

# Oral but not transdermal estrogen replacement therapy reduced level of tissue factor pathway inhibitor: Cross-over designed study

Tomas FAIT<sup>1</sup>, Michal VRABLIK<sup>2</sup>, David CIBULA<sup>1</sup>, Jaromir MASATA<sup>1</sup>, Martin HILL<sup>3</sup>  
& Bohuslava TRNKOVA<sup>4</sup>

<sup>1</sup> Department of Obstetrics and Gynaecology, General Faculty Hospital Prague and 1<sup>st</sup> Faculty of Medicine, Charles University,

<sup>2</sup> 3<sup>rd</sup> Medical Department, General Faculty Hospital Prague and 1<sup>st</sup> Faculty of Medicine, Charles University

<sup>3</sup> Institute of Endocrinology, Prague

<sup>4</sup> Institute of Clinical Biochemistry, General Faculty Hospital Prague and 1<sup>st</sup> Medical Faculty, Charles University

*Correspondence to:* Tomas Fait M.D.  
Dept. Obstet. Gynecol. General Faculty Hospital and 1<sup>st</sup> Faculty of Medicine  
Apolinarska 18, 128 53 Praha 2, CZECH REPUBLIC  
EMAIL: tfait@seznam.cz

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## Abstract

**OBJECTIVES:** The purpose of the present study was to determine changes of biochemical risk factors of thromboembolism especially of TFPI during the use of oral versus transdermal administration of early estrogen replacement therapy.

**METHODS:** In a 12-week prospective, randomized, cross-over trial, oestradiol was administered orally in a dose of 2 mg daily or transdermally in an equivalent dose of 0.05 mg daily. Forty-five healthy early postmenopausal women were included into the study within 12 weeks after the hysterectomy and ovariectomy (surgical castration). Forty-one women completed the study and their data were analyzed. The average age was of  $49 \pm 6$  years.

**RESULTS:** After an oral therapy, the average value of TFPI decreased significantly ( $p < 0.0001$ ) from  $87.5 \pm 38.5$  ng/ml to  $68 \pm 37$  ng/ml but remained stable in transdermal group ( $81.9 \pm 36.8$  ng/ml). No significant changes occurred in D-dimers. In both groups AT – III and fibrinogen decreased without exceeding their physiological ranges.

**CONCLUSIONS:** The oral therapy reduced significantly TFPI levels compared to transdermal route of administration. The results confirm more pronounced impact of oral estrogen on hemostasis variables.

## Introduction

The effect of hormonal replacement therapy (HRT) on the risk of thromboembolism is generally recognized. Not only composition but also dose, administration route and timing are of importance in determining the risk (1, 2).

Tissue factor pathway inhibitor (TFPI) is the main inhibitor of the complex tissue factor – activated factor VII of the coagulation cascade and thus also a considerable modulator of thrombogenesis (3, 4). The average plasma concentration in the normal

population is  $60 \pm 13$  mg/l (5). Low level of free TFPI increases the relative risk of thromboembolism up to its doubled value (6).

This is the first randomised study with cross-over design which follow changes of TFPI during oral versus transdermal route of estradiol administration.

### The group of patients and methods

The prospective study was conducted from September 2003 to March 2005. Forty-five healthy women with manifestation of acute climacteric syndrome were included; 41 completed the study and their data were analyzed. The treatment started within 6 to 12 weeks after the ovariectomy and hysterectomy. Any of women showed symptoms of oestrogen deficiency before the surgery. The average age of the whole group was of 49  $\pm$  6 years (32 – 57 years).

In the cross-over design, the women were randomized for the oral or transdermal estrogen replacement therapy (ERT) administered for 12 weeks with changing the route of administration after an one-week wash-out period. Either 2 mg of 17beta-oestradiol (E2) daily (Estrofem tbl, Novo Nordisk, Denmark) or its equivalent in a transdermal therapeutic system (Climara emp, Schering, Germany) were administered every week and releasing 0,05 mg of E2 daily.

The blood samples were taken at the beginning of the study, after 12 weeks and at the end of the protocol after 25 weeks. The blood plasma was separated from the venous blood taken between 8 and 9 a.m. into test tubes with EDTA and stored at  $-80^{\circ}\text{C}$ .

The kit IMUBIND<sup>R</sup> Total TFPI ELISA test, American Diagnostica, Germany was employed for the TFPI determination. This is an enzyme sandwich method with a lower detection limit of 0.18 ng/ml. Pairs of analyses were provided for each sample.

The other analytes (fibrinogen, AT III, D-dimers) were routinely determined on an automatic analyzer.

Repeated measures ANOVA model with factors Stage and Subject was used to evaluate the changes during

the study. The design enabled to separate the variability associated with changes during the experiment from inter-individual variability and unexplained variability (variability of residuals). Due to non-constant variance and lack of symmetry in the data as in the residuals, a power- or logarithmic transformation of dependent variables was applied before the statistical testing. The approach resulted in approximation to Gaussian distribution and stabilization of the variance. Non-homogeneities were detected using diagnostic residual plots. The experimental points with absolute values of studentized residuals greater than 3 were excluded from the analysis. The proportion of such points never exceeded 5% of the original number. The differences between individual stages of the study were evaluated using Bonferroni multiple comparisons on the probability level  $p < 0.05$ . Statistical software Statgraphics Plus version 5.1 was used for the analysis.

The study was approved by the Ethical Committee of the General Faculty Hospital Prague.

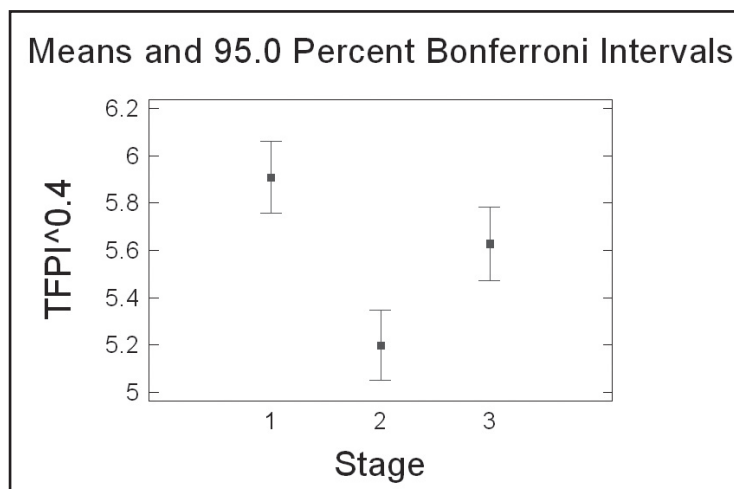
### Results

After the oral ERT, the average TFPI value decreased significantly ( $p < 0.0001$ ) ( $87.5 \pm 38.5$  ng/ml vs.  $68 \pm 37$  ng/ml). This change was also significantly different ( $p = 0.03$ ) compared with the non-significant decrease after the transdermal therapy ( $81.9 \pm 36.8$  ng/ml) (Table 1).

There were no significant changes in D-dimers after both oral ( $217 \pm 125$  g/l vs.  $221.6 \pm 125$  g/l,  $p = 0.711$ ) and transdermal ( $241.7 \pm 162.6$  g/l,  $p = 0.123$ ) ERT.

The AT-III level decreased significantly after both oral ( $110.2 \pm 10.6$  % vs.  $103.3 \pm 8.1$ ,  $p = 0.001$ ) and transdermal ( $106.1 \pm 11$ ,  $p = 0.047$ ) treatment; there was no significant difference between the two types of the intervention ( $p = 0.116$ ).

Fibrinogen decreased more significantly ( $p = 0.022$ ) after the oral treatment ( $3.4 \pm 0,8$  g/l vs.  $3.0 \pm 0,6$  g/l,  $p = 0,001$ ) compared to the transdermal administration ( $3.2 \pm 0,6$  g/l,  $p = 0.022$ ) (Table 2).



**Table 1:** TFPI levels (analysis summary) – 1- before therapy, 2 – after oral administration, 3 – after transdermal administration.

**Least Squares Means for TFPI<sup>0.4</sup> with 95.0 Percent Confidence Intervals**

Level	Count	Mean	Std. Error	Lower Limit	Upper Limit
GRAND MEAN					
		119	5.57823		
Stage					
1		40	5.90886	0.0869756	5.73563
2		41	5.1985	0.0853885	5.02844
3		38	5.62733	0.0903053	5.44747

**Table 2:** Characteristics of study groups and changes of hemostasis variables (LQ –lower quartile, UQ – upper quartile, SD – statistic difference, Med – Median, BMI – body mass index, INR – international normalized ratio, APTT - activated partial thromboplastin time, TT - thrombin time, TFPI – tissue factor pathway inhibitor)

Variable	Baseline					Oral					Transdermal				
	Mean	SD	Med	LQ	UQ	Mean	SD	Med	LQ	UQ	Mean	SD	Med	LQ	UQ
Age	49,05	5,441	50	48	52										
BMI	26,81	4,916	26,89	22,94	29,98	26,87	4,878	26,71	22,28	30,1	27,08	4,991	27,07	22,87	30,49
INR	0,907	0,078	0,9	0,9	0,9	0,902	0,056	0,9	0,9	0,9	0,912	0,08	0,9	0,9	0,9
APTT	29,41	3,015	28,8	27,3	31,2	29,36	3,031	29,4	27,3	30,6	31,42	3,837	30,4	28,7	33,8
TT	13,53	1,265	13,7	12,5	14,5	13,34	1,919	12,8	12,1	14,2	13,24	1,555	12,8	12,2	14,6
Fbg	3,425	0,766	3,3	3	3,725	2,959	0,654	2,8	2,4	3,3	3,205	0,623	3,2	2,9	3,4
ATIII	110,2	10,57	111	104	117	103,3	8,055	104	97	110	106,1	10,97	106	98	114
D-dimer	217	123,4	190	126	259	221,6	145,3	192	121	260	241,7	160,6	205	160	276
TFPI	87,47	38,53	83,6	63,1	104	68,04	37,03	61,2	42,3	76,6	81,94	36,84	78,4	53,9	105

## Discussion

We found significantly different changes of TFPI during oral versus transdermal ERT.

In accordance with data from the literature, the chosen duration of the intervention of 12 weeks is sufficient, since changes in coagulation variables after the HRT are obvious after 3 months of the treatment and the values are no more changed after that (7).

Several principal differences between oral and transdermal administration of the hormonal therapy have been described. In the transdermal therapy, more stable oestrogen plasma levels are achieved and the metabolic load to the liver first-pass effect is thus reduced. Between the two modalities there is no difference in the effect on the acute climacteric syndrome, urogenital atrophy and osteoporosis prevention (8). The results of multicentre hospital-based case-control study ESTHER (9) (155 consecutive cases and 381 controls) showed a significant relative risk of thromboembolism in women receiving the oral ERT in comparison to untreated women (RR 3.5), but almost equal to transdermal ERT (RR 4).

Tissue factor pathway inhibitor (TFPI) is the main inhibitor of the complex tissue factor – activated factor VII of the coagulation cascade. A significant decrease in the TFPI level was demonstrated after an oral HRT

(10) independently of the type and regimen of gestagen (11) even after administration for five years (12). The decrease reached about 25%. In a group of women with the history of thromboembolism, these values were decreased even by 30 to 50% (13). In these women, low TFPI is considered a risk factor of the recurrence of thromboembolic events (14). There are currently no data available concerning the effect of different routes of administration on TFPI. We evaluated in our study hemostatic variables in cross over design allowing direct comparison of the two routes of administration in each subject with a minimal influence by confounding factors. Changes of TFPI were significantly different between the two groups, decreased by 22% during oral oestrogen therapy but remained unchanged in transdermal group.

D-dimers are the breakdown products of a fibrin mesh that has been stabilised by Factor XIII. D-dimers has become an important test performed in patients suspected of thrombotic disorders. Negative result practically rules out thrombosis and activation of coagulation cascade. A positive result can indicate thrombosis but also has other potential causes (liver disease, high rheumatoid factor, inflammation, malignancy, trauma, pregnancy, recent surgery). In a 12-week study on 27 postmenopausal women, placebo and oestradiol in oral and transdermal forms were compared. The oral treatment induced

increase in D-dimers while there were no changes on transdermal therapy and placebo (15). Scarabin SR. et al. (7) in group of forty-five healthy postmenopausal women, aged 45 to 64 years, assigned randomly to one of the three following groups: cyclic oral or transdermal estradiol, both combined with progesterone, or no hormonal treatment have found no differences in D-dimers after a 6-month period. In our study we also did not find significant changes in either group.

Antithrombin is a serpin (serine protease inhibitor) that inactivates a number of enzymes from the coagulation system, namely the activated forms of Factor X, Factor IX, Factor II (thrombin), Factor VII, Factor XI, and Factor XII. Its affinity for these molecules (i.e. its effectivity) is enhanced by heparin. In a prospective, open three-months study on 45 postmenopausal women comparing values of haemostatic markers during the use of transdermal oestradiol 0.05 mg/day with controls without treatment, the level of AT III were not significantly changed (16). When compared with post-menopausal non-users group, current HRT users had lower mean levels of AT III (17). Oral administration induced a significant decrease in AT III, but in case of transdermal therapy no significant change was found (18). We were able to confirm significant decrease in AT III after an oral treatment, but the identical decrease was observed during transdermal administration. There was no significant difference between the two types of the intervention. It can be speculated, that this discrepancy is a consequence of early start of therapy in our study.

Discrepant data concerning fibrinogen changes during hormonal treatment have been published to date. In the PEPI trial, placebo resulted in a significantly greater increase in mean fibrinogen than any active treatments (19). In meta-analysis of 46 studies, HRT was associated with significantly decreased levels of fibrinogen and antithrombin. The same highly significant decrease of fibrinogen levels was shown with oral and transdermal therapy. In our study we found a significant decrease in fibrinogen during the oral treatment.

## Conclusion:

In our study, we demonstrated significant differences in TFPI changes between oral and transdermal estradiol treatment in equivalent doses. Values of TFPI were reduced significantly during oral but remained unchanged while on transdermal administration.

Significant changes in TFPI, AT III and fibrinogen found after oral ERT are in accordance with literature. Discrepancies in AT III during transdermal therapy could be explained by early start of therapy in our study.

With respect to the consensual recommendations of the choice of the HRT, which is neutral with respect to the haemostatic system (20), our results suggest that the oral form of the ERT induce more pronounced changes when evaluating equivalent dosage of 2 mg oestradiol orally and 0.05 mg transdermally.

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