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Risk of venous thromboembolic disease in postmenopausal women taking oral or transdermal hormone replacement therapy*

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Abstract: Objective: The influence of hormone replacement therapy (HRT) on hemostasis processes depends on the type of hormone, the combination of doses, the time of taking HRT, and the route of administration (oral, transdermal, implanted). The aim of the current study was to assess some parameters of coagulation, especially tissue factor pathway inhibitor (TFPI) and tissue factor (TF) in postmenopausal women using oral or transdermal HRT. Methods: The study was conducted on 76 healthy women, including 46 women aged 44–58 years who were taking oral (26) or transdermal (20) HRT, and 30 women aged 44–54 years who did not take HRT as the control group. Plasma concentrations of TF, TFPI, thrombin-antithrombin complex (TAT), and D-dimer were performed by enzyme-linked immunosorbent assay (ELISA). Moreover, the concentration of fibrinogen and activity of protein C were measured by chromogenic and chronometric methods. Results: We observed a significantly higher concentration of TF and a significantly lower concentration of TFPI in women taking oral and transdermal HRT in comparison with the control group. We also found a significantly lower concentration of fibrinogen in women taking oral HRT vs. the control group. Moreover, no statistically significant changes in concentrations of TAT and D-dimer, or activity of protein C were noted. Conclusions: In this study, the occurrence of an increased TF concentration simultaneously with a decreased concentration of TFPI in women taking HRT indicates hypercoagulability. No significant modification of TAT or D-dimer occurred, and thus there may not be increased risk of thrombosis.

Key words: Menopause, Coagulation, Hormone replacement therapy
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1 Introduction

According to the world health organization (WHO), menopause is the time in a woman's life when the reproductive capacity ceases. A woman reaches menopause when she has not had a period for 12 months (WHO, 1996). In menopause, the ovaries gradually and inevitably stop functioning, which in turn results in a hypoestrogenism state. On the other hand, the secretion of gonadotrophic follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the hypophysis is increased not only during, but also after, menopause. The basis of hormone imbalance in perimenopausal women is disorders of the efficiency of the hypothalamus-hypophysis-ovary axis. Sex hormone disorders in perimenopausal women lead to metabolic changes in various tissues and organs. Before menopause, women are reasonably protected against cardiovascular disease (CVD) and thromboembolism by their circulating estrogens.

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Nevertheless, this protection ends after menopause (Hale *et al*., 2007).

Estrogen and progestogen are administered in hormone replacement therapy (HRT) to postmenopausal women with the purpose of preventing the symptoms of menopause, such as hot flashes, nocturnal sweat, mood swings, insomnia, palpitation, atrophy of urogenital system, higher risk of ischemic heart disease, osteoporosis, insulin-resistance, and atherosclerosis (Callejon *et al*., 2005; Guimarães *et al*., 2009). HRT should be individually adjusted—"tailored hormonal therapy". HRT can be applied in three regimens: estrogen alone and estrogen plus continuous or cyclical progestin. Because unconstrained estrogen replacement is associated with the higher risk of endometrial cancer, it is the best treatment for women after a hysterectomy. Estrogen combined with progesterone in HRT is a choice for women with a uterus (Gomes and Deitcher, 2004).

Moreover, HRT brings 2–4-fold risk of venous thromboembolism (VTE) which includes deep vein thrombosis, pulmonary embolism, and stroke. The risk of VTE is higher in the first 6–12 months of HRT and gradually decreases over several years (Peverill, 2003; Gomes and Deitcher, 2004; Guimarães *et al*., 2009; Stevenson, 2009). This increased risk of VTE may occur due to the activation of coagulation or inhibition of fibrinolysis. The effects of HRT on hemostasis parameters may depend on the type of estrogen, dosage, the route of administration, and the time period of usage (Callejon *et al*., 2005). In addition, progesterone may modify the effect of estrogens (Post *et al*., 2003).

