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# Estetrol (E4) is a Unique Native Estrogen that does not modify Coagulation Markers in Postmenopausal Women and maintains Sensitivity to Activated Protein C (APC)

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## Introduction

Combined oral contraceptives (COC) or hormonal replacement therapy (HRT) increase 3 to 6 fold the risk of venous thromboembolism (VTE). This risk increase is largely explained by induction by the estrogens of resistance towards the physiological anticoagulant called Activated Protein C (APC) [1–3] (Figure 1) .

Estetrol (E4) is a promising natural estrogen in development by Mithra Pharmaceuticals. Unlike other estrogens, E4 blocks the activation of the membrane estrogen receptor α (ERα).This property of E4 is the basis for its tissue specific action and its unique pharmacodynamic profile. Data in pre- and postmenopausal women show that E4 alone or in combination with a progestin has minimal stimulatory effects on triglycerides, sex hormone binding globulin, corticosteroid-binding globulin, and angiotensinogen.

In this study, we sought to determine the effects of E4 on the resistance to APC in postmenopausal women.

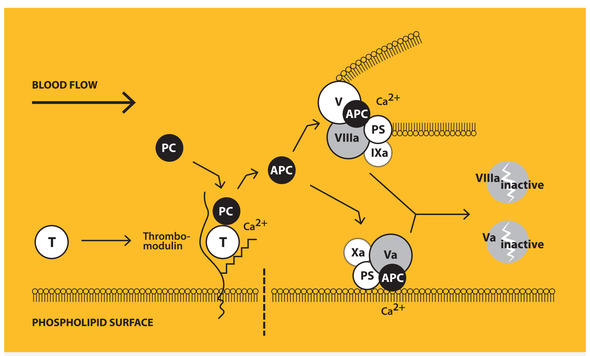


**Figure 1**: **Protein C Anticoagulant Pathway**

Thrombin (T), once activated by the extrinsic or intrinsic pathways of the coagulation, binds to its receptor thrombomodulin on the intact cell surface. As a result, this complex loses the procoagulant properties of thrombin and instead becomes a potent activator of protein C. APC will then form with protein S a complex that functions as a circulating anticoagulant, which specifically degrades and inactivates the phospholipid-bound factors Va and VIIIa. This effectively down-regulates the coagulation process and limits clot extension..

T = Thrombin, PC = Protein C, APC = Activated Protein C, PS = Protein S

## Design and methods

- Multicenter, randomized, placebo-controlled, double-blind, dose-finding study
- 257 postmenopausal women
- 2.5, 5, 10, or 15 mg E4; or placebo once daily for 12 weeks
- Assessments: changes from baseline of coagulation factors, hemostasis biomarkers, and ETP-based normalized APC sensitivity ratio (nAPCsr)

The global clotting capacity of plasma called the endogenous thrombin potential (ETP), is determined with the thrombin generation test (TGT). The TGT quantifies thrombin generation after triggering the extrinsic pathway of the coagulation using tissue factor in presence of phospholipids. To assess APC resistance with this test, the amounts of thrombin generated in the absence and presence of exogenous APC are compared. The concentration of APC added to plasma is chosen to obtain 90% inhibition of the ETP in individuals not taking COCs, HRT, nor having thrombotic abnormalities. Resistance to APC induced by COCs and/or HRT reduces the percentage inhibition of the ETP by APC. The relative resistance of the patient's plasma is then compared to that of a reference plasma. This is the basis of the well-known ETP-based normalized APC sensitivity ratio (nAPCsr).

## ETP-based nAPCsr

- ETP based APCsr was determined as described previously [7]. Venous blood was collected from the antecubital vein into 0.109 M sodium citrate tubes without corn trypsin inhibitor. Platelet poor plasma (PPP) was obtained by centrifugation within 30 minutes of blood withdrawal. PPP was aliquoted, snap frozen, and subsequently stored in liquid nitrogen.
- Frozen samples were thawed and performed within 4 hours after thawing. The ETP-based APC resistance assay was conducted using the Calibrated Automated Thrombogram (CAT) and the Thrombinoscope software (Thrombinoscope bv, Maastricht, the Netherlands).
- Concentration of APC was calculated to ensure 90% inhibition of ETP (equation 1) on reference plasma. ETP-based APCr was also expressed as the nAPCsr according to equation 2. APCsr is defined as the ratio of ETP in the presence and absence of exogenous APC in the respective plasma sample, divided by the same ratio of a control plasma.

$$\text{Inhibition \%} = \left(1 - \frac{\text{sample ETP (+APC)}}{\text{sample ETP (–APC)}}\right) \times 100 \qquad \text{[Equation 1]}$$

$$\text{nAPCsr} = \frac{\text{sample ETP (+APC)} \,/\, \text{sample ETP (–APC)}}{\text{Reference plasma ETP (+APC)} \,/\, \text{Reference plasma ETP (–APC)}} \qquad \text{[Equation 2]}$$

## Results

- No change from baseline in any of the hemostasis markers including fibrinogen, prothrombin, prothrombin fragment 1+2, D-dimer, factor VIII activity, protein C, antithrombin, and tissue factor pathway inhibitor
- Non-clinically meaningful, but statistically significant, small decrease of free protein S (5.9%) at the highest E4 dose
- Relative changes from baseline of the ETP-based nAPCsr are displayed in (Figure 2)
- The similar relative change with E4 15 mg in postmenopausal women was comparable to the increase observed in younger women receiving E4 15 mg/DRSP 3 mg, but is in marked contrast to the increases on EE 20 µg/DRSP 3 mg (219%) and EE 30 µg / LNG 150 µg (165%) (Figure 3)

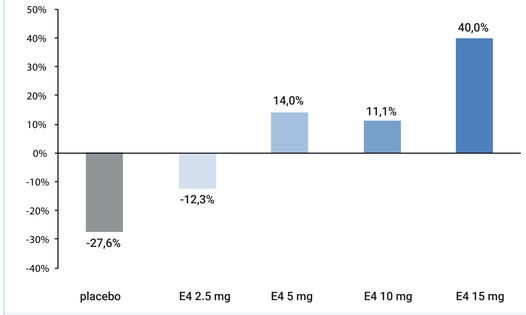


**Figure 2**: Relative changes from baseline of the ETP-based nAPCsr in postmenopausal women receiving different doses of E4 or placebo

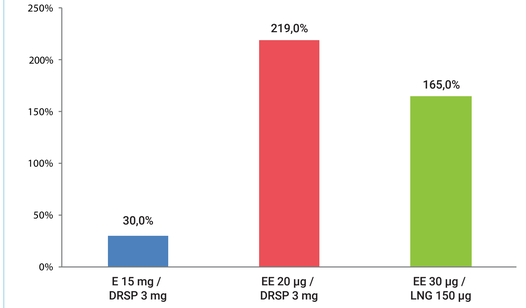


**Figure 3**: Relative changes from baseline of the ETP-based nAPCsr in premenopausal women receiving 15 mg E4/DRSP, EE 20 µg/DRSP3 mg, or EE 30 µg/LNG 150 µg [4]

## Conclusions

- The observed hemostasis changes with E4 and E4/DRSP in the old and younger populations included in postmenopausal and contraceptive trials can be considered as clinically non-significant. These hemostasis changes were less than those observed with other HRT products containing estradiol or conjugated equine estrogens
- These data on ETP-based nAPCsr and the other hemostasis data from previous trials support the notion that E4 may be associated with a lower risk of thrombosis, strengthening the position of E4 as a promising new treatment option for postmenopausal women

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