Transdermal estrogen therapy effects on fibrinogen levels in women with a past history of venous thromboembolism: a pilot study

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Summary

Objective: To evaluate thromboelastographic parameters and fibrinogen levels in women treated with transdermal 17β estradiol. *Methods:* 29 menopausal women with a history of venous thromboembolic disease were included. Nine patients composed the treatment (HT) group and 20 the control group. Coagulation was assessed by thromboelastography in samples of whole blood and platelet-poor plasma (PPP). The following thromboelastographic variables were measured: time for initial coagulation (R), blood clotting speed (K and the α angle), clot tensile strength (MA and G), global index of coagulation (CI) and fibrinolysis (LY30) and fibrinogen levels. *Results:* There were no differences in the other parameters comparing both groups. Fibrinogen levels showed a 13.77 ± 19.94% reduction in the HT group and a 5.51 ± 8.09% increase in the control group after 6 months. *Conclusions:* Our data suggested that transdermal estrogen may not increase blood coagulability, but that it reduces fibrinogen levels in HT women.

Key words: Hormone therapy; Menopause; Venous thromboembolism; Blood coagulation; Fibrinogen.

Introduction

Hormone therapy (HT) can be used to treat symptoms associated with the depletion of estrogen observed during the menopausal years and to prevent osteoporosis in postmenopausal women [1]. Although early observational studies indicated that HT had a beneficial effect on the cardiovascular system, controlled trials and systematic reviews instead found a higher risk of coronary heart disease (CHD) and venous thromboembolism (VTE) in women using HT [2, 3]. It seems that changes in the coagulation system are at the basis of the mechanisms that explain this phenomenon [4]. Women undergoing hormone therapy have a 2-4-fold increased risk of VTE when compared with nonusers [5] and, classically, recent thrombosis is a contraindication for the therapy [6, 7]. However, an important number of patients under special conditions, such as a history of venous thromboembolism, do need HT to alleviate vasomotor and urogenital symptoms [8].

A growing body of evidence has suggested that the risk of thrombosis during HT varies according to the type of estrogen used, route of administration, and presence of other predisposing factors [9]. Although oral hormone therapy has been shown to increases the risk of venous thromboembolism [10], there are studies suggesting that the transdermal route does not do so [11-14]. Moreover, increased levels of some coagulation factors have already been identified as independent risk factors for VTE [10, 11], and menopause and HT may play a role in the levels [15-17]. In addition, the study of coagulation markers may not reflect the whole coagulation system status at that moment. The only method that seems to be able to show the global tendency of coagulation is thromboelastography (TEG). This is a low-cost method that is more sensitive than routine coagulation assays for the detection of hypercoagulable states [12, 18-23].

Considering that there is no information about transdermal estrogen use in women at high risk of VTE, we conducted a pilot study to evaluate the effects of a 6-month regimen of a transdermal 17-β estradiol formulation on thromboelastographic parameters and levels of fibrinogen in women with a past history of VTE.

Subjects and Methods

The Institutional Review Board of the General Hospital of the Medical School of the University of São Paulo approved the present study. A total of 29 women with menopausal symptoms and a history of venous thromboembolic disease (VTE) were enrolled in the Endocrine Gynecology Division of the General Hospital for this study. A written informed consent was obtained from each individual.

On the screening visit, the women filled out a standardized questionnaire with demographic characteristics including age, ethnicity, and education level. Women were also queried about menopausal symptoms, gynecologic history, age at menopause, use of selected medications, cigarette smoking history, frequency of alcohol use, physical activity, education status, and dietary and nutritional habits.

