Hormone therapy and hemostasis among postmenopausal women: a review.

Marianne Canonico

Hormone therapy and hemostasis among postmenopausal women: a review.

Marianne Canonico

Hormone therapy and haemostasis among postmenopausal women

A review

Running title: Hormones and haemostasis in menopausal women

Marianne Canonico [1-2]

[1] Centre for Research in Epidemiology and Population Health, U1018, Hormones and Cardiovascular Disease, Villejuif, France

[2] Univ Paris-Sud, UMR-S 1018, Villejuif, France

Conflicts of interest: none

Fundings: none

Abstract

Objective. Postmenopausal hormone therapy (MHT) which consists of exogenous estrogens with or without combined progestogens remains the most effective treatment to correct climacteric symptoms. Depending on its characteristics, it may nevertheless increases the risk of venous thromboembolism and its effect on haemostasis has been studied for several decades. The aim of this review was to summarize the current knowledge on the effect of MHT on haemostasis, taking into account the route of estrogen administration, the estrogens daily doses and chemical structures as well as the pharmacological classes of progestogens.

Methods. We selected data from randomized controlled trials which included a control group (either placebo or no treatment) and we conducted our analysis on different biomarkers generations.

Results. Overall, studies showed a haemostasis imbalance among oral estrogens users with a decrease in coagulation inhibitors and an increase in markers of activation coagulation leading to a global enhanced thrombin generation. By contrast, transdermal estrogens use was associated with less change in

haemostasis variables and did not activate coagulation and fibrinolysis. No clear difference in MHT effect on haemostasis was highlighted between estrogens daily doses and compounds as well as between pharmacological classes of progestogens

Conclusions. Changes in haemostasis are in accordance with clinical results that show an increased thrombotic risk with oral but not transdermal estrogens use.

Key words. Postmenopausal hormone therapy, haemostasis, randomized controlled trials, estrogens, progestogens.

Introduction

Women are subject to steroid sex hormones exposure from puberty to menopause and other times if prescribed postmenopausal hormone therapy (MHT). This treatment remains the most effective treatment of climacteric symptoms [1] but may increase the risk of venous thromboembolism (VTE) [2, 3]. Nevertheless, there is now compelling evidence that thrombotic MHT effect may strongly depend on the route of estrogen administration [4-9], the estrogens daily doses [8] and chemical structure [10, 11] as well as the pharmacological class of progestogens [5, 7, 9].

The MHT effects on haemostasis have been studied for several decades with a dual interest. On one hand, investigating the coagulation balance or imbalance among MHT users provides a biological support to the thrombogenic potential of specific treatments [12-15]. On the other hand, some haemostatic variables have been validated as surrogate marker markers of VTE [16-18] and can be used to investigate the pharmacologic effect of MHT on thrombosis [19]. During the early 80', factors and inhibitors of coagulation and fibrinolysis were the only haemostatic variables that have been investigated in response to MHT use. Few years later, activation markers have been found to be helpful as markers of coagulation and/or fibrinolysis. More recently, some global assays used to test a hypercoagulability state have been validated as surrogate markers of VTE risk.

Using exclusively the data from randomized trials that included a control arm (either placebo or no treatment), we propose here to summarize the results of MHT and haemostasis, taking into account the route of estrogen administration, the estrogens daily doses and chemical structure as well as the pharmacological class of progestogens [12, 13, 20-49].

Haemostasis and thrombosis biomarkers

Haemostasis is a physiological state that includes both coagulation and fibrinolysis (figure 1). Together with the platelets, the haemostatic system maintains blood in a fluid state under normal conditions and can activate controlled mechanisms to prevent blood loss [50, 51].

Coagulation cascade is a set of initiation and amplification processes that lead in its final phase to the fibrin clot after the conversion of fibrinogen by the thrombin, the key enzyme of the system. After activation of several coagulation factors, both intrinsic and extrinsic pathways conduct to activated factor X that is responsible, together with activated factor V, for thrombin generation after a proteolytical cleavage of prothrombin and releasing of prothrombin fragment 1+2. Accretion of fibrin depends on both activity of the coagulation cascade and fibrinolysis which causes degradation of fibrin. Plasminogen, the central protein of the fibrinolytic system, is converted into plasmin which cleaves fibrin clot into D-Dimers, soluble degradation products [52-54].

For decades, several generations of biomarkers have been used to investigate the thrombotic process (table 1). During the early years, factors and inhibitors of both coagulation and fibrinolysis were investigated with clear direct relationship neither with an activation of coagulation and fibrinolysis nor with the clinical risk of VTE [4, 55]. Then, specific activation markers of coagulation and fibrinolysis have been identified and were used to highlight the initiation of a physiological process that conducts to the fibrin clot formation and degradation. Nevertheless, this second generation of biomarkers has not been consistently related to VTE risk [19].

More recently, global tests of a hypercoagulability state have been validated as surrogate markers of VTE [16-18] and can be used for both investigating the pharmacological effect of MHT and as a plausible biological reason for the potential thrombogenic role of MHT. This global test of thrombin generation is an *in vitro* assay of the ability for a plasma sample to generate thrombin following a coagulation activation. This thrombin generation reflects therefore all the integrated pro and anticoagulant reactions that can be implicated and can regulate the formation and inhibition of thrombin [56].

Characteristics of postmenopausal hormone therapy

Postmenopausal MHT has been introduced in the early 50' for counteracting climacteric symptoms including hot flushes, vaginal dryness and depression. It is also efficient to prevent osteoporosis. MHT initially consisted of an estrogenic compound administrated alone but, in the 70', progestogens were added to estrogens to reduce the increased risk of endometrial hyperplasia and cancer associated with estrogen therapy [57].

There are now several types of MHT that differ according to the chemical structure, the daily doses and the route of administration. The characteristics of the main marketed MHT are summarized in the figure 2.

The estrogenic compound of estrogen therapy (ET) consists of either 17β-estradiol, the bio-identical compound, or synthetic ones including estradiol valerate and conjugated equine estrogens (CEE). The natural 17β-estradiol can be administered orally and non-orally. Non oral 17β-estradiol can be given transdermally but rarely nasallyly [4, 58]. Estradiol valerate is a synthetic compound administered by oral route, the composition of which and therefore the effects are very close to those of 17β-estradiol [59, 60]. Finally, CEE is synthetized from urine of gravid mares and contains more than 10 known biologically active estrogen compounds including mainly estrone sulfate and equilin sulfate. It is always orally administered [10].

