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Increased thrombin generation among postmenopausal women using hormone therapy: importance of the route of estrogen administration and progestogens

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Abstract

Objective: Increased thrombin generation has emerged as a new surrogate marker of venous thromboembolism. Using calibrated automated thrombography, we tested the influence of the route of estrogen administration and progestogens on thrombin generation among postmenopausal women using hormone therapy.

Methods: Baseline thrombin generation, together with clotting factors and inhibitors, was determined in plasma from 115 healthy postmenopausal women. Women were classified by the use of hormone therapy into three groups: nonusers (n = 38), users of oral estrogens (n = 38), and users of transdermal estrogens (n = 39).

Results: Oral estrogens dose dependently increased thrombin generation. Thrombin generation was increased among users of transdermal estrogens combined with progestins but was similar to nonusers among women using transdermal estrogens plus progesterone. Prothrombin was the main determinant of thrombin generation and explained a part of these differences. However, single clotting factors and inhibitors contributed little to the hormone-related changes in thrombin generation.

Conclusions: Increased thrombin generation can be detected in women using hormone therapy, but this hypercoagulable phenotype depends both on the route of estrogen administration and the type of progestogens. These findings are consistent with current data on the risk of venous thromboembolism related to hormone therapy.

Key Words: Hormone therapy – Menopause – Hemostatic variables – Thrombin generation.

Venous thromboembolism (VTE) is a major harmful effect of hormone therapy (HT) among postmenopausal women.[1-3] Observational data showed that oral but not transdermal estrogens were associated with an increased VTE risk,[4] especially among postmenopausal women at high risk for a first VTE event.[5,6] The difference in VTE risk between oral and transdermal estrogens has been recently confirmed in two large cohort studies,[7,8] and an updated meta-analysis of current data showed that transdermal estrogens did not expose participants to an excess risk of VTE in contrast to oral estrogens.[9] In addition, one recent cohort study provided the first epidemiological evidence that transdermal estrogens might be well tolerated with respect to VTE recurrence among postmenopausal women with a history of VTE.[10] Data on VTE risk related to HT according to the type of progestogens are scarce, but recent studies suggested that women using combined estrogen-progestin had higher VTE risk than did users of estrogen alone or combined with micronized progesterone.[7,11,12]

The difference in VTE risk between oral and transdermal estrogen users is believed to be due to a hepatic first-pass effect of estrogens, which may be responsible for an impaired biosynthesis and clearance of proteins involved in hemostasis.[4] Previous studies showed that oral estrogens could activate the coagulation cascade, whereas transdermal estrogens had little or no effect on hemostasis.[13-15] In addition, the differential effects of HT by the route of estrogen administration on thrombin generation in the presence of activated protein C (APC) were demonstrated among postmenopausal women. Indeed, several randomized trials showed a substantial acquired APC resistance among postmenopausal women using oral estrogens but not in users of transdermal estrogens.[16-18] Although APC resistance has been shown to be a predictor of VTE risk,[19] an increased thrombin generation in the absence of APC has emerged as a new surrogate marker of VTE.[20,21] However, data regarding the influence of HT on baseline thrombin generation are lacking. Therefore, we tested

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the influence of the route of estrogen administration and progestogens on thrombin generation among healthy HT users.

METHODS

Population and study design

Healthy female volunteers aged 25 to 65 years were consecutively recruited in a healthcare center (Investigations Pré-Cliniques, Paris, France) as described.[22] The main purpose of this cross-sectional study was to compare the effects of different types of oral contraceptives and postmenopausal HT used in France on thrombin generation as well as clotting factors and inhibitors.

All participants answered a standardized questionnaire concerning demographic background, medical history, drug use, and personal habits such as smoking and alcohol consumption. Menopause was defined as amenorrhea for more than 12 months with an intact uterus. Postmenopausal women were classified into three groups according to the presence or absence of HT and to the type of HT. Women who did not use HT during the 3 last months were included as nonusers. Current users who used HT at the time of blood sampling were included as oral estrogen users or transdermal estrogen users. Current users of HT were matched with nonusers according to age (age categories, 2 y) and obesity status (normal weight, overweight, obesity). Details of HT including duration of current use, dose of estrogens, and type and dose of progestogens were systematically recorded.

Blood pressure was measured three times on the right arm after a 10-minute rest, and the mean of three measurements was used for analysis. Height and weight of the participants were systematically measured. Body mass index was expressed as the ratio of the weight to the square of the height.

Exclusion criteria were anticoagulant treatment, previous thrombotic events (self-reported history of deep venous thrombosis or pulmonary embolism), and cardiovascular disease (self-reported history of myocardial infarction, coronary insufficiency, stroke, arterial occlusive disease, or malignancy).

The study protocol was approved by an ethics committee. Written consent was obtained from all participants.

Blood samples

Venous blood was drawn between 8 and 10 AM after overnight fasting and a 10-minute rest. Cholesterol, triglycerides, and glucose measurements were performed immediately. For coagulation measurements, venous blood (9 vol) was collected in 5-mL Vacutainer tubes containing 0.105 M/L trisodium citrate (1 vol). Platelet-poor plasma was obtained by two centrifugation steps at 2500*g* for 15 minutes at 15°C. Aliquots were transferred into plastic tubes, quickly frozen, and stored at −40°C. Long-term stability of coagulation factors in frozen-thawed plasma samples has been previously demonstrated.[23] Venous blood was also collected in tubes containing 0.084 mL 15% EDTA for DNA extraction.

Hemostasis measurements

At the time of the assays, frozen plasma samples were transferred into a water bath at 37°C for 5 minutes and then were handled at room temperature. Thrombin generation in platelet-poor plasma was measured with the calibrated automated thrombogram[24] using a PPP reagent, thrombin calibrator, and fluorogenic substrate FluCa (Thrombinoscope BV, Maastricht, the Netherlands), according to the manufacturer's instructions. Briefly, thrombin generation was triggered in 80 μL citrated plasma by the addition of 20 μL PPP reagent and 20 μL of the fluorogenic substrate FluCa. The final concentration of tissue factor in the mixture was 4.8 pM. Each thrombin generation measurement was calibrated against the fluorescence curve obtained under the same conditions, except for the PPP reagent, which was replaced by a thrombin calibrator (Thrombinoscope BV). Three parameters were derived from the thrombin generation curves: lag time (in minutes); area under the curve (endogenous thrombin potential [ETP]), which represents the total amount of active thrombin formed (in nanomolar per minute); and peak height (in nanomolar). Methods for measuring clotting factors and inhibitors have been described.[21] Factor VIIIc, factor IIc, factor II, fibrinogen, antithrombin activity, and protein S activity were measured with an STA analyzer using reagents (factor VIII–, factor VII–, or factor II–deficient plasmas and Neoplastine, STA fibrinogen, STA Stachrom ATIII, STA Staclot protein S) from Stago. Commercially available kits based on enzyme-linked immunosorbent assay methods were used for measuring D-dimers (Fibrinostika FBDP, Organon Teknika) and free tissue factor pathway inhibitor (TFPI) (Asserachrom free TFPI; Stago). Coefficients of variation for hemostatic variables were ranged from 3% (fibrinogen) to 8% (lag time).[13,23]

DNA isolation and genotyping

Genomic DNA was extracted from EDTA blood samples using a standard method. Screening for the FV Leiden mutation and the G20210A prothrombin mutation was performed as previously described.[22]

Statistical analysis

Statistical analysis used procedures available in SAS software (SAS Institute, Inc., Cary, NC). Analysis of variance and the χ^2 test were used to compare the baseline characteristics of participants and to assess the differences in hemostatic variables between HT groups. Data are given as mean and SD. Stepwise multiple linear regressions were used to assess the relative contribution of clotting factors and inhibitors to the prediction of thrombin generation parameters. For each dependent variable (ETP, peak height, and lag time), R^2 and the standardized regression coefficients of the independent variables were calculated. The standardized regression coefficients provide the changes in the dependent variable expressed in SD when the independent variable is subject to a similar change of 1 SD and all other variables in the model remain unchanged. Finally, unadjusted and adjusted regression coefficients were used to estimate the potential mediating role of hemostatic variables in the HT-related changes in thrombin generation.

Based on previous studies,[13,16] we estimated that 40 women per group were needed to detect a difference between groups

TABLE 1. *General characteristics of postmenopausal women by hormone therapy use*

	Nonuser (n = 38)	Oral estrogen (n = 38)	Transdermal estrogen (n = 39)
Age, y	57.0 (4.4)	55.1 (4.5)	55.0 (4.7)
Smokers, n (%)	6 (15.8)	3 (7.9)	7 (17.9)
BMI, kg/m^2	24.1 (3.4)	23.2 (3.2)	23.7 (3.0)
BMI categories, n (%)			
Normal weight	27 (71.1)	30 (78.9)	25 (64.1)
Overweight	10 (26.3)	6 (15.8)	14 (35.9)
Obesity	1 (2.6)	2 (5.3)	0 (0.0)
Total cholesterol, mg/100 mL	230 (40)	211 (26)[a]	224 (34)
HDL cholesterol, mg/100 mL	75 (18)	76 (19)	71 (17)
LDL cholesterol, mg/100 mL	137 (36)	118 (29)[a]	138 (34)[b]
Triglycerides, mg/100 mL	91 (45)	86 (53)	75 (32)
SBP, mm Hg	136 (18)	140 (16)	132 (21)
DBP, mm Hg	81 (10)	81 (8)	78 (13)
Dose,[c] n (%)			
Low	–	8 (21.0)	21 (53.9)
Intermediate	–	6 (15.8)	10 (25.6)
High	–	19 (50.0)	3 (7.7)
Unknown	–	5 (13.2)	5 (12.8)
Duration of use, mo	–	40.6 (35.3)	39.5 (32.5)
Type of hormone therapy, n (%)			
Estrogen + progesterone	–	5 (13.2)	17 (43.6)
Estrogen + progestin	–	32 (84.2)	19 (48.7)
Unknown	–	1 (2.6)	3 (7.7)
Regimen n (%)			
Continuous		26 (68.4)	25 (64.1)
Cyclic	–	12 (31.6)	13 (33.3)
Unknown	–	–	1 (2.6)

Values are mean values (SD).
BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[a]Comparison of oral estrogen users and nonusers: $P < 0.05$.
[b]Comparison of oral estrogen and transdermal estrogen: $P < 0.05$.
[c]Low, <1.5 mg; medium, 1.5 mg; high, ≥2 mg.

of about two-thirds SD for a normally distributed hemostatic variable with a 5% two-sided level and a 80% statistical power.

RESULTS

After exclusion of factor V Leiden and 20210 G>A prothrombin mutation carriers (n = 3 and n = 3, respectively), there were 115 women available for analysis. Table 1 shows the baseline characteristics of women according to HT use. Thirty-eight women were nonusers, 38 women used oral estrogens, and 39 women used transdermal estrogens. All the HT users used 17β-estradiol combined with a progestogen. Most transdermal estrogen users used a gel (n = 33), whereas only six women used a patch. Progestogens encompass both progesterone and progestins. Progestin refers to all synthetic steroids. Among the users of transdermal estrogens, women used micronized progesterone (n = 17), pregnane derivatives (n = 6), or norpregnane derivatives (n = 13). Among the users of oral estrogens, women used micronized progesterone (n = 5), pregnane derivatives (n = 4), norpregnane derivatives (n = 14), medroxyprogesterone acetate (n = 3), nortestosterone derivatives (n = 8), or cyproterone acetate (n = 3). Cardiovascular risk factors were similar among HT users and nonusers except for total cholesterol and low-density lipoprotein cholesterol levels, which were significantly lower in oral estrogen users compared with nonusers ($P < 0.05$).

Thrombin generation and other hemostatic variables by HT use

Mean levels of thrombin generation parameters and other hemostatic variables by HT group are given in Table 2. Oral estrogen users showed significantly higher peak height and

TABLE 2. *Thrombin generation parameters and other hemostatic variables by hormone therapy use*

			Transdermal estrogen		
	Nonuser (n = 38)	Oral estrogen (n = 38)	All (n = 39)	+Progesterone (n = 17)	+Progestin (n = 19)
ETP, nM·min	1843 (279)	2043 (374)[a]	1924 (348)	1834 (360)	2021 (345)[b]
Peak, nM	355 (67)	399 (60)[a]	353 (61)[c]	329 (53)	381 (61)[d]
Lag time, min	3.43 (0.66)	2.88 (0.62)[a]	3.40 (0.83)[c]	3.28 (0.70)	3.42 (0.94)
Protein C, %	128 (22)	112 (20)[e]	109 (16)[e]	110 (13)	109 (15)
Protein S activity, %	111 (16)	105 (27)	110 (19)	111 (20)	105 (17)
Free protein S, %	96 (11)	92 (16)	92 (13)	94 (13)	88 (13)
Total protein S, %	101 (10)	89 (13)[e]	94 (15)[f,g]	93 (12)	95 (17)
FV, %	103 (16)	102 (15)	108 (15)[g]	103 (12)	107 (13)
FVIII, %	148 (39)	139 (42)	129 (24)[f]	123 (21)	139 (23)
FII, %	106 (7)	108 (11)	107 (12)	103 (7)	111 (13)[h]
TFPI, %	9.7 (2.8)	7.3 (1.8)[e]	8.3 (1.7)[a,i]	8.7 (1.5)	7.8 (1.9)
Fibrinogen, g·L^{-1}	3.16 (0.43)	2.78 (0.41)[e]	2.88 (0.41)[a]	2.76 (0.40)	3.00 (0.42)[h]
Antithrombin, %	104 (7)	100 (9)[a]	103 (7)[g]	102 (6)	102 (6)

Values are means (SD).
ETP, endogenous thrombin potential; FII, prothrombin; FV, factor V; FVIII, factor VIII; TFPI, tissue factor pathway inhibitor.
[a]Comparison with nonuser: $P \leq 0.01$.
[b]Comparison of transdermal progestin and transdermal progesterone: $P < 0.10$.
[c]Comparison of transdermal estrogen and oral estrogen: $P \leq 0.01$.
[d]Comparison of transdermal progestin and transdermal progesterone: $P \leq 0.01$.
[e]Comparison with nonuser: $P \leq 0.001$.
[f]Comparison with nonuser: $P < 0.05$.
[g]Comparison of transdermal estrogen and oral estrogen: $P < 0.10$.
[h]Comparison of transdermal progestin with transdermal progesterone: $P < 0.05$.
[i]Comparison of transdermal estrogen and oral estrogen: $P < 0.05$.

shorter lag times than did both nonusers and transdermal estrogen users ($P < 0.01$). Similarly, oral estrogen users had higher ETP levels than did both nonusers ($P < 0.01$) and transdermal estrogen users ($P = 0.2$). Women using transdermal estrogens combined with progestins had higher ETP and peak levels than did users of transdermal estrogens combined with progesterone ($P < 0.10$ and $P < 0.01$, respectively). With regard to clotting factors and inhibitors, protein C activity, total protein S, free TFPI, and fibrinogen levels were significantly lower in both oral and transdermal estrogen users compared with nonusers ($P < 0.05$). The decrease in total protein S and TFPI levels was more pronounced in oral estrogen users than in transdermal estrogen users ($P < 0.10$ and $P < 0.05$, respectively). Antithrombin activity was significantly lower in oral estrogen users than in nonusers ($P < 0.01$). Women using transdermal estrogens combined with progestins had significantly higher factor II levels than the users of transdermal estrogens combined with progesterone ($P < 0.01$).

Table 3 shows the mean levels of thrombin generation parameters according to the dose of estrogens by the route of estrogen administration. ETP and peak levels were positively and significantly correlated with the dose of estrogens among oral estrogen users ($P < 0.05$ and $P < 0.01$, respectively) but not among transdermal estrogen users. There was no significant association between the dose of estrogen and the levels of coagulation factors and inhibitors in both oral and transdermal estrogen users.

Determinants of thrombin generation

Table 4 shows the standardized regression coefficients of coagulation factors and inhibitors for each thrombin generation parameter. Factor II level was the main positive determinant of ETP ($P < 0.001$) and explained 23% of the total variability of ETP. Fibrinogen, antithrombin, and TFPI levels did not contribute significantly to the prediction of ETP, and taken together, clotting factors and inhibitors explained about one third of the total variance of ETP ($R^2 = 0.32$). Peak height was mainly dependent on factor II and factor VIII levels (positive determinants, $P < 0.001$ and $P < 0.01$, respectively), both factors explaining 22% of peak variance. Finally, protein S and TFPI activities were significant predictors (positive determinants, $P < 0.001$ and $P < 0.01$, respectively) of lag time, and they explained 20% of the total variance of this parameter.

TABLE 4. *Determinants of thrombin generation among 115 healthy postmenopausal women*

		β		
Determinant	SD	ETP: 1937 (343)	Peak: 369 (66)	Lag time: 3.24 (0.75)
R^2		0.32	0.34	0.29
Adjusted R^2		0.25	0.27	0.22
Protein C	21	−0.21	−0.12	−0.05
Protein S activity	11	0.05	−0.18	0.35[a]
Total protein S	14	0.07	0.02	0.03
FV	15	−0.04	−0.13	−0.05
FII	10	0.47[a]	0.34[a]	0.02
FVIII	37	0.17	0.29[b]	0.05
TFPI	2.4	0.05	−0.01	0.28[b]
Fibrinogen	0.44	0.06	0.10	0.10
Antithrombin	8	−0.10	−0.006	0.06

β in the standardized regression coefficients indicates the change in thrombin generation parameters (in SD) per 1 SD increase in determinant.
ETP, endogenous thrombin potential; FII, prothrombin; FV, factor V; FVIII, factor VIII.
[a] $P \leq 0.001$.
[b] $P \leq 0.01$.

Potential mediating role of clotting factors and inhibitors

Comparisons between regression models unadjusted and adjusted for clotting factors and inhibitors showed that factor II accounted for only 10% of the difference in ETP levels between oral estrogen users and nonusers. This mediating effect increased up to 40% when the women using transdermal estrogens combined with progesterone were taken as a reference group. Similarly, prothrombin levels explained 10% and 40%, respectively, of higher peak among oral estrogen users. TFPI levels had a mediating effect close to 30% with respect to a shorter lag time in oral estrogen users. Changes in other clotting factors and inhibitors contributed little to the differences in thrombin generation parameters between oral and transdermal estrogen users. Finally, prothrombin explained 61% of the higher ETP in women using transdermal estrogens combined with progestins compared with the users of transdermal estrogens combined with progesterone.

