Vascular effects of estrogens: arterial protection versus venous thrombotic risk

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Mechanisms by which estrogen reduces the risk of arterial disease, while simultaneously increasing the risk of venous thrombosis in postmenopausal women, are not clearly understood. In addition to providing beneficial arterial effects on the lipid profile, estrogen both increases production of nitric oxide and decreases production of endothelin-1 from arterial endothelium, decreases intracellular calcium in arterial smooth muscle and might favor fibrinolysis. All of these effects could act in concert to protect against development of arterial occlusive disease. However, comparable effects on venous endothelium and smooth muscle have not been studied systematically, and although blood elements such as platelets and leukocytes contain estrogen receptors, much remains to be learned about the effect that dose and duration of estrogen-treatment might have upon these cells. An integrative approach to understanding the actions of estrogen on the venous system and the interaction of blood elements with the vascular wall is necessary before new therapeutic interventions will provide arterial protection with no risk of venous thrombosis.

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Cardiovascular diseases are the leading cause of death for women and men in the USA (Ref. 1). Premenopausal women have a lower incidence of cardiovascular disease (CVD) than do men of the same age, although after menopause, this incidence increases to levels seen in age-matched men. The risk of CVD is decreased in healthy women taking estrogen as part of hormone replacement for the symptoms of menopause[2–4]. In spite of some concerns of healthy-user bias, compliance bias and surveillance bias that are associated with these studies[5], circulating estrogen is still thought to have a 'primary preventive' role against development of arterial CVD in women. However, estrogen replacement is also associated with increased risk of venous thrombosis in both women and men[6–8], and estrogen treatment in women with ongoing CVD increased the incidence of myocardial infarction (MI) and venous thrombosis within the 3–4 years of study[9,10].

Mechanisms by which estrogen reduces development of arterial vascular disease in healthy women, while simultaneously increasing the risk of adverse events when CVD is present and of venous thrombotic events in healthy women and women with CVD, are not clearly understood. Much experimental work has focused on mechanisms of action of estrogen on limiting development of arterial lesions in healthy experimental animals[11–14]. Currently, there are only sparse data on the effects of estrogen on the venous circulation, or on the interaction of blood elements with the venous wall or components of existing atherosclerotic plaques. This review takes an integrative approach in discussing effects of estrogen on components of the vascular wall and blood elements that might participate in thrombotic and remodeling processes. It also identifies areas where research is needed to understand the apparent paradox of how estrogen can provide primary protection against development of arterial vascular disease and at the same time increase the risk of venous thrombosis and adverse events in the presence of existing CVD.

Venous thrombosis: Virchow's triad revisited

Over a century ago, the German physician and scientist Rudolph Virchow postulated that thrombosis resulted from a combination of reduction in blood flow (venous stasis), changes in anatomy of the vessel wall and changes in the ability of the blood to coagulate, a theory known as Virchow's triad[15] (Fig. 1). Experimental data support this theory and extend it to suggest a complex interaction among the components. The endothelium provides an interface between the venous wall and the blood, and releases factors that modulate (1) tone of vascular smooth muscle, thus influencing blood flow and therefore stasis, (2) proliferation and migration of the underlying smooth muscle (vessel anatomy) and (3) activation of platelets and leukocytes in the blood and, therefore, the ability of the blood to coagulate. For example, an intact endothelium releases prostacyclin, nitric oxide (NO) and endothelin-1 (ET-1), which inhibit coagulation and platelet aggregation (Fig. 2). Release of these factors is regulated by shear stress to the endothelial surface[16–18]. Although the threshold shear that modulates release of endothelium-derived factors in veins is not known, stasis initiated by an external stimulus (bedrest or immobility, pregnancy, or tight or heavy belts) might establish a positive feedback condition, whereby reduction in flow reduces release of anticoagulant endothelium-derived factors. Although significant anatomical damage has not been seen in veins of patients with thrombosis[19], functional or biochemical 'damage' that has yet to be appreciated might be a prerequisite (Fig. 2).

Estrogen receptors, ligands and mechanism of action

Estrogen, like other steroid hormones, affects all components of Virchow's triad.