Progestogens compound of estrogen/progestogen therapy (EPT) include both progesterone, the bio-identical compound synthesized and secreted by ovary, and synthetic ones named progestins which derived from either progesterone (pregnanes and 19-norpregnanes) or testosterone (19-nortestosterone). Pregnane derivatives consist of different molecules including dydrogesterone, medrogestone, chlormadinone acetate, cyproterone acetate and medroxyprogesterone acetate (MPA). Norpregnane derivatives include nomegestrol acetate, promegestone, trimegestone and nestorone. Finally, nortestosterone derivatives consist of ethinylated derivatives, non ethinylated derivatives, spironolactone derivatives and tibolone. Nortestosterone ethinylated derivatives are composed of estranes, including especially norethisterone acetate (NETA) and of gonanes which are preferentially used in contraceptive pills. Nortestosterone non ethinylated derivative (dienogest) and the spironolactone

derivative (drospirenone) are also used in contraception. As an EPT compound, progestogens are always combined with estrogens and almost exclusively administered by oral route [57].

According to the countries, medical practices regarding postmenopausal MHT use may present important differences in term of chemical structure and route of administration. In France, women are preferentially prescribed 17β-estradiol transdermally administered and combined with micronized progesterone. By contrast, oral CEE combined with MPA are often used in USA.

Estrogens-induced changes in haemostasis

MHT that consists of exogenous estrogens with or without combined progestogens has long been believed to have little effect on VTE risk. However, it is known for several decades to induce changes in haemostasis. Nevertheless, these modifications in coagulation and fibrinolysis may depend on several MHT characteristics, including especially the route of estrogens administration (table 2).

Route of estrogen administration

Within the twenty-eight selected randomized controlled trials of MHT and haemostasis which included a control group (either placebo or no treatment), a large majority investigated only the effect of oral estrogens [20-24, 26-32, 34-36, 39, 42, 43, 45, 46, 48, 49]. Some of them included both oral and transdermal arms [12, 13, 37, 38, 44] and only five trials had a transdermal but not an oral group [25, 33, 40, 41, 47].

Oral estrogens

Overall, there is no clear effect of oral estrogens on coagulation factors, including fibrinogen, factor VII, factor VIII, thrombin and prothrombin. Until the end of the 90', all the studies but one [22] found no change in these parameters among oral estrogens users as compared to non-users or to users of placebo [12, 20, 23, 27]. From 2001, several studies showed that some of these parameters, especially fibrinogen, factors VII and VIII decreased in oral groups without a clear biological explanation for this reduction [28, 29, 31, 34, 37-39, 43, 45, 46]. This time-dependent discrepancy between early and recent studies may be in part explained by lack of standardization in laboratory methods that lead to important differences in biological assays. With respect to inhibitors of coagulation, most of the studies reported a significant decrease in anti-thrombin and/or protein C and protein S among users of oral estrogens, suggesting a haemostatic imbalance due to these treatments [12, 13, 21, 27, 29, 31, 35, 37-39, 43].

Evidence for an effect of oral estrogens on fibrinolysis factors emerged neither for plasminogen nor for tissue plasminogen activator with studies showing no change and others an increase or a decrease in one or both factors [12, 20, 23, 27, 29,

[32, 35-38, 45, 46]. By contrast, plasminogen activator inhibitor 1, as a fibrinolysis inhibitor, has been consistently found to decrease with use of oral estrogens that may thus promote a fibrinolytic process in response to the initial coagulation activation [12, 26, 27, 29, 32, 35-39, 45, 46].

Overall, these results on factors and inhibitors of coagulation and fibrinolysis in users of oral estrogens are consistent with studies that found on the whole an increase in activation markers of coagulation and therefore "in response" fibrinolysis, as shown by changes in prothrombin fragment 1+2 and D-dimers concentrations [12, 13, 21, 26, 27, 31, 35, 37, 38].

Since 2001, several studies used haemostatic global tests, including baseline thrombin generation and thrombin generation with or without activated protein C, to evaluate the effect of MHT on a hypercoagulability state [13, 28-30, 34, 37, 38, 43, 48, 49]. Most of the studies found an important and significant increase in APC resistance or baseline thrombin generation associated with oral estrogens use [13, 30, 34, 37, 38, 48, 49]. Nevertheless, the biological mechanism for such APC resistance in postmenopausal women using oral estrogens remains largely not understood. Some studies have found that changes in levels of protein S and Tissue Factor Pathway Inhibitor might have important roles in determining APC resistance in women receiving oral estrogens [15, 61]. Others reported that soluble form of endothelial protein C receptor which has been reported to inhibit APC anticoagulant activity might therefore be a candidate to explain APC resistance [62]. The three studies which showed no APC resistance with oral estrogens [28, 29, 43] used a biological method which has been demonstrated to be sensitive to Factor V Leiden mutation but not to exogenous estrogens [13].

Important is to remark that these results concern estrogens alone or administered in combination with a progestogen. Since women who prescribe ET have to be hysterectomized and account in industrialized countries for less than 20% of HT users, only some studies included an unopposed arm to evidence the main effect of estrogens, irrespective of progestogens [21, 27, 29, 32, 34, 37, 38, 44-46]. Nevertheless, restricted analysis to ET led to similar results.

Taken together, these results show that oral estrogens cause a haemostatic imbalance that leads to a blood coagulation activation and a hypercoagulability state. This biological process is consistent with clinical studies that found an increase thrombotic risk among users of oral estrogens [63].

Transdermal estrogens

Effect of transdermal estrogens use on haemostasis has been assessed for the first time in a randomized controlled trial in 1997 [12].

Inconsistency remains in the results regarding coagulation factors with studies having shown no change in transdermal estrogens users [12, 25, 33, 47] and others which found a decrease in fibrinogen [41], factor VII [38, 41] or factor VIII [38, 40, 41]. Similarly, while inhibitors of coagulation have been found to decrease in four randomized controlled trials [13, 25, 37, 41], there was no change is other investigations [12, 47].

Results were more coherent regarding factors and inhibitors of fibrinolysis where all studies but one [41] found no change in these parameters between users of transdermal estrogens and non-users or users of placebo [12, 25, 33, 37, 40, 47].

In the same way, most of the studies showed no effect of transdermal estrogens on markers of coagulation and fibrinolysis [12, 13, 25, 33, 38, 40] and this maintenance of a haemostasis balance was confirmed in studies by Oger et al. and Post et al., who both found no APC resistance in transdermal estrogens users as compared to placebo users [13, 37].

Altogether, results consistently showed that transdermal estrogens have little or no effect on haemostasis. Also in this case, this biological effect is consistent with results of clinical studies that found a similar thrombotic risk between users of transdermal estrogens and MHT non-users [63].

Oral versus transdermal estrogens

Only three studies included together oral and transdermal estrogens arms to directly compare the effect of the route of administration [12, 13, 37, 38]. In the IMEP trial, Scarabin et al. reported a significant increase in prothrombin fragment 1+2 with oral estrogens use as compared to transdermal estrogens use [12] and few years later, Oger et al. confirmed this result in the SARAH trial [13]. In addition, this latest study, taken together with the investigation by Post, showed that the increase in APC resistance with oral estrogens use was significant when compared to transdermal estrogens use [13, 37]. One more time, biological and clinical results are consistent [63].