Medication use was validated during the study. To be eligible for the study, women had to have been in menopause for at least 12 months, could not have undergone any type of hormone treatment for the previous 12 months and could not be consuming lipid-lowering drugs, diabetes medicine, soybean-derived products or herbal supplements [13]. Other inclusion criteria

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were serum follicle-stimulating hormone (FSH) levels exceeding 25 U/ml, estradiol levels lower than 20 pg/ml and presence of hot flushes. Furthermore, all the included subjects had a history of VTE diagnosed by clinical and laboratory findings confirmed by venous Doppler, flebography and chest computed tomography (CT). Women with a history of uncontrolled hypertension, stroke or transient ischemic attack or previous myocardial infarction were excluded from the study. Subjects with arterial thromboembolic disease, recent VTE (less than six months), any malignancies or use of anticoagulants were also excluded from this study. This was an open-label, randomized pilot study in which the women were sequentially assigned to receive or not transdermal estrogen therapy. All the subjects had their body weight (kg), height (m), blood pressure (mmHg) and body mass index (kg/m^2) assessed before and after the intervention. The average age of the women taking HT and that of the control group was 53.7 ± 5.9 yrs [46-60 yrs] and 50.9 ± 7.3 [40-63 yrs], respectively (p = 0.315). The average body mass index (BMI) of the HT group was 26.7 ± 2.4 kg/m^2 [23.88-32.48 kg/m^2] and that of the control group was 29.2 ± 5.8 [19.33-44.44 kg/m^2] (p = 0.109). Associated conditions such as high blood pressure, diabetes, obesity and tobacco use were also noted.

The women in the HT group used weekly replaced patches containing 50 μg of 17β estradiol. The women with an intact uterus took 10 mg of medroxyprogesterone acetate orally for ten days every three months for endometrial protection. The women in the control group were followed up according to the same schedule as that of the treatment group.

Blood samples were collected between 08:00 and 09:00 a.m. after a 12-hour overnight fast and a 40-minute rest in the supine position. Blood was withdrawn from an antecubital vein into tubes containing 3.2% sodium citrate, and the first sample was used solely for an analysis exclusive for hemostatic factors [14]. Plasma was separated within 30 min of collection by centrifugation at 1200 rpm for 15 min at 4°C, and the plasma aliquots were then frozen and stored at –70°C for subsequent simultaneous analyses in duplicate of all the samples, thus avoiding interassay variations.

In order to exclude any bleeding disorders, prothrombin time (PT) [15] (presented as percentage of activity and international normalized ratio - (INR)), thrombin time (TT) [16] (presented in seconds), and activated partial thromboplastin time (aPTT) [17] were evaluated. Clot formation was analyzed by a coagulometer (MTX - Organon Teknika). Time ratios between 0.76 and 1.16 were considered normal for aPTT. Also, platelets were scored manually according to standard protocols as described by Brecher and Cronkite using a light microscope with Neubauer's chamber [18].

TEG was performed immediately after blood collection with a computerized Thromboelastograph 3000 coagulation analyzer. To evaluate the differential participation of platelets or plasma factors in the observed changes, whole blood (WB) and platelet-poor plasma (PPP) samples were studied following blood centrifugation at 1220 g for 15 min. From each sample, 200 μl were pipetted into the cuvette of the Thromboelastograph, which had been previously warmed to 37°C, and 60 μl of caolin at 0.2% were added and the mixture was incubated for 3 min. The sample was then recalcified with 0.025 M calcium chloride. The TEG parameters of both groups at the start were compared with the samples collected at the blood bank from 30 healthy individuals between 40 and 60 years to find out whether the first women to be selected were normocoagulants or hypercoagulants.