DISCUSSION

Our study shows that oral estrogens shorten the lag time and dose dependently increases both ETP and the thrombin peak among postmenopausal women using HT. Both ETP and peak increase among the users of transdermal estrogens plus

TABLE 3. *Mean levels of thrombin generation parameters according to the dose of estrogen[a] by the route of estrogen administration*

	ETP		Peak		Lag time	
Dose	Oral (n = 33)	Transdermal (n = 34)	Oral (n = 33)	Transdermal (n = 34)	Oral (n = 33)	Transdermal (n = 34)
Low	1881 (200)	1999 (267)	362 (41)	368 (56)	2.92 (0.89)	3.47 (0.77)
Intermediate	1967 (292)	1982 (347)	382 (61)	363 (63)	2.78 (0.51)	3.42 (0.66)
High	2187 (436)	1774 (649)	427 (60)	317 (37)	2.89 (0.47)	3.13 (1.60)
P linear trend	0.04	0.37	0.006	0.23	0.97	0.55

Values are means (SD).
ETP, endogenous thrombin potential.
[a] Dose of estrogen: low, <1.5 mg; medium, 1.5 mg; high, ≥2 mg.

progestins when compared with women using transdermal estrogens plus progesterone. Except for prothrombin, there is no significant rise in procoagulant factors or fall in inhibitors to which this increase can be specifically attributed.

Previous studies consistently showed that oral estrogen use induced a hypercoagulable state among postmenopausal women.[15] Early findings were based on the measurement of single procoagulant or anticoagulant factors as well as specific markers of activated coagulation and/or fibrinolysis in vivo such as prothrombin fragment 1 + 2, fibrinopeptide A, and D-dimers.[13-15] However, procoagulant changes in the hemostatic system of women using HT were not a consistent finding in these studies. Moreover, these first-generation hemostatic biomarkers have not been validated against VTE risk. Because thrombin is the central enzyme in blood coagulation, the capacity of plasma to generate thrombin may be an attractive tool to assess the overall plasma coagulability in vitro. Thrombin generation in vitro measures the potential of generating thrombin, whereas the in vivo tests measure whether there is actually ongoing thrombin generation in circulating blood. Thrombin generation tests have therefore been developed to detect a hypercoagulable phenotype, and APC resistance was initially demonstrated in women using oral contraceptives[25] as well as in postmenopausal women using oral estrogens.[16-18]

Our data show that the thrombin generation test in the absence of APC is sensitive to HT use in postmenopausal women. In addition, we report for the first time that ETP and peak both depend on both the route of estrogen administration and on the type of progestogen. Because increased thrombin generation in the absence of APC has been consistently related to increased VTE risk,[21] our findings suggest that baseline ETP and peak can be used to assess the thrombogenic potential of different types of HT in postmenopausal women. One study has just reported similar differential associations of thrombin generation with oral and transdermal estrogens among HT users.[26] Interestingly, thrombin peak was positively correlated with plasma estrone levels among the oral estrogen users but not among the transdermal estrogen users. This finding suggests that changes in thrombin generation among oral estrogen users are mediated through the hepatic first-pass metabolism of estrone, the main metabolite of oral estradiol, which is avoided by transdermal estrogens.[26] Another important new finding relates to the dose-dependent increases in both ETP and peak among oral estrogen users. Previous studies generally failed to show any correlation between the dose of estrogens among oral estrogen users and either VTE risk or hemostatic variables, except for a recent cohort study that showed VTE risk to increase with estrogen dose in oral but not transdermal estrogen users.[8] In addition, it was reported that HT users taking low-dose oral estrogens tended to be less APC resistant than did women using the conventional dose.[27] Our data suggest that lower doses of oral estrogens may confer less VTE risk than higher doses, and they add to the rationale of the current guidelines regarding HT prescription that recommend the lowest possible dose of estrogens for the shortest possible period.[28]