Chemical structures

Oral 17β-estradiol and oral CEE were included together and then compared only in one randomized controlled trial but statistical analysis did not allow comparing their respective effect on haemostasis [42]. Nevertheless, an indirect comparison of several other selected studies suggests no difference in MHT effect on haemostasis between 17β-estradiol, estradiol valerate and CEE. Since transdermal administration exclusively concerns 17β-estradiol, no potential effect of chemical structure can be assessed for transdermal estrogens.

Daily dose

Studies having investigated different doses of a same estrogenic compound are scarce and only concern oral route of administration [21, 23, 32, 48]. In his study, Caine et al. found a dose effect of oral estrogens that remained borderline for prothrombin fragment 1+2 and fibrinopeptid A but reached the significance for antithrombin, protein S and protein C [21]. Few years later, Conard et al. found no effect of oral estrogens whatever the daily doses [23] and Lobo et al. who investigated three doses of CEE with or without MPA did not highlight a significant dose effect of oral estrogens on haemostasis [32]. Recently, Rousseau et al. found no significant difference between two daily doses of oral 17β-estradiol [48].

Overall, there is no clear evidence for a significant dose effect of oral estrogens on haemostatic variables.

Effect of progestogens on haemostasis

Progestogens that consist of several compounds with different pharmacological properties [64] are systematically added to estrogens for women with an intact uterus to reduce the increased risk of endometrial hyperplasia and cancer associated with ET [57].

Chemical structures

Randomized controlled trials able to assess the main effect of a progestogen or to compare different progestogens are scarce and only concern progestogens combined with oral estrogens [22, 27, 32, 34, 37, 38].

In the PEPI trial, MPA was administered either sequentially or continuously and these two hormone regimens can be compared to micronized progesterone or to no progestogen [22]. Results showed similar changes in fibrinogen across the different active groups with neither an effect of adding a progestogen nor a specific effect of different chemical structures [22]. Few years later, Lobo conducted a large trial with different doses of CEE alone or combined with MPA [32] and did not highlight any evidence for a specific effect of MPA on haemostatic parameters [32]. During the same time, Van Baal and Post investigated the impact of dydrogesterone and trimegestone, a pregnane and a norpregnane derivative, respectively [27, 34]. Here too, they found no difference in their effect on haemostasis and pooled the two groups receiving opposed oral estrogens for some specific analyses [27, 34]. In another study, the main effect of gestodene, a testosterone derivative, was assessed by comparing changes in haemostatic parameters between two arms consisting of oral estrogens either alone or combined with this progestin [37, 38]. This study was the only one that found a decrease in Protein C in opposed oral estrogens group but not in the estrogens alone group [37, 38].

Overall, randomized controlled trials did not consistently detect any specific effect of progestogen on haemostasis among postmenopausal women using oral estrogens. Nevertheless, this absence of association does not necessarily imply that progestogens have no effect. It could be partly explained by a lack of statistical power and/or a dilution effect due to the concomitant use of oral estrogens that activate blood coagulation by themselves and might then hide the

specific effect of progestogens. By the way, a cross-sectional study on postmenopausal EPT and haemostasis suggested that norpregnane derivatives and micronized progesterone could have a differential effect on APC resistance and blood coagulation activation when combined with transdermal estrogens [15]. In addition, clinical data support a differential effect of pharmacological classes of progestogens on thrombotic risk [5, 7]. Further data on the biological and clinical effect of progestogens are therefore needed, especially in the context of transdermal estrogens use.

Daily dose

Only two randomized controlled trials assessed the differential effects of two progestogen doses and both concerned oral estrogens use [23, 32]. On the one hand, Conard et al. found no difference in progestogen effect on haemostasis between the two doses of nomegestrol acetate but did not find any more an effect of oral estrogens [23]. We can therefore wonder about the compliance of the participants and/or the validity of biological assays. In the other hand, use of opposed CEE led to similar 13-month changes in haemostasis irrespective of the dose of combined MPA [32].

Conclusion

This review is a summary of randomized controlled including a control group and having investigated the effect of postmenopausal MHT on haemostasis. This comprehensive analysis was conducted taking into account the route of estrogen administration, the estrogens daily dose and chemical structure, as well as, the progestogens. There is compelling evidence for a differential effect of estrogens according to the route of administration with a blood coagulation activation and a hypercoagulability state among oral estrogens users and a definite haemostatic balance for transdermal estrogens users. This difference would depend on neither the estrogenic compound nor the daily dose. Clinical results showing an increased VTE risk among oral but not transdermal estrogens users are absolutely consistent with these biological results. By contrast, while some clinical studies suggested that progestogens could be another important determinant of thrombotic risk, no clear effect of the different progestogens on haemostasis was highlighted.

References

1. Rymer J, Wilson R, Ballard K. Making decisions about hormone replacement therapy. *Bmj*. 2003;326:322-326
2. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the women's health initiative randomized controlled trial. *Jama*. 2002;288:321-333
3. Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, Bonds D, Brunner R, Brzyski R, Caan B, Chlebowski R, Curb D, Gass M, Hays J, Heiss G, Hendrix S, Howard BV, Hsia J, Hubbell A, Jackson R, Johnson KC, Judd H, Kotchen JM, Kuller L, LaCroix AZ, Lane D, Langer RD, Lasser N, Lewis CE, Manson J, Margolis K, Ockene J, O'Sullivan MJ, Phillips L, Prentice RL, Ritenbaugh C, Robbins J, Rossouw JE, Sarto G, Stefanick ML, Van Horn L, Wactawski-Wende J, Wallace R, Wassertheil-Smoller S. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: The women's health initiative randomized controlled trial. *Jama*. 2004;291:1701-1712
4. Scarabin PY, Oger E, Plu-Bureau G. Differential association of oral and transdermal oestrogen-replacement therapy with venous thromboembolism risk. *Lancet*. 2003;362:428-432
5. Canonico M, Oger E, Plu-Bureau G, Conard J, Meyer G, Levesque H, Trillot N, Barrellier MT, Wahl D, Emmerich J, Scarabin PY, Estrogen, Thromboembolism Risk Study G. Hormone therapy and venous thromboembolism among postmenopausal women: Impact of the route of estrogen administration and progestogens: The esther study. *Circulation*. 2007;115:840-845
6. Canonico M, Plu-Bureau G, Lowe GD, Scarabin PY. Hormone replacement therapy and risk of venous thromboembolism in postmenopausal women: Systematic review and meta-analysis. *BMJ*. 2008;336:1227-1231
7. Canonico M, Fournier A, Carcaillon L, Olie V, Plu-Bureau G, Oger E, Mesrine S, Boutron-Ruault MC, Clavel-Chapelon F, Scarabin PY. Postmenopausal hormone therapy and risk of idiopathic venous thromboembolism: Results from the e3n cohort study. *Arteriosclerosis, thrombosis, and vascular biology*. 2010;30:340-345
8. Renoux C, Dell'Aniello S, Suissa S. Hormone replacement therapy and the risk of venous thromboembolism: A population-based study. *J Thromb Haemost*. 2010;8:979-986
9. Sweetland S, Beral V, Balkwill A, Liu B, Benson VS, Canonico M, Green J, Reeves GK, Million Women Study C. Venous thromboembolism risk in relation to use of different types of postmenopausal hormone therapy in a large prospective study. *J Thromb Haemost*. 2012;10:2277-2286
10. Smith NL, Heckbert SR, Lemaitre RN, Reiner AP, Lumley T, Weiss NS, Larson EB, Rosendaal FR, Psaty BM. Esterified estrogens and conjugated equine estrogens and the risk of venous thrombosis. *Jama*. 2004;292:1581-1587
11. Smith NL, Blondon M, Wiggins KL, Harrington LB, van Hylckama Vlieg A, Floyd JS, Hwang M, Bis JC, McKnight B, Rice KM, Lumley T, Rosendaal

FR, Heckbert SR, Psaty BM. Lower risk of cardiovascular events in postmenopausal women taking oral estradiol compared with oral conjugated equine estrogens. *JAMA internal medicine*. 2014;174:25-31
12. Scarabin PY, Alhenc-Gelas M, Plu-Bureau G, Taisne P, Agher R, Aiach M. Effects of oral and transdermal estrogen/progesterone regimens on blood coagulation and fibrinolysis in postmenopausal women. A randomized controlled trial. *Arteriosclerosis, thrombosis, and vascular biology*. 1997;17:3071-3078
13. Oger E, Alhenc-Gelas M, Lacut K, Blouch MT, Roudaut N, Kerlan V, Collet M, Abgrall JF, Aiach M, Scarabin PY, Mottier D. Differential effects of oral and transdermal estrogen/progesterone regimens on sensitivity to activated protein c among postmenopausal women: A randomized trial. *Arteriosclerosis, thrombosis, and vascular biology*. 2003;23:1671-1676
14. Smith NL, Heckbert SR, Doggen CJ, Lemaitre RN, Reiner AP, Lumley T, Meijers JC, Psaty BM, Rosendaal FR. The differential association of conjugated equine estrogen and esterified estrogen with activated protein c resistance in postmenopausal women. *J Thromb Haemost*. 2006;4:1701-1706
15. Canonico M, Alhenc-Gelas M, Plu-Bureau G, Olie V, Scarabin PY. Activated protein c resistance among postmenopausal women using transdermal estrogens: Importance of progestogen. *Menopause*. 2010;17:1122-1127
16. de Visser MC, Rosendaal FR, Bertina RM. A reduced sensitivity for activated protein c in the absence of factor v leiden increases the risk of venous thrombosis. *Blood*. 1999;93:1271-1276
17. Tans G, van Hylckama Vlieg A, Thomassen MC, Curvers J, Bertina RM, Rosing J, Rosendaal FR. Activated protein c resistance determined with a thrombin generation-based test predicts for venous thrombosis in men and women. *British journal of haematology*. 2003;122:465-470
18. Hron G, Kollars M, Binder BR, Eichinger S, Kyrle PA. Identification of patients at low risk for recurrent venous thromboembolism by measuring thrombin generation. *Jama*. 2006;296:397-402
19. Vasan RS. Biomarkers of cardiovascular disease: Molecular basis and practical considerations. *Circulation*. 2006;113:2335-2362
20. Notelovitz M, Kitchens C, Ware M, Hirschberg K, Coone L. Combination estrogen and progestogen replacement therapy does not adversely affect coagulation. *Obstetrics and gynecology*. 1983;62:596-600
21. Caine YG, Bauer KA, Barzegar S, ten Cate H, Sacks FM, Walsh BW, Schiff I, Rosenberg RD. Coagulation activation following estrogen administration to postmenopausal women. *Thromb Haemost*. 1992;68:392-395
22. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The postmenopausal estrogen/progestin interventions (pepi) trial. The writing group for the pepi trial. *Jama*. 1995;273:199-208
23. Conard J, Basdevant A, Thomas JL, Ochsenbein E, Denis C, Guyene TT, Degrelle H. Cardiovascular risk factors and combined estrogen-progestin replacement therapy: A placebo-controlled study with nomegestrol acetate and estradiol. *Fertility and sterility*. 1995;64:957-962
24. Nozaki M, Ogata R, Koera K, Hashimoto K, Nakano H. Changes in coagulation factors and fibrinolytic components of postmenopausal women

receiving continuous hormone replacement therapy. *Climacteric*. 1999;2:124-130
25. Hoibraaten E, Os I, Seljeflot I, Andersen TO, Hofstad A, Sandset PM. The effects of hormone replacement therapy on hemostatic variables in women with angiographically verified coronary artery disease: Results from the estrogen in women with atherosclerosis study. *Thrombosis research*. 2000;98:19-27
26. Teede HJ, McGrath BP, Smolich JJ, Malan E, Kotsopoulos D, Liang YL, Peverill RE. Postmenopausal hormone replacement therapy increases coagulation activity and fibrinolysis. *Arteriosclerosis, thrombosis, and vascular biology*. 2000;20:1404-1409
27. van Baal WM, Emeis JJ, van der Mooren MJ, Kessel H, Kenemans P, Stehouwer CD. Impaired procoagulant-anticoagulant balance during hormone replacement therapy? A randomised, placebo-controlled 12-week study. *Thromb Haemost*. 2000;83:29-34
28. Demirol A, Baykal C, Kirazli S, Ayhan A. Effects of hormone replacement on hemostasis in spontaneous menopause. *Menopause*. 2001;8:135-140
29. Gottsater A, Rendell M, Hulthen UL, Berntorp E, Mattiasson I. Hormone replacement therapy in healthy postmenopausal women: A randomized, placebo-controlled study of effects on coagulation and fibrinolytic factors. *Journal of internal medicine*. 2001;249:237-246
30. Hoibraaten E, Mowinckel MC, de Ronde H, Bertina RM, Sandset PM. Hormone replacement therapy and acquired resistance to activated protein c: Results of a randomized, double-blind, placebo-controlled trial. *British journal of haematology*. 2001;115:415-420
31. Hoibraaten E, Qvigstad E, Andersen TO, Mowinckel MC, Sandset PM. The effects of hormone replacement therapy (hrt) on hemostatic variables in women with previous venous thromboembolism--results from a randomized, double-blind, clinical trial. *Thromb Haemost*. 2001;85:775-781
32. Lobo RA, Bush T, Carr BR, Pickar JH. Effects of lower doses of conjugated equine estrogens and medroxyprogesterone acetate on plasma lipids and lipoproteins, coagulation factors, and carbohydrate metabolism. *Fertility and sterility*. 2001;76:13-24
33. Perera M, Sattar N, Petrie JR, Hillier C, Small M, Connell JM, Lowe GD, Lumsden MA. The effects of transdermal estradiol in combination with oral norethisterone on lipoproteins, coagulation, and endothelial markers in postmenopausal women with type 2 diabetes: A randomized, placebo-controlled study. *The Journal of clinical endocrinology and metabolism*. 2001;86:1140-1143
34. Post MS, Rosing J, Van Der Mooren MJ, Zweegman S, Van Baal WM, Kenemans P, Stehouwer CD. Increased resistance to activated protein c after short-term oral hormone replacement therapy in healthy post-menopausal women. *British journal of haematology*. 2002;119:1017-1023
35. Salobir BG, Keber I, Vrabic L. A randomized, placebo-controlled trial of the effects of continuous combined hormone replacement therapy on coagulation and fibrinolytic systems in healthy postmenopausal women. *Fertility and sterility*. 2002;78:1178-1183
36. Madsen JS, Kristensen SR, Gram J, Bladbjerg EM, Henriksen FL, Gram J, Christensen K, Jespersen J. Positive impact of hormone replacement therapy on the fibrinolytic system: A long-term randomized controlled