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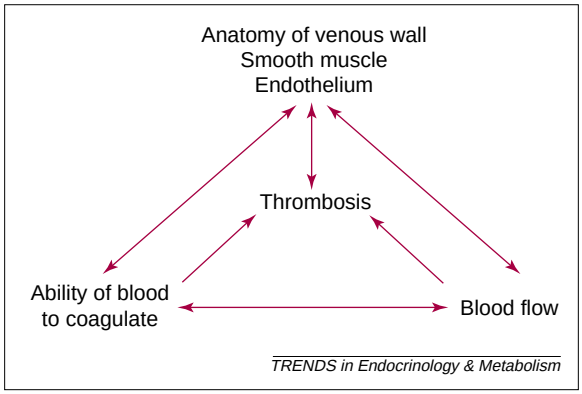

Fig. 1. Virchow's triad[15]. The key component of the vascular wall that might affect blood flow by determining vessel diameter and the ability of the blood to coagulate is the endothelium, which produces endothelium-derived factors. These factors cause relaxation of the underlying smooth muscle and inhibit platelet aggregation and leukocyte adhesion. Changes in blood flow-induced shear stress affects production of factors from the endothelium such that a decrease in blood flow promotes coagulation. Platelets also release factors that stimulate release of endothelium-derived factors, therefore influencing blood flow, but which also have direct effects on remodeling of the smooth muscle.

Estrogen receptors and their distribution

There are two genetically distinct estrogen receptors (ERs): ERα and ERβ. Both have six functional domains characteristic of the family of steroid receptors: two coactivator or ligand-independent transcriptional activation domains, a DNA-binding domain, a hinge region, a hormone-binding domain and an estrogen-distinguishing domain. There is a high degree of homology between receptors at the DNA-binding domain and least homology at the ligand-independent transcription activation and ligand-binding domains. The areas of least homology define differences in ligand-binding affinity and cell specificity. In the absence of ligand, ERs are associated with several polypeptides belonging to the heat shock protein (HSP) family[20]. These proteins keep the receptor in a conformation that has high affinity for the hormone.

ERα and ERβ have been localized in the nucleus and cytoplasm of human arterial and venous endothelial cells and of human vascular smooth muscle cells (VSMCs). ERβ is the predominant type of receptor in arteries and veins in both sexes[24] (Fig. 3), although both receptors might be important for mediating vascular effects of estrogen. This conclusion is supported by several observations. Expression of ERα is reduced in atherosclerotic coronary arteries of premenopausal women[23] and accelerated atherosclerosis was observed in a man lacking ERα (Ref. 25). However, estrogen treatment reduced medial thickening after arterial endothelial denudation in mice lacking either ERα or ERβ (Refs 13,14). More work is needed to define regulation of ERα- and ERβ-expression in different vascular beds during normal and pathological states. Understanding regulation and distribution of ERs is important for the development of tissue-specific ER ligands such as SERMS (selective ER modulators).

ERs are also present in platelets[26], monocytes[27] and neutrophils[28], all of which can affect the ability of the blood to coagulate. Estrogen affects secretion, aggregation, adhesion and migration of these cells[28–31], which are involved in thrombus formation and inflammation. In addition, platelet–leukocyte interactions in the blood might modify the activation of each type of cell, thereby affecting the ability of the blood to coagulate[32]. Cellular functions modulated by estrogens might initiate changes in secretion and activation of platelets and leukocytes that could facilitate clot formation in the venous circulation. Effects of estrogens on blood elements might even provide some insight into the adverse events of the HERS (heart and estrogen/progestin replacement study) trial[9,10]. Leukocytic infiltration is an important component of atherosclerotic plaques. Changes in cellular functions mediated by estrogens might facilitate remodeling of plaques, which could make them unstable, and thus contribute to the increase in adverse events in women with active disease early in the treatment regimen[9]. Important associations between inflammation and coagulation are beginning to be understood: evidence suggests that infections are important in the etiology of atherosclerotic disease[33]. Indeed, C-reactive protein (CRP), a component of the acute phase response, increases with atherosclerosis and with estrogen replacement therapy[34,35]. CRP might influence migration of monocytes, thereby influencing remodeling of atherosclerotic plaques[36]. Effects of CRP on venous function are unknown. These observations suggest that estrogen affects vascular