The HRT influence on the coagulation system is very wide. For instance, oral estrogen decreases several clotting factors such as fibrinogen, tissue factor pathway inhibitor (TFPI), protein S, protein C, and antithrombin. On the other hand, HRT increases levels of the procoagulant factors: VII, X, XII, XIII, and prothrombin fragments 1+2 (F_{1+2}) as a marker of thrombin generation (Bladbjerg *et al*., 2003; Peverill, 2003; Guimarães *et al*., 2009). These facts are associated with an increase in activation of coagulation. It is likely due to the hepatic first-pass effect of oral administration, and is dose-dependent. Nevertheless, during transdermal HRT, no changes in F_{1+2}, thrombin-antithrombin complex (TAT), or D-dimer were observed (Høibraaten *et al*., 2000). The impact on activation of coagulation may be avoided by using the transdermal HRT or by reduced doses of oral estrogen (Callejon *et al*., 2005; Stevenson, 2009). Transdermal HRT does not exert a harmful influence on the hepatocytes. The hormone reaches the target organ and avoids the portal circulation. Transdermal HRT is more physiological than oral HRT (Callejon *et al*., 2005; Gracia *et al*., 2005).

The levels of relieved perimenopausal symptoms and the character and intensity of adverse effects are different and depend on the route of HRT administration (oral or transdermal), the kind of hormone, pattern (continuous or cyclic), and the time of HRT usage. Determination of these parameters should lead to the best effectiveness of HRT and minimize adverse effects.

Several studies have examined the influence of HRT on hemostatic parameters, but the results are still controversial (Høibraaten *et al*., 2000; Callejon *et al*., 2005; Guimarães *et al*., 2009). The aim of the study was to assess the concentrations of TFPI, tissue factor (TF), TAT, fibrinogen, and D-dimer, and the activity of protein C in postmenopausal women who were receiving oral or transdermal HRT.

2 Materials and methods

2.1 Subjects

A total of 76 healthy, nonsmoking women, who were 1–2 years postmenopausal, are included in this study. Forty-six women aged 44–58 years (mean age 52 years) received oral or transdermal HRT, which was taken daily during continuous treatment with estrogen-progesterone combinations. Among them, 26 women used oral HRT [2 mg 17 β-estradiol (E_2) and 1 mg norethisterone acetate (NETA) (Kliogest, Novo Nordisk Pharma, Poland)] and 20 women used transdermal HRT [50 μg E_2 and 170 μg NETA (SYSTEN® Conti, Janssen-Cilag, Warsaw, Poland)]. Those subjects had been using HRT for 6–14 months. Persistent climacteric symptoms, like heavy and regular hot flashes with drenching sweat, were the main indication for HRT. The control group consisted of 30 healthy, nonsmoking, postmenopausal women, aged 44–54 years (mean age 49 years), who did not take HRT.

Women in both groups had neither diabetes mellitus nor glucose intolerance. They had no prior

thromboses or systemic illnesses. None of them were taking any other medication that might have interfered with coagulation system. All women included in the study had a complete gynecological examination, cytology smear, breast examination, mammography, and biochemical examinations (lipid and hormone profiles) (Table 1).

As mentioned in Ruszkowska *et al*. (2010), a total of 4.5 ml venous blood for hemostatic tests was collected in a fasting state into cooled tubes (Becton Dickinson Vacutainer® System, Plymouth, UK) containing 0.13 mol/L trisodium citrate (the final blood-anticoagulant ratio was 9:1) after 30 min of rest between 7:30 and 9:30 am and after a 12-h overnight fast. The blood samples were immediately mixed and centrifuged at 3000×*g* at 4 °C for 20 min. The obtained platelet-poor plasma was divided into 200 μl Eppendorf-type tubes and then samples were frozen at −86 °C until assayed, but no longer than six months. Blood for serum lipids and hormone tests was collected in a tube without anticoagulant (Becton Dickinson Vacutainer® 17490, Plymouth, UK) and the serum was separated by centrifuging at 2500×*g* for 15 min and kept at 4 °C until analyzed.

Women in both groups were selected in the Outpatient Gynecology Centre of the University Hospital in Bydgoszcz. Written informed consent was obtained from each participant before entering the study. The study was approved by the Bioethics Committee of Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń (No. KB/305/2004).

2.2 Hemostatic assays

The concentration of TFPI was determined by enzyme-linked immunosorbent assay (ELISA) IMUBIND® total TFPI (American Diagnostica, Żory, Poland), TF by ELISA IMUBIND® TF (American Diagnostica), TAT by ELISA ENZYGNOST® TAT micro (Behring, Marburg, France), and D-dimer by ELISA ASSERACHROM® D-DI (Diagnostica Stago, Asnières, France). The concentration of fibrinogen and activity of protein C were performed in an automated coagulometer CC-3003 apparatus and reagents were purchased from Bio-Ksel Co., Grudziądź, Poland.

2.3 Hormone assays

Levels of serum FSH and estradiol were determined by standardized micromolecular immunoenzymatic test (Microparticle Enzyme Immunoassay (MEIA), AXSYM® SYSTEM, Abbott laboratories, Diagnostics Division, Abbott Park, IL, USA).

2.4 Lipid assays

Levels of serum total cholesterols, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, and triglycerides were determined by elimination method. Apparatus and reagents were purchased from Abbott laboratories.

2.5 Statistical analysis

Statistical analysis was performed by using Statistica 6.0 software (StatStoft®, Cracow, Poland). Shapiro-Wilk test was used to assess normality of the distribution. We used the classical Student's *t*-test to analyze the normal distribution, and U-Mann-Whitney rank-sum test to analyze abnormal distribution. For variables with normal distribution, the mean and standard deviations (SD) were determined. The median, lower quartile (Q_1), and upper quartile (Q_3) were used to express the values with abnormal distribution. The P values <0.05 were considered statistically significant.

3 Results

We have compared the biochemical data in the study and control groups. As shown in Table 1, there was no significant difference observed within all the study groups.

Table 2 shows concentrations of TF, TFPI, TAT, fibrinogen, and D-dimer, and activity of protein C in women taking oral or transdermal HRT and in the control group. In the study, we observed significantly higher concentrations of TF in women using oral or transdermal HRT in comparison with the control group (P<0.001). Women taking oral or transdermal HRT had significantly lower concentrations of TFPI than women in the control group (P<0.001). A significantly lower concentration of fibrinogen in women taking oral HRT vs. the control group was also found (P<0.05). Moreover, no statistically significant changes in concentrations of TAT and D-dimer, and activity of protein C were noted.

Table 1 Clinical and biochemical data concerning women in this study

Group	FSH (U/L)	Estradiol (pmol/L)	Total cholesterol (mmol/L)	HDL-cholesterol (mmol/L)	LDL-cholesterol (mmol/L)	Triglycerides (mmol/L)
Oral (I)	67.20±23.97	71.21±24.34	6.05±1.25	1.68±0.33	3.62±0.91	1.58±0.81
Transdermal (II)	61.60±26.35	67.62±25.88	5.84±0.99	1.56±0.37	3.57±1.03	1.54±0.64
Control (III)	70.37±28.23	66.99±27.13	5.63±0.94	1.63±0.32	3.27±0.94	1.42±0.51
P	I vs. II, 0.58 I vs. III, 0.82 II vs. III, 0.55	I vs. II, 0.73 I vs. III, 0.72 II vs. III, 0.94	I vs. II, 0.55 I vs. III, 0.09 II vs. III, 0.29	I vs. II, 0.27 I vs. III, 0.60 II vs. III, 0.48	I vs. II, 0.84 I vs. III, 0.20 II vs. III, 0.30	I vs. II, 0.80 I vs. III, 0.21 II vs. III, 0.30