Running time for both samples was one hour. An assessment was made of the parameters to be utilized as markers of the hemostatic system activity: R time – measured in millimeters, which is the time from the beginning of the trace to a one-millimeter divergence in the TEG trace; it is influenced by the concentration of thrombin [24, 25]; K time – measured in minutes, which is the period of time between the endpoint of R and a 20-mm divergence in the trace; it represents the formation speed of the clot, and reflects thrombin activity as well as fibrin formation [24, 25]; α Angle – measured in degrees, which is the angle formed by a straight line starting at the endpoint of R and tangent to the curve of the TEG trace and the straight line initiating at the same point and running along the middle of the TEG tracing; it also represents the speed of clot formation, and its values are directly dependent on fibrin [24]; Maximum amplitude (MA) – expressed in millimeters, which is the measure of the maximum strength developed by the clot and it depends on platelets and fibrin [24, 25]; G parameter – expressed in dynes/cm^2, which measures clot firmness and is more sensitive to variations in clot firmness than MA [24, 25]; Coagulation index (CI) – which describes the global state of coagulation and derives from the R, K, MA and angle variables. It is a parameter provided by TEG with normalcy values in the -3.0 to 3.0 range. Values above the upper limit (CI > 3.0) indicate a state of hypercoagulability, whereas values below the lower limit (CI < -3.0) point to a state of hypocoagulability [24-26].

Since a few thromboelastographic variables, chiefly MA and its dependents are a function of the number and activity of platelets and fibrinogen concentration, the latter was measured to evaluate its participation in possible changes in TEG. The fibrinogen levels were determined by the modified Clauss method [19]. Clot formation was analyzed by a coagulometer (MTX – Organon Teknika).

The fibrinogen levels were analyzed using the two-way ANOVA for time and groups separately and the Bonferroni correction when appropriate. For clinical and anthropometric features the Student's t-test was used. TEG coagulability parameters were assessed using analysis of variance (ANOVA) for repeated measures on two parameters [27], and the factors were the group, the times and the samples (repetition parameter); Bonferroni's multiple comparisons were also used [28]. The analyses were performed using SAS 8.0 and SPSS 13.0 software and Microsoft Excel 2000™. A p value of < 0.05 was considered to be significant, and the results are presented as the mean ± SE.

Results

Subjects were followed up for six months and there was no medical complication related to their medication. Once enrolled, all the women were evaluated clinically and had their laboratory samples analyzed. Ten women dropped out of the study, meaning that 19 finished the entire 6-month study period, nine in the HT group and ten in the control group. Clinical information on the subjects enrolled in this study is listed in Table 1. Hemostatic parameters (platelets, PT, TT, aPTT) were evaluated and compared within groups. There were no differences between the groups. It was assumed that none of the groups presented any bleeding disorders.

The R parameter was statistically significant between the blood samples and PPP ($p < 0.001$); the mean value of R in the blood was higher than in PPP by 30 min. Behavior of the R parameter between the groups during the study as shown by the mean and the standard error.

Table 1. — *Clinical parameters and hemostatic profile*.

	Case (n = 9)	Control (n = 20)	*p*
Age	53.78 ± 5.97	50.90 ± 7.38	0.315
BMI (Kg/m²)	26.69 ± 2.45	29.25 ± 5.86	0.109
SIST (mmHg)	125.78 ± 12.35	126.05 ± 16.51	0.965
DIAST (mmHg)	74.33 ± 17.18	79.65 ± 10.17	0.305
Hypertension	44.4% (4)	40.0% (8)	0.691
Diabetes mellitus	0	5.0% (1)	1.000
Smoking	22.2% (2)	10.0% (2)	0.555
Varicose veins	22.2% (2)	5.0% (1)	0.188
Obesity	11.1% (1)	40.0% (8)	0.214
PT - INR	0.93 ± 0.08	0.97 ± 0.08	0.568
Activity (%)	1.04 ± 0.09	1.01 ± 0.08	0.706
TT	0.93 ± 0.06	0.94 ± 0.05	0.791
TTPA	1.01 ± 0.18	0.96 ± 0.13	0.336
Platelets (n/mm3)	242 375 ± 79 104	213 842 ± 59 028	0.502

BMI = body mass index; SIST = Maximum blood pressure; DIAST = Minimum blood pressure.

The K parameter was smaller in the HT and control groups than in the normal group [control = -2.78 min ($p < 0.001$); HT = -3.17 min ($p < 0.001$)]. There were no differences in the K means throughout the study ($p = 0.854$), nor was there any variation in K in the course of time in any of the groups ($p = 0.567$).