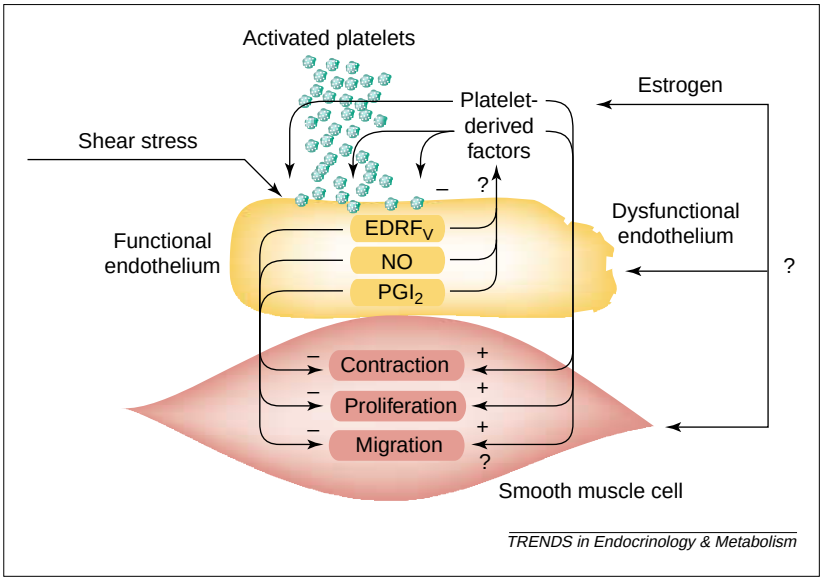

Fig. 2. Interactions of estrogen with the venous wall that might contribute to development of venous thrombosis. Estrogen might affect production of coagulation proteins in the liver and the aggregation of platelets. Aggregating platelets release platelet-derived factors including ADP, serotonin, prostacyclin and thromboxane, which cause release of factors from the endothelium, and in the absence of a functional endothelium, direct stimulation of the venous smooth muscle. Factors released from the endothelium such as prostacyclin, NO and endothelin I alter platelet aggregation and cause changes in venous tone. Release of endothelium-derived factors is reduced with decreases in blood flow (stasis), perhaps providing an environment for increased platelet aggregation. Disruption of the endothelium by mechanical damage or a change in biochemical function will influence interaction of platelets with the vein wall. Estrogen affects functions of platelets, leukocytes and coagulation factors in the blood, and also proliferation and migration of the endothelium and smooth muscle. Abbreviations: $EDRF_V$, endothelium-derived relaxing factor of veins; NO, nitric oxide; PGI_2, prostaglandin I_2; question marks refer to interactions and pathways that remain to be defined in the venous system.

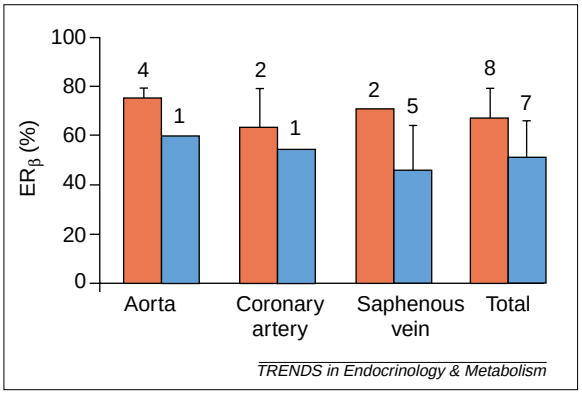

Fig. 3. Quantification of estrogen receptors (ERs) in human arteries and veins. Data represent mean ± SD percentage of total ER that are ERβ and are derived from Table 1 of Ref. 24. Blue, men; red, women. The numbers represent the number of specimens studied from different individuals.

responses directly through actions on endothelial cells and VSMCs, and indirectly through modulation of blood elements that interact with the vascular wall.

Estrogen also affects other components of the coagulation cascade that contribute to changes in the ability of the blood to coagulate. These components include fibrinogen, Factors VII, IX, and X, anti-thrombin III, protein S and tissue factor pathway inhibitor (TFPI) (Ref. 37). Because oral estrogen carries a greater risk of venous thrombosis than does transdermal estrogen, changes in these coagulation factors probably reflect effects of estrogen on the liver. However, studies on the effects of estrogen treatment on coagulation parameters in humans show variable results, probably because of differences in formulations of estrogen, dose and duration of treatment, and participants with multiple risk factors, including smoking and existing disease[9,38–41]. Relative activation and distribution of estrogen receptors in the liver under these various conditions are not known.

Ligands for ERs

There are three sources of ligands for ERs: those derived from animal tissue, those derived from plant tissue (phytoestrogens) and synthetic compounds. The main circulating ovarian hormone in premenopausal women is 17β-estradiol (E_2). After menopause, E_2 concentrations decline and the main hormone in circulation is estrone, which is 50–70% less active than is E_2. Estriol, the third estrogen present in the female circulation, is only about one-tenth as active as E_2. These three natural estrogens exert their activity by binding to ERs, with E_2 having the highest affinity, followed by estrone and then estriol (Table 1). E_2 binds with similar affinity to both types of ER, whereas estrone binds to ERα with high affinity (although with a lower affinity than E_2) and estriol binds weakly to ERβ (Ref. 42). E_2 is converted to the less potent estrone through the action of 17β-hydroxysteroid dehydrogenase (17β-HSD). This reaction is reversible, but estrone formation is favored. It is important to consider these metabolites of E_2 as biologically active substances. For example, 17β-HSD is present in endothelial cells, and steroid sulfatase, which hydrolyzes estrone sulfate, is present in VSMCