, we found that CEE use was associated with a more prothrombotic biologic hemostatic profile than E2 use. These biological findings provide further evidence and possible mechanisms for previously reported differences in clinical risk of users of these oral estrogen treatments.

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Table 1

Characteristics of participants.

	Use of hormone therapy		
	Oral E2 (n=93)	**Oral CEE (n=48)**	**None (n=141)**
Age, years (mean, SD)	63.8 (10.6)	65.3 (8.9)	64.0 (10.0)
White, n (%)	83 (89.3%)	48 (100.0%)	130 (92.2%)
Good to excellent self-reported health status, n (%)	76 (82.6%)	37 (80.4%)	121 (88.3%)
BMI, kg/m^2 (mean, SD)	28.6 (6.1)	28.0 (5.6)	29.9 (7.6)
Current smoking, n (%)	8 (8.6%)	1 (2.1%)	14 (9.9%)
Diabetes, n (%)	4 (4.3%)	4 (8.3%)	19 (13.5%)
Hyperlipidemia, n (%)	9 (9.7%)	4 (8.3%)	25 (17.7%)
Total cholesterol, mg/dl (mean, SD)	212.3 (37.3)	235.2 (40.0)	209.2 (40.4)
Hypertension, n (%)	53 (57.0%)	26 (54.2%)	77 (54.6%)
SBP, mmHg (mean, SD)	132.1 (18.9)	136.6 (23.6)	131.4 (17.6)
History of cardiovascular disease*, n (%)	5 (5.4%)	3 (6.3%)	11 (7.8%)
Statin Use, n (%)	11 (11.8%)	8 (16.7%)	25 (17.7%)
Cancer <= 5 years prior, n (%)	2 (2.2%)	1 (2.1%)	2 (1.4%)
Factor V Leiden †, n (%)	7 (10.9%)	1 (2.1%)	3 (2.8%)
Estrogen daily dose, n (%)			
CEE <0.625mg or E2<1mg	35 (37.6%)	9 (18.8%)	
CEE 0.625mg or E2 1mg	51 (54.9%)	31 (65.6%)	
CEE >0.625mg or E2>1mg	7 (7.5%)	8 (16.7%)	
Progestin (MPA) use, n (%)	28 (30.1%)	10 (20.8%)	

*defined as a history of myocardial infarction, angina, coronary artery bypass grafting, angioplasty, stroke, carotid endarterectomy, claudication, or peripheral vascular bypass

†among participants with non-missing FVL status (77%). FVL missing in 35 non-HT users (24.8%), 29 E2 users (31.2%), and 1 CEE user (2.1%).

E2 = estradiol; CEE = conjugated equine estrogen; SBP = systolic blood pressure; MPA = medroxyprogesterone acetate

Table 2

Unadjusted means and adjusted differences in hemostatic biomarkers between users of CEE and E2.

		Unadjusted means (SD)		Adjusted absolute difference between CEE users and E2 users *		
		E2 users (n=93)	CEE users (n=48)	E2 users (n=93)	CEE users (n=48)	P-value
Thrombin Generation	Peak value (nM)	262.5 (62.1)	316.0 (85.8)	Reference	+49.8 (21.0 to 78.6)	0.001
	ETP (nMxMin)	1183.5 (255.7)	1337.0 (361.1)	Reference	+175.0 (54.4 to 295.7)	0.005
	Lag-time (min)	2.1 (0.34)	2.0 (0.89)	Reference	−0.01 (−.26 to 0.25)	0.97
	Time to peak (min)	4.1 (0.7)	3.8 (1.1)	Reference	−0.2 (−0.5 to 0.2)	0.27
Factor VII (%)		136.0 % (38.8)	135.3 % (36.3)	Reference	−8.7 (−23.0 to 5.6)	0.23
Antithrombin (%)		104.5 % (12.7)	102.8 % (17.5)	Reference	−2.7 (−8.4 to 3.0)	0.35
Total protein S (%)		105.9 % (18.2)	92.3 % (16.6)	Reference	−13.4 (−19.8 to −6.9)	<0.001

* Adjusted for age, race, BMI, cancer, FVL, use of statins, diabetes, use/dose of progestogen, dose of estrogen

Missing values: Factor VII (n=7), Antithrombin (n=10), Protein S (n=1).

ETP= endogenous thrombin potential

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Table 3

Adjusted difference of hemostatic measurements between users of CEE and E2, stratified by the use of progestogen.

		Adjusted absolute differences (95%CI) [CEE-E2]							
		Progestogen use				Estrogen dose¶			
		Unopposed estrogens (without progestogen) §		Opposed estrogens (with progestogen) †		Low estrogen dose (CEE ≤0.3125mg or E2 ≤0.5mg/d)		Standard estrogen dose (CEE=0.625mg or E2=1mg/d)	
		E2 users (n=65)	CEE users (n=38)	E2 + MPA users (n=28)	CEE + MPA users (n=10)	E2 users (n=35)	CEE users (n=9)	E2 users (n=51)	CEE users (n=35)
Thrombin Generation	**Peak value (nM)**	Ref.	+42.8 ** (11.8 to 73.7)	Ref.	+92.4 * (4.1 to 180.8)	Ref.	+24.1 (−1.9 to 70.0)	Ref.	+65.3 ** (29.3 to 101.2)
	ETP (nMxMin)	Ref.	+140.0 * (5.9 to 274.0)	Ref.	+400.4 * (65.5 to 735.4)	Ref.	−21.2 (−177.7 to 135.3)	Ref.	+227.0 *** (78.8 to 377.1)
	Lag-time (min)	Ref.	+ 0.0 (−.3 to 0.4)	Ref.	− 0.10 (−0.6 to 0.4)	Ref.	−0.4 * (−0.7 to −0.1)	Ref.	+0.1 (−0.3 to 0.5)
	Time to peak (min)	Ref.	−0.1 (−0.5 to 0.3)	Ref.	−0.3 (−1.0 to 0.3)	Ref.	−0.8 * (−1.4 to −0.1)	Ref.	−0.2 (−0.7 to 0.3)
Factor VII (%)		Ref.	−10.6 (−29.2 to 7.9)	Ref.	−2.1 (−20.4 to 16.2)	Ref.	−1.8 (−30.0 to 26.3)	Ref.	−6.4 (−26.7 to 13.9)
Antithrombin (%)		Ref.	−0.4 (−6.6 to 5.8)	Ref.	−6.7 (−18.2 to 4.8)	Ref.	−7.8 (−20.6 to 5.0)	Ref.	+0.7 (−6.0 to 7.4)
Total protein S (%)		Ref.	−13.7 *** (−21.6 to −5.7)	Ref.	−9.0 (−22.6 to 4.7)	Ref.	−15.1 * (−26.5 yo −3.6)	Ref.	−12.9 ** (−21.9 to −3.9)

§ Adjusted for age, race, BMI, cancer, FVL, use of statins, diabetes, dose of estrogen

† Adjusted for age, race, BMI, cancer, FVL, use of statins, diabetes, dose of estrogen, dose of medroxyprogesterone acetate (progestogen)

¶ Excluding high-dose estrogen (n=15) and adjusted for age, race, BMI, cancer, FVL, use of statins, diabetes, use of medroxyprogesterone acetate.

All P-values for interaction by progestogen use or estrogen dose were >0.05 except the P-value by estrogen dose for the measurement of lag-time (p=0.014).

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* $p<0.05$ / ** $p<0.01$ / *** $p<0.001$;

ETP= endogenous thrombin potential

Table 4

Unadjusted means and adjusted differences in hemostatic biomarkers between non-HT users and users of CEE or users of E2.

		Unadjusted means (SD)			Adjusted absolute difference between E2 or CEE users and non-users *				
		Non HT users (n=141)	E2 users (n=93)	CEE users (n=48)	Non HT users (n=141)	E2 users (n=93)	P-value	CEE users (n=48)	P-value
Thrombin generation	Peak value (nM)	262.2 (56.6)	262.5 (62.1)	316.0 (85.8)	Ref.	+2.4 (−12.9 to 17.7)	0.76	+56.4 (30.3 to 82.5)	<0.001
	ETP (nMxMin)	1212.7 (265.3)	1183.5 (255.7)	1337.0 (361.1)	Ref.	−28.4 (−95.3 to 38.6)	0.41	+137.5 (26.3 to 248.7)	0.016
	Lag-time (min)	2.3 (0.6)	2.1 (0.3)	2.0 (0.9)	Ref.	−0.2 (−0.3 to −0.1)	0.004	−0.2 (−0.5 to 0.0)	0.09
	Time to peak (min)	4.4 (0.9)	4.1 (0.7)	3.8 (1.1)	Ref.	− 0.3 (−0.5 to −0.1)	0.004	−0.6 (−0.9 to −0.2)	0.001
Factor VII (%)		131.1 (35.7)	136.0 (38.8)	135.9 (36.3)	Ref.	+8.0 (−2.2 to 18.2)	0.12	+5.8 (−6.3 to 17.8)	0.35
Antithrombin (%)		106.5 (13.6)	104.5 (12.7)	102.8 (17.5)	Ref.	−1.9 (−5.6 to 1.8)	0.32	−4.1 (−9.4 to 1.3)	0.14
Total protein S (%)		109.4 (18.7)	105.9 (18.2)	92.3 (16.6)	Ref.	−2.6 (−7.3 to 2.2)	0.29	−16.1 (−21.6 to −10.7)	<0.001

*Adjusted for age, race, BMI, cancer, FVL, use of statins, diabetes.

Missing values: Factor VII (n=7), Antithrombin (n=10), Protein S (n=1).

ETP= endogenous thrombin potential