The α angle was wider in the HT and control groups than in the normal group in the total blood samples [HT = + 30.7% ($p = 0.004$); control = + 25.3% ($p = 0.002$)]. As for the PPP samples, the initial differences were 18.3% between the HT and normal groups and 1.7% only between the control and normal groups. The global evaluation between the total blood and PPP samples showed a statistically significant difference with total blood sample 7.06 degrees narrower than the PPP sample ($p < 0.001$). There were no statistically significant changes in the comparison between the HT and control groups throughout the study.

There were variations in the MA measure between the samples and between the groups. The total blood and the PPP samples differed in about 19.45 mm ($p < 0.001$). The median difference in MA between the HT and the normal groups and the control and the normal groups was 5.68 mm ($p < 0.001$) and 3.67 mm ($p < 0.001$), respectively. There was no difference in MA between the HT and control groups. Nor was any significant variation detected between the groups or between the samples in the course of time.

The G parameter values were higher in the HT and control groups; the observable difference between the HT and normal groups equaled 0.51 dynes/cm² ($p < 0.001$) and between the control and normal groups, 0.29 dynes/cm² ($p = 0.003$). Sample variation was detected throughout the study ($p = 0.020$).

The difference between the samples (total blood and PPP) was significant at the three times in the study ($p < 0.001$), thus the behavior of the G parameter was similar to that of the MA parameter, but with greater sensitivity to reveal differences throughout the study. The CI was always negative between the groups under investigation, and there were no differences between them. No variation in CI was observed throughout the study, nor was there any difference between the groups over time. Fibrinogen levels showed different behaviors throughout the study, with a 13.77 ± 19.94% reduction in the HT group and a 5.51 ± 8.09% increase in the control group ($p = 0.011$) after six months of follow-up. There was no difference in the percentile variation of fibrinogen levels between three months and six months ($p = 0.344$).

Discussion

HT for menopausal symptoms under special conditions has been a challenge in the field of women's health. Women with a history of venous VTE and intense menopausal symptoms with poor response to nonhormonal therapy are a major clinical problem [20]. Here we suggest that transdermal hormonal therapy might not be a critical option for those cases. VTE is now recognized as a multicausal disease which results from interactions of multiple genetic and environmental factors and in which coagulation plays a central role [21]. Among the acquired risk factors, age increases the disease exponentially [28-31]. Other clinical conditions contribute to increase the risk of VTE, namely obesity [22-24], varicose veins [24], trauma [24], thrombophlebitis [22], surgeries, stroke, pregnancy, puerperium, cancer, oral contraceptives, tobacco use, heart failure and others [22, 25, 26]. Besides these clinical conditions, high levels of coagulation factors such as fibrinogen have been described as independent risk factors for VTE [27].

Plasma fibrinogen concentration increases with age, and in women it occurs mainly after menopause[28]. Reduction in plasmatic levels of fibrinogen has been seen with the use of both oral and transdermal estrogen treatments [28-30] in menopausal women. These studies suggest that both the oral and transdermal routes are effective in reducing plasma fibrinogen levels, although their effects may be impaired by the use of a progestogen. Lindoff *et al*. [31] measured fibrinogen as a clottable protein and observed a 6% decrease in fibrinogen concentrations among transdermal estradiol users. Zegura *et al*. [32] observed a statistically significant 11.6% diminution of plasma fibrinogen levels after a 6-month treatment with transdermal estrogen. This is in agreement with our findings, where a 13.77% reduction in plasma fibrinogen levels was verified in the treated women. In fact, the use of estrogen therapy by women with a history of venous thromboembolism may increase their chances of a recurrence of the disease. Swiatkiewicz *et al*., who compared subjects with recurrent VTE and healthy patients, found higher concentrations of plasma fibrinogen, as well as other coagulation factors, in the VTE group, suggesting a permanent hypercoagulable state in these women. According to other studies, high fibrinogen levels may predispose to hypercoagulability by increasing blood viscosity and stepping up the production of platelet aggregates. Although different at baseline, the fibrinogen levels were within the normal range, as defined by the laboratory method, meaning that none of the groups had high lev-

els of fibrinogen. On the other hand, transdermal therapy was effective in diminishing the fibrinogen levels in the HT group. Further research is needed to clarify the main problems and benefits of this therapy and to explore related leads, such as acute VTE, risk indicators and therapy length.

References

[1] Writing Group for the PEPI Trial: "Effects of hormone replacement therapy on bone mineral density: results fromthe postmenopausal estrogen/progestin interventions (PEPI) trial". *JAMA,* 1996, *276,* 1389.

[2] Rossouw J.E.: "Coronary heart disease in menopausal women: Implications of primary and secondary prevention trials of hormones". *Maturitas,* 2005, *51,* 51.

[3] Writing Group for the Women's Health Initiative Investigators: "Principal results from the women's health initiative randomized controlled trial". *JAMA,* 2002, *288,* 321.

[4] Lowe G.D.O.: "Hormone replacement therapy and cardiovascular disease: increased risks of venous thromboembolism and stroke, and no protection from coronary heart disease". *J. Int. Med*., 2004, *256*, 361.

[5] Wu O.: "Postmenopausal hormone replacement therapy and venous thromboembolism". *Gender Med.,* 2005, *2* (suppl. 1), S18.

[6] Lip G.Y., Blann A.D., Jones A.F., Beevers D.G.: "Effects of hormone-replacement therapy on hemostatic factors, lipid factors and endothelial function in women undergoing surgical menopause: Implications for prevention of atherosclerosis". *Am. Heart J*., 1997, *134,* 764.

[7] Gomes M.P.V., Deitcher S.R.: "Risk of venous thromboembolic disease associated with hormonal contraceptives and hormone replacement therapy: a clinical review". *Arch. Intern. Med*., 2004, *164*, 1965.

[8] Speroff L., Gilbert R.D., Ellman H. for the estradiol acetate Investigator Group: "Efficacy of a new, oral estradiol acetate formulation for relief of menopause symptoms". *Menopause*, 2006, *13,* 442.

[9] Modena M.G., Sismondi P., Mueck A.O., Kuttenn F., Lignières, B., Verhaeghe J. *et al*.: "New evidence regarding hormone replacement therapies is urgently required transdermal postmenopausal hormone therapy differs from oral hormone therapy in risks and benefits". *Maturitas,* 2005, *52*, 1.

[10] Bertina R.M.: "Elevated clotting factor levels and venous thrombosis". *Pathophysiol haemost. thromb*., 2004, *33*, 395.

[11] Poort S.R., Rosendaal F.R., Reitsma P.H., Bertina R.M.: "A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and increase in venous thrombosis". *Blood,* 1996, *88,* 3698.

[12] Schreiber M.A., Differding J., Thorborg P., Mayberry J.C., Mullins R.J.: "Hypercoagulability is most prevalent early after injury and in female patients". *J. Trauma,* 2005, *58*, 475.

[13] Han K.K., Soares J.M. Jr, Haidar M.A., de Lima G.R., Baracat E.C.: "Benefits of soy isoflavone therapeutic regimen on menopausal symptoms". *Obstet. Gynecol.,* 2002, *99,* 389.

[14] Sztejnsznajd C., Silva M.E., Nussbacher A., Gebara O.E., D'AmicoE.A., Rocha D.M. *et al*.: "Estrogen treatment improves arterial distensibility, fibrinolysis, and metabolic profile in postmenopausal women with type 2 diabetes mellitus". *Metabolism*, 2006, *55,* 953.

[15] Quick A.J., Stanley-Brown M., Bancroft F.W.: "A study of the coagulation defect in hemophilia and jaundice". *Am. J. Med. Science,* 1935, *190*, 501.

[16] Ingram G.F., Matchett M.O.: "The serial thrombin time method for measuring fibrinogenolytic activity in plasma". *Nature,* 1960, *188*, 674.

[17] Bell W.N., Alton H.C.: "A brain extract as a substitute for platelet suspension in the thromboplastin generation test". *Nature,* 1954, *174,* 880.

[18] Brecher G., Cronkite E.P.: "Morphology and enumeration of human blood platelets". *J. Appl. Physiol*., 1950, *3,* 365.

[19] Clauss A.: "Rapid physiological coagulation method for the determination of fibrinogen". *Acta Haemat.,* 1957, *17,* 237.

[20] NIH State-of-the-Science Panel: "National Institutes of Health State-of-the-Science Conference Statements: Management of menopause-Related Symptoms". *Ann. Intern. Med*., 2005, *142,* 1003.

[21] Rosendaal F.R.: "Venous thrombosis: a multicausal disease". *Lancet*, 1999, *353*, 1167.

[22] Heit J.A., Silverstein M.D., Mohr D.N., Petterson T.M., Lohse C.M., O'Fallon, W.M., Melton L.J. 3rd: "The epidemiology of venous thromboembolism in the community". *Thromb. Haemost.,* 2001, *86*, 452.

[23] Jick H., Derby L.E., Myers M.W., Vasilakis C., Newton K.M.: "Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens". *Lancet*, 1996, *348*, 981.

[24] Varas-Lorenzo C., García-Rodríguez L.A., Cattaruzzi C., Troncon M.G., Agostinis L., Perez-Gutthann S.: "Hormone replacement therapy and the risk of hospitalization for venous thromboembolism: a population-based study in southern Europe". *Am. J. Epidemiol*., 1998, *147,* 387.

[25] Task Force on Pulmonary Embolism, European Society of Cardiology: "Guidelines on diagnosis and management of acute pulmonary embolism" *Eur. Heart J*., 2000, *21,* 1301.

[26] Seligsohn U., Lubetsky A.: "Genetic susceptibility to venous thrombosis". *N. Engl. J. Med*., 2001, *344*, 1222.

[27] Koster T., Rosendaal F.R., Reitsma P.H., van der Velden P.A., Briët E., Vandenbroucke J.P.: "Factor VII and fibrinogen levels as risk factors for venous thrombosis. A case-control study of plasma levels and DNA polymorphisms–the Leiden Thrombophilia Study (LETS)". *Thromb. Haemost.,* 1994, *71*, 719.

[28] Lowe G.D., Rumley A., Woodward M., Morrison C.E., Philippou H., Lane D.A., Tunstall-Pedoe H.: "Epidemiology of coagulation factors, inhibitors and activation markers: The Third Glasgow MONICA Survey. I. Illustrative reference ranges by age, sex and hormone use". *Br. J. Haematol*., 1997, *97,* 775.

[29] Zegura B., Keber I., Sebestjen M., Koenig W.: "Double blind, randomized study of estradiol replacement therapy on markers of inflammation, coagulation and fibrinolysis". *Atherosclerosis,* 2003, *168*, 123.

[30] Taner M.Z., Ozpolat E., Taskiran C., Onan M.A., Gursel T., Karabulut E. *et al*.: "Effects of four different regimens of hormone replacement therapy on hemostatic parameters: A prospective randomized study". *Maturitas*, 2006, *53,* 267.

[31] Lindoff C., Peterson F., Lecander I., Martinsson G., Astedt B.: "Transdermal estrogen replacement therapy: beneficial effects on hemostatic risk factors for cardiovascular disease". *Maturitas*, 1996, *24,* 43.

[32] Zegura B., Guzic-Salobir B., Sebestjen M., Keber I.: "The effect of various menopausal hormone therapies on markers of inflammation, coagulation, fibrinolysis, lipids, and lipoproteins in healthy postmenopausal women". *Menopause,* 2006, *3*, 643.

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