Values of biochemical parameters are presented as mean±SD

Table 2 Concentrations of TF, TFPI, TAT, fibrinogen, and D-dimer, and activity of protein C in women in this study

Group	TF (pg/ml)	TFPI (ng/ml)	TAT (ng/ml)	Fibrinogen (g/L)	D-dimer* (ng/ml)	Activity of protein C (%)
Oral (I)	212.03±57.74	46.18 (41.76/52.92)	3.88 (2.82/4.74)	3.24 (2.99/3.85)	338.25 (218.52/473.13)	135.00 (124.00/148.00)
Transdermal (II)	240.37±98.06	44.00 (37.98/57.62)	3.40 (2.56/5.01)	3.67 (3.33/4.17)	371.81 (265.16/431.54)	127.00 (117.00/144.00)
Control (III)	114.64±57.56	96.24 (73.64/123.00)	3.25 (2.46/4.25)	3.74 (3.37/4.07)	325.70 (279.11/467.47)	124.50 (114.00/130.00)
P	I vs. II, NS I vs. III, <0.001 II vs. III, <0.001	I vs. II, NS I vs. III, <0.001 II vs. III, <0.001	I vs. II, NS I vs. III, NS II vs. III, NS	I vs. II, NS I vs. III, <0.05 II vs. III, NS	I vs. II, NS I vs. III, NS II vs. III, NS	I vs. II, NS I vs. III, NS II vs. III, NS

Values of hemostasis parameters are shown as mean±SD or median (Q_1/Q_3). NS: not significant. * Fibrinogen equivalent units

4 Discussion

VTE is the major adverse effect associated with HRT (Peverill, 2003; Guimarães *et al*., 2009). The state of hypercoagulability is associated with the activation of coagulation simultaneously with lower potential of coagulation inhibitors (Callejon *et al*., 2005). Estrogen combined with progesterone has been suggested as a treatment in postmenopausal women having a uterus to protect the endometrial hyperplasia against malignancy (Koh *et al*., 2001).

Our results showed a significantly higher concentration of TF in women using oral or transdermal HRT in comparison with the control group. Women using oral or transdermal HRT had a significantly lower concentration of TFPI.

The role of TF in blood coagulation is well established as the main factor engaged in the activation of coagulation system. Moreover, TF is associated with different processes, such as inflammatory, sepsis, angiogenesis, metastasis, and atherosclerosis. High levels of TF contribute to the higher risk of thrombosis and myocardial infarction (Versteeg *et al*., 2001).

Koh *et al*. (2001; 2003) observed significantly decreased TF antigen and increased TF activity in women who received micronized progesterone (MP) 200 mg/d or medroxyprogesterone acetate (MPA) 2.5 mg/d for 10 d with conjugated equine estrogens (CEE) 0.625 mg/d for HRT, suggesting activation of the coagulation pathway.

Human TFPI is synthesized in microvascular endothelial cells, monocytes, megakaryocytes, and macrophages. Increased TFPI concentration was found during pregnancy, sepsis, disseminated intravascular coagulation (DIC), and solid tumors (Bajaj *et al*., 2001). Harris *et al*. (1999) have found that, in women taking oral contraceptives, a significant lowering in TFPI plasma levels occurs. Low plasma levels of TFPI indicate an increased risk of VTE (Bajaj *et al*., 2001).

The results of the current study are in accordance with Høibraaten *et al*. (2000). They described significantly increased levels of TF and a decrease in TFPI concentration in women taking oral HRT, but they observed a slight impact of transdermal HRT on plasma levels of TFPI. Bladbjerg *et al*. (2002) noted a highly significant decrease in TFPI concentration in all hormone groups regardless of the type of progestin regimen. Luyer *et al*. (2001) have also observed reduced concentrations of TFPI when women were

using estrogen replacement for only three months. Peverill *et al*. (2001) found that six weeks of estradiol and norethisterone resulted in a 26% decrease in TFPI antigen levels. Bladbjerg *et al*. (2003) have observed that after 5–6 years of HRT/ERT, plasma concentration of TFPI in postmenopausal women was significantly reduced. The impact on TFPI was present in all genotypes of the TFPI-287T/C polymorphism.

In our study, the results suggest that oral and transdermal treatments can induce coagulatory activation through the significant increase in TF concentration, which binds with factor VIIa. This complex TF:VIIa is a main initiator of the coagulation system in the extrinsic pathway. We also observed the significant decrease in TFPI level, which is a major inhibitor of the TF:VIIa complex. Nevertheless, there was no increase in the risk of coagulation activity because no significant increase in the level of TAT complex or D-dimer was noted. A normal D-dimer concentration, which we had obtained in our study, proves that in both groups there was no increase in the formation of thrombin and fibrin. Thus we did not observe the dissolving stabilized fibrin.

A decrease in the level of TFPI, which lowers the inhibition of the extrinsic pathway, results in downstream activation of coagulation and an increase in thrombin generation (Peverill *et al*., 2001; Bladbjerg *et al*., 2003). The observed lower TFPI levels in all studies may contribute to increased coronary heart disease (CHD) risk associated with HRT, mainly in subgroups of women with atherosclerosis and with an increased risk of intimal TF expression (Bladbjerg *et al*., 2003).

Results of our study showed a decrease in the concentration of fibrinogen in the women taking oral HRT in comparison with the control group. The concentration of fibrinogen increases with age. Additionally, it is higher during pregnancy and menopause, in obesity, and in women taking contraceptives (Post *et al*., 2003). Oral and transdermal estrogens decrease fibrinogen concentration, but in many studies, differences of fibrinogen concentration were not found while using HRT (Iacoviello *et al*., 2001; Ruszkowska *et al*., 2010). The postmenopausal estrogen/progestin interventions-trial (PEPI) research showed a slight decrease in fibrinogen concentration when taking only estrogen replacement (mix of horse estrogens of a natural origin-conjugate estrogens in a dose of 0.625 mg/d) (The Writing Group for the PEPI Trial, 1995; Ruszkowska *et al*., 2010). The results of our study are in accordance with the results of Andersen *et al*. (1999). They show a significant decrease in fibrinogen concentration when taking oral estrogen/progesterone therapy, which consists of estradiol valerate (2 mg/d) and cyproterone acetate (1 mg/d) taken in the second phase of the menstrual cycle. The reduction of the fibrinogen level could be beneficial because it lowers the quantity of substrate for fibrin formation and causes the associated reduction in blood viscosity (Peverill, 2003). Moreover, decreased fibrinogen concentration protects from the development of atherosclerosis in menopausal women and demonstrates a higher effectiveness of oral HRT in this scope. In addition, there have been several studies which indicate that transdermal HRT may have a slight unfavorable impact on hemostasis compared with oral HRT (Peverill, 2003).

During qualifying women for HRT, both indications and contraindications must be considered. HRT ought to start as soon as possible when the first symptoms of hypoestrogenism appear. It has been accepted worldwide that the HRT has a positive impact on the reduction of menopause symptoms. When HRT is applied early, it reduces the risks of ischemic heart disease, venous thrombosis, pulmonary embolism, and death. On the other hand, long-term HRT increases the potential risks of cancer development and thromboembolic disease. The present knowledge on this issue is still insufficient; however, we are able to explain what different factors may cause the development of thrombosis in some women taking HRT. Thus, it must not be forgotten that every patient must be treated on an individual basis (LaCroix, 2005; Dębski, 2006; Guimarães *et al*., 2009).

5 Conclusions

In this study, the occurrence of increased TF concentration simultaneously with a decreased concentration of TFPI in women taking HRT indicates hypercoagulability. However, no significant modification of TAT complex or D-dimer occurred, and thus there may not be an increased risk of thrombosis. Larger trials are required to further confirm our results.

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