There are few data on the influence of progestogens on hemostatic variables among postmenopausal women using HT. Previous studies have shown no significant change in either traditional hemostatic biomarkers[13-15] or APC sensitivity[16,17] among women using transdermal estrogens combined with micronized progesterone compared with nonusers. In contrast, a recent study reported activated coagulation and APC resistance among women using transdermal estrogens combined with norpregnane derivatives.[29] Data on the influence of progestogens on thrombin generation in the absence of APC among HT users are lacking. However, one recent study showed no difference in thrombin generation between opposed and nonopposed estrogen use.[26] Our data suggest that progestins as a whole may induce unfavorable changes in thrombin generation. However, among transdermal estrogen users, concomitant progestins encompassed only pregnane and norpregnane derivatives, and our findings may not be relevant to other types of progestins, including medroxyprogesterone acetate and nortestosterone derivatives. In addition, it remains unclear whether the observed differences are caused by direct pharmacological effects of progestins or result from a prescription bias leading to select postmenopausal women with hyperestrogenic symptoms.[7,11,29] There was no significant difference in thrombin generation parameters between progestogen subgroups among oral estrogen users. However, because oral estrogens increased thrombin generation, this negative finding may be due to the dilution of the potential effects of progestogens. Overall, our results are consistent with the clinical data on hormone-related changes in VTE risk among postmenopausal women.[7,11] However, the extent to which progestins may impact both VTE risk and hemostatic variables requires clearly further investigations.

In our study, prothrombin was the main determinant of both ETP and peak height, whereas protein S and TFPI activity were significant correlates of lag time. These results are consistent with previous data on the determinants of thrombin generation in the absence of APC among women using or not using oral contraceptives.[30,31] However, in our study, anticoagulant proteins, especially antithrombin, contributed little to the prediction of either ETP or peak height, and this result was unexpected. The reasons for this discrepancy are unclear.

The mechanisms underlying the HT-related changes in thrombin generation in postmenopausal women are poorly understood. Single clotting factors and inhibitors had a limited mediating role in the present study. However, prothrombin explained a part of the differences in thrombin generation between HT user subgroups. Because prothrombin is synthesized by the liver, this finding may be relevant to the differential VTE risk by the route of estrogen administration, which is generally attributed to the hepatic first-pass effect of oral estrogens. The reduction in total protein S and TFPI activity in both oral and transdermal estrogen users, although more pronounced among oral estrogen users, could be largely unrelated to the hepatic first-pass effect because a substantial part of these proteins are synthesized in endothelial cells. By contrast

with higher VTE risk in pill users, which has been explained by decreased protein S and TFPI via APC resistance,[32] our data suggest that hepatic proteins including prothrombin and other potential factors, which were not measured in this study, may play an important role in determining VTE risk among HT users. Small changes in a number of clotting factors and inhibitors may all work in the same direction and therefore lead to an increase in a global test. It is one of the advantages of an overall test of the complete thrombin-generating system that such combined effects can be detected.[33]

There are some limitations in our study. First, cross-sectional design implies that any causal inferences should be made with caution. Despite matching on age and obesity status, residual confounding by cardiovascular risk factors could affect the comparison between HT users and nonusers. Prescription bias may also have occurred in the comparison between oral and transdermal estrogens. Few women were beginning to use HT, and no woman with a personal history of VTE was included in the study. Thus, our results may not be relevant to women who are most susceptible to thrombosis. Second, a relatively small population sample was used, and a lack of statistical power may explain some negative results especially relating to the type of progestogens. Third, there were many comparisons in this study, and thus, some significant results may be due to chance and require confirmation in larger studies. Finally, findings vary with experimental conditions, and the thrombin generation test used in this study might not have been optimally targeted to investigate postmenopausal HT.

CONCLUSIONS

Increased thrombin generation in the absence of APC or thrombomodulin can be detected in women using HT, but this hypercoagulable phenotype depends on the route of estrogen administration and type of progestogens. These findings add to epidemiological evidence that women using oral estrogens are at higher VTE risk than are women using transdermal estrogens. The impact of progestins on surrogate markers of VTE warrants further data. Mechanistic studies are also needed to investigate how and to which extent increased thrombin generation contributes to VTE excess risk among HT users.

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