study in healthy postmenopausal women. *J Thromb Haemost*. 2003;1:1984-1991
37. Post MS, Christella M, Thomassen LG, van der Mooren MJ, van Baal WM, Rosing J, Kenemans P, Stehouwer CD. Effect of oral and transdermal estrogen replacement therapy on hemostatic variables associated with venous thrombosis: A randomized, placebo-controlled study in postmenopausal women. *Arteriosclerosis, thrombosis, and vascular biology*. 2003;23:1116-1121
38. Post MS, van der Mooren MJ, van Baal WM, Blankenstein MA, Merkus HM, Kroeks MV, Franke HR, Kenemans P, Stehouwer CD. Effects of low-dose oral and transdermal estrogen replacement therapy on hemostatic factors in healthy postmenopausal women: A randomized placebo-controlled study. *American journal of obstetrics and gynecology*. 2003;189:1221-1227
39. Borgfeldt C, Li C, Samsioe G. Low-dose oral combination of 17beta-estradiol and norethisterone acetate in postmenopausal women decreases factor vii, fibrinogen, antithrombin and plasminogen activator inhibitor-1. *Climacteric*. 2004;7:78-85
40. Stevenson JC, Oladipo A, Manassiev N, Whitehead MI, Guilford S, Proudler AJ. Randomized trial of effect of transdermal continuous combined hormone replacement therapy on cardiovascular risk markers. *British journal of haematology*. 2004;124:802-808
41. Martinez C, Basurto L, Zarate A, Saucedo R, Gaminio E, Collazo J. Transdermal estradiol does not impair hemostatic biomarkers in postmenopausal women. *Maturitas*. 2005;50:39-43
42. Osmanagaoglu MA, Osmanagaoglu S, Osmanagaoglu T, Okumus B, Bozkaya H. Effect of different preparations of hormone therapy on lipid and glucose metabolism, coagulation factors, and bone mineral density in overweight and obese postmenopausal women. *Fertility and sterility*. 2005;84:384-393
43. Collins P, Flather M, Lees B, Mister R, Proudler AJ, Stevenson JC. Randomized trial of effects of continuous combined hrt on markers of lipids and coagulation in women with acute coronary syndromes: Whisp pilot study. *European heart journal*. 2006;27:2046-2053
44. 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;13:643-650
45. Kooperberg C, Cushman M, Hsia J, Robinson JG, Aragaki AK, Lynch JK, Baird AE, Johnson KC, Kuller LH, Beresford SA, Rodriguez B. Can biomarkers identify women at increased stroke risk? The women's health initiative hormone trials. *PLoS clinical trials*. 2007;2:e28
46. Rossouw JE, Cushman M, Greenland P, Lloyd-Jones DM, Bray P, Kooperberg C, Pettinger M, Robinson J, Hendrix S, Hsia J. Inflammatory, lipid, thrombotic, and genetic markers of coronary heart disease risk in the women's health initiative trials of hormone therapy. *Archives of internal medicine*. 2008;168:2245-2253
47. Perrone G, Capri O, Galoppi P, Brunelli R, Bevilacqua E, Ceci F, Ciarla MV, Strom R. Effects of either tibolone or continuous combined transdermal estradiol with medroxyprogesterone acetate on coagulatory

factors and lipoprotein(a) in menopause. *Gynecologic and obstetric investigation*. 2009;68:33-39
48. Rousseau A, Robert A, Gerotziafas G, Torchin D, Zannad F, Lacut K, Libersa C, Dasque E, Demolis JL, Elalamy I, Simon T. Effect of two oral doses of 17beta-estradiol associated with dydrogesterone on thrombin generation in healthy menopausal women: A randomized double-blind placebo-controlled study. *Fundamental & clinical pharmacology*. 2010;24:239-245
49. Rossouw JE, Johnson KC, Pettinger M, Cushman M, Sandset PM, Kuller L, Rosendaal F, Rosing J, Wasserthal-Smoller S, Martin LW, Manson JE, Lakshminarayan K, Merino JG, Lynch J. Tissue factor pathway inhibitor, activated protein c resistance, and risk of ischemic stroke due to postmenopausal hormone therapy. *Stroke; a journal of cerebral circulation*. 2012;43:952-957
50. Hillarp A, Zoller B, Svensson PJ, Dahlback B. The 20210 a allele of the prothrombin gene is a common risk factor among swedish outpatients with verified deep venous thrombosis. *Thromb Haemost*. 1997;78:990-992
51. Mackman N, Tilley RE, Key NS. Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. *Arteriosclerosis, thrombosis, and vascular biology*. 2007;27:1687-1693
52. Macfarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature*. 1964;202:498-499
53. Davie EW, Ratnoff OD. Waterfall sequence for intrinsic blood clotting. *Science*. 1964;145:1310-1312
54. Kumar A, Kar S, Fay WP. Thrombosis, physical activity, and acute coronary syndromes. *Journal of applied physiology*. 2011;111:599-605
55. Miller J, Chan BK, Nelson HD. Postmenopausal estrogen replacement and risk for venous thromboembolism: A systematic review and meta-analysis for the u.S. Preventive services task force. *Annals of internal medicine*. 2002;136:680-690
56. Castoldi E, Rosing J. Thrombin generation tests. *Thrombosis research*. 2011;127 Suppl 3:S21-25
57. Role of progestogen in hormone therapy for postmenopausal women: Position statement of the north american menopause society. *Menopause*. 2003;10:113-132
58. Basurto L, Saucedo R, Zarate A, Martinez C, Gaminio E, Reyes E, Hernandez M. Effect of pulsed estrogen therapy on hemostatic markers in comparison with oral estrogen regimen in postmenopausal women. *Gynecologic and obstetric investigation*. 2006;61:61-64
59. Koninckx PR, Spielmann D. A comparative 2-year study of the effects of sequential regimens of 1 mg 17beta-estradiol and trimegestone with a regimen containing estradiol valerate and norethisterone on the bleeding profile and endometrial safety in postmenopausal women. *Gynecol Endocrinol*. 2005;21:82-89
60. Pornel B, Spielmann D. A study of the control of climacteric symptoms in postmenopausal women following sequential regimens of 1 mg 17beta-estradiol and trimegestone compared with a regimen containing 1 mg estradiol valerate and norethisterone over a two-year period. *Gynecol Endocrinol*. 2005;21:74-81
61. Scarabin PY, Hemker HC, Clement C, Soisson V, Alhenc-Gelas M. Increased thrombin generation among postmenopausal women using

hormone therapy: Importance of the route of estrogen administration and progestogens. *Menopause*. 2011;18:873-879
62. Regan LM, Stearns-Kurosawa DJ, Kurosawa S, Mollica J, Fukudome K, Esmon CT. The endothelial cell protein c receptor. Inhibition of activated protein c anticoagulant function without modulation of reaction with proteinase inhibitors. *The Journal of biological chemistry*. 1996;271:17499-17503
63. Olie V, Canonico M, Scarabin PY. Risk of venous thrombosis with oral versus transdermal estrogen therapy among postmenopausal women. *Current opinion in hematology*. 2010;17:457-463
64. Canonico M, Plu-Bureau G, Scarabin PY. Progestogens and venous thromboembolism among postmenopausal women using hormone therapy. *Maturitas*. 2011;70:354-360

Table

Table 1. Haemostatic variables

	Coagulation	Fibrinolysis
Factors	Prothrombin (ProT) Thrombin (T) Fibrinogen (Fg) Factor VII Factor VIII	Plasminogen (Plg) Tissu Plasminogen Activator (t-PA)
Inhibitors	Protein C (PC) Protein S (PS) Antithrombin (AT)	Plasminogen Activator Inhibitor 1 (PAI-1) Antiplasmin (AP)
Activation Markers	Fragment 1+2 (F1+2) Thrombin-Antithrombin complex (T-AT) Fibrinopeptide A (FpA) Fibrinopeptide B (FpB) Ddimers (DD)	Ddimers (DD) Plasmin-Antiplasmin (P-AP)
Global tests	Baseline Thrombin generation (TG) Thrombin generation with and without activated protein C (rPCA)	

Table

Table 2. Effect of hormone therapy on haemostatic variables among postmenopausal women in randomized controlled trials

Author, year	Follow-up	Treatment, daily dose	Number of subjects	Coagulation		Fibrinolysis		Activation markers		Global tests
				Factors	Inhibitors	Factors	Inhibitors	Coagulation	Fibrinolysis	
Notelovitz, 1983 [20]	18 mth	No treatment	18	**Fg:** no diff	**AT:** no diff	**Plg:** no diff				
		O CEE 0.625 mg + MPA 10 mg	20							
		O CEE 1.25 mg + MPA 10 mg								
Caine, 1992 [21]	3 mth (cross-over)	Placebo	29		**PC:** increase in CEE 1.25 mg			**F1+2:** increase in O gps		
		O CEE 0.625 mg			**PS:** decrease in O gps			**FpA:** increase in O gps		
		O CEE 1.25 mg			**AT:** decrease in O gps					
Conard, 1995 [23]	3 mth	Placebo	19	**Fg:** no diff	**PC:** no diff	**Plg:** increase in O gps		**F1+2:** no diff		
		O 17βE2 1 mg + NOMAC 2.5 mg	19		**PS:** no diff					
		O 17βE2 1.5 mg + NOMAC 3.75 mg	19		**AT:** no diff					
PEPI group, 1995 [22]	36 mth	Placebo	174	**Fg:** increase in placebo gp						
		O CEE 0.625 mg	175							
		O CEE 0.625 mg + MPA 2.5 mg*	174							
		O CEE 0.625 mg + MPA 2.5 mg	174							
		O CEE 0.625 mg + prog 200 mg	178							
Scarabin, 1997 [12]	6 mth	No treatment	14	**Fg:** no diff	**PC:** no diff	**Plg:** no diff	**PAI-1**: decrease in O gp	**DD:** no diff	**DD:** no diff	
		T 17βE2 50 µg + prog 200 mg	14	**fVII:** no diff	**AT:** decrease in O gp	**t-PA**: decrease in O gp		**F1+2:** increase in O gp		
		O 17βE2 2 mg + prog 200 mg	15	**fVIII:** no diff						
Nozaki, 1999 [24]	6 mth	Placebo	47	No comparison of changes in haemostatic variables between treatment groups						
		O CEE 0.625 mg	39							
		O CEE 0.625 mg + MPA 2.5 mg	48							

Van Baal, 2000 [27] and Post, 2002 [34]	3 mth	Placebo O 17βE2 2 mg O 17βE2 2 mg + dydro 10 mg O 17βE2 2 mg + trime 0.5 mg	16 16 14 14	**fVII:** no diff **fVIII:** no diff	**PC:** decrease in O gps **PS:** decrease in O gps **AT:** decrease in O gps	**Plg:** increase in E2-only gp	**PAI-1**: decrease in O gps	**T-AT**: no diff **F1+2**: increase in O gps	**P-AP**: no diff	**APCr:** increase in O gps
Hoibraaten, 2000 [25]	12 mth	No treatement T 17βE2 50 µg + MPA 5 mg	58 60	**Fg:** no diff **fVII:** no diff	**PC:** decrease in T gp **PS:** decrease in T gp **AT:** decrease in T gp	**t-PA**: no diff	**PAI-1**: decrease in T gp	**F1+2:** no diff **T-AT**: no diff **DD**: no diff	**DD:** no diff	
Teede, 2000 [26]	1.5 mth	Placebo O 17βE2 2 mg + NETA 1 mg	20 22				**PAI-1**: decrease in O gp	**F1+2**: increase in O gp **DD:** increase in O gp	**DD:** increase in O gp	
Gottsater, 2001 [29]	3 mth	Placebo O E2V 2 mg	24 27	**fVII:** no diff **fVIII:** no diff **Fg:** decrease in O gp	**PC:** no diff **PS:** decrease in O gp **AT:** decrease in O gp	**t-PA**: no diff	**PAI-1**: decrease in O gp	**F1+2**: no diff		**APCr:** no diff
Hoibraaten, 2001 and 2001 [30, 31]	24 mth	Placebo O 17βE2 2 mg + NETA 1 mg	69 71	**Fg:** no diff **fVII:** decrease in O gp	**PC:** decrease in O gp **PS:** no diff **AT:** decrease in O gp			**F1+2:** no diff **T-AT**: no diff **DD:** increase in O gp	**DD:** increase in O gp	**APCr:** increase in O gp

Lobo, 2001 [32]	12 mth	Placebo O CEE 0.625 mg O CEE 0.625 mg + MPA 2.5 mg O CEE 0.45 mg O CEE 0.45 mg + MPA 2.5 mg O CEE 0.45 mg + MPA 1.5 mg O CEE 0.3 mg O CEE 0.3 mg + MPA 1.5 mg	94 97 86 95 96 94 89 98		**PC:** no diff **AT:** no diff	**Plg:** increase in all O gps	**PAI-1**: decrease in O gps except in CEE 0.3 mg			
Perera, 2001 [33]	6 mth	Placebo T 17βE2 80 µg + NETA 1 mg	21 22	**Fg:** no diff **fVII:** decrease in T gp **fVIII:** decrease in T gp		**t-PA**: no diff		**DD:** no diff	**DD:** no diff	
Demirol, 2001 [28]	6 mth	Placebo O CEE 0.625 mg + MPA 5 mg	55 55	**Fg:** decrease in O gp **fVII:** no diff **fVIII:** decrease in O gp	**AT:** increase in O gp					**APCr:** decrease in O gp
Salobir, 2002 [35]	6 mth	Placebo O 17βE2 2 mg + NETA 1 mg	30 31		**PC:** decrease in O gp **AT:** decrease in O gp	**t-PA**: decrease in O gp	**PAI-1**: decrease in O gp	**DD:** increase in O gp	**DD:** increase in O gp	
Oger, 2003 [13]	6 mth	Placebo O 17βE2 1 mg + prog 100 mg T 17βE2 50 µg + prog 100 mg	65 63 68		**PS:** decrease in O and T gps			**F1+2**: increase in O gp		**APCr:** increase in O gp

Madsen, 2003 [36]	5 yr	Placebo O 17βE2 2 mg + NETA 1 mg	126 122					**t-PA:** decrease in O gp	**PAI-1**: decrease in O gp			
Post, 2003 and 2003 [37, 38]	13 mth	Placebo T 17βE2 50 µg O 17βE2 1 mg O 17βE2 1 mg + gesto 25 µg	49 33 37 33	**Fg:** no diff **ProT:** no diff **fVII:** decrease in all active gps	**PC**: decreased in O + gesto gp **PS:** decrease in all active gps			**t-PA:** decrease in O gp	**PAI-1**: decrease in O gps	**F1+2**: no diff **T-AT**: no diff **DD:** Increase in O gps	**DD:** increase in O gps **P-AP**: no diff	**APCr:** increase in O gps
Stevenson, 2004 [40]	6 mth	Placebo T 17βE2 50 µg + NETA 125 µg	27 28	**Fg:** no diff **fVII:** decrease in T gp **fVIII:** no diff					**PAI-1**: no diff	**F1+2:** decrease in placebo gp **DD:** no diff	**DD:** no diff	
Borgfeldt, 2004 [39]	12 mth	Placebo O17βE2 1 mg + NETA 250 µg O 17βE2 1 mg + NETA 500 µg	40 40 40	**fVII:** decrease in O gps	**AT:** decrease in O gps				**PAI-1**: decrease in O gps			
Martinez, 2005 [41]	3 mth	No treatment T 17βE2 50 µg	29 30	**Fg:** decrease in T gp **fVII:** decrease in T group	**PC:** no diff **PS:** no diff **AT:** decrease in T pg	**Plg:** no diff **t-PA**: increase in T gp		**PAI-1**: increase in T gp				
Osmanagaoglu, 2005 [42]	6 mth	No treatment Tibolone 2.5 mg O 17βE2 2 mg + NETA 1 mg O CEE 0.625 mg + MPA 2.5 mg	88 90 84 90	No comparison of changes in haemostatic variables between treatment groups								

Collins, 2006 [43]	6 mth	Placebo O 17βE2 1 mg + NETA 0.5 mg	51 49	**Fg:** no diff **fVII:** decrease in O gps	**AT:** decrease in O gps			**F1+2**: no diff **DD:** no diff	**DD:** no diff	**APCr:** no diff
Zegura, 2006 [44]	6 mth	Placebo O 17βE2 2 mg O 17βE2 2 mg + NETA 1 mg T 17βE2 50 µg	30 20 31 21	No comparison of changes in haemostatic variables between treatment groups						
Kooperberg, 2007 [45]; Rossouw, 2008 [46]; Rossouw, 2012 [49]	12 mth	Placebo O CEE 0.625 mg Placebo O CEE 0.625 mg + MPA 2.5 mg	** 	**Fg:** decrease in active gps **fVII:** no diff **fVIII:** no diff			**PAI-1:** decrease in active gps	**F1+2**: no diff **DD** : no diff	**P-AP**: increase in active gps **DD** : no diff	**APCr**: increase in active gps
Perrone, 2009 [47]	12 mth	Placebo T 17βE2 50 µg + MPA 10 mg Tibolone 2,5 mg	19 20 21	**Fg:** no diff **fVII:** no diff	**AT:** no diff	**Plg:** no diff				
Rousseau, 2010 [48]	2 mth	Placebo O 17βE2 1 mg+ dydro 10 mg O 17βE2 2 mg + dydro 10 mg	22 24 26	**ProT:** no diff **fVII:** no diff **fVIII:** no diff						**TG:** increase in O gps

O: oral route of estrogen administration
T: transdermal route of estrogen administration
diff: difference
CEE: Conjugated Equine Estrogens
17βE2: 17β-estradiol
E2V: estradiol valerate
MPA: medroxyprogesterone acetate
NOMAC: nomegestrol acetate
Prog: micronised progesterone
Dydro: dydrogesterone
Trime: trimegestone
NETA: norethisterone acetate
Gesto: gestodene
gp: group
mth: month
yr: year
Fg: fibrinogen
fVII: facteur VII
fVIII: facteur VIII
ProT: prothrombin
PC: protein C
PS: protein S
FpA: fibrinopeptid A
T-AT: thrombin-antithombin
AT: antithrombin
Plg: plasminogen
t-PA: tissu plasminogen activator
PAI-1: plasminogen activator inhibitor
F1+2: prothombin fragment 1+2
DD: D-dimers
P-AP: plasmin-antiplasmin
APCr: Activated Protein C resistance
TG: thombin generation
* cyclic ** number of subjects par group depends on the nested case/control study

Figure

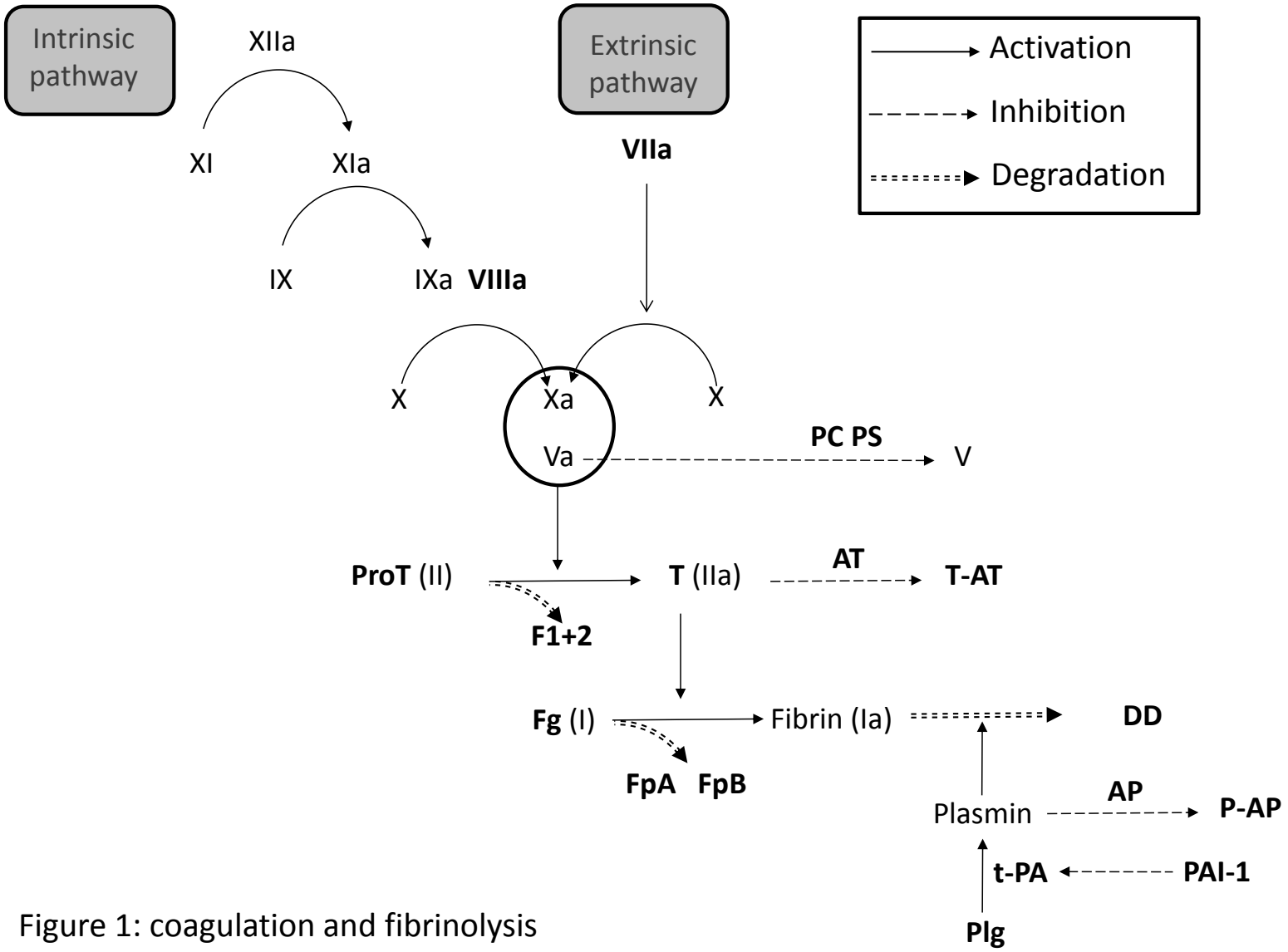

Figure 1: coagulation and fibrinolysis

Figure

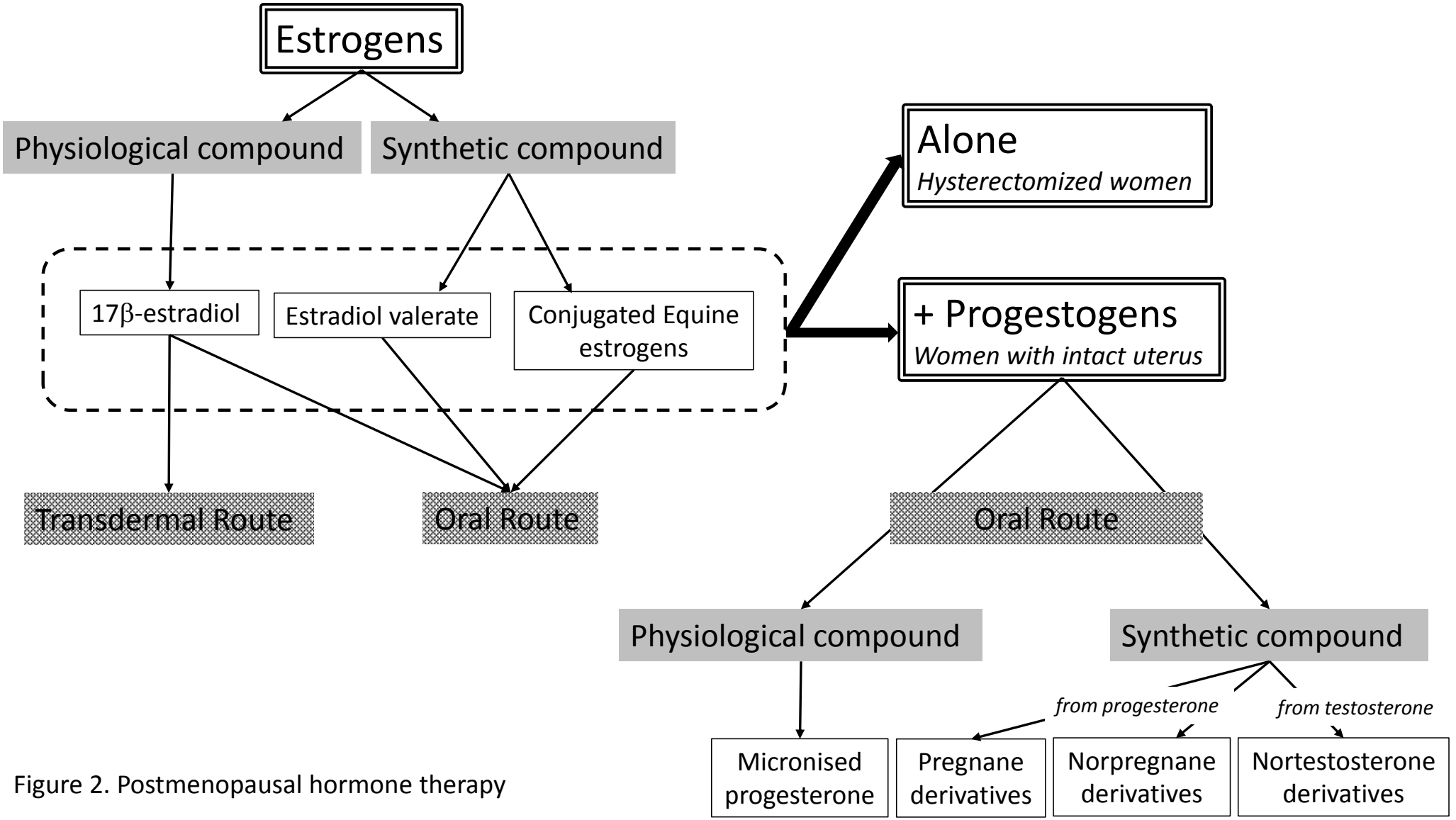

Figure 2. Postmenopausal hormone therapy

Copyright Transfer and Disclosure Form

Click here to download Copyright Transfer and Disclosure Form: Current CTA 2013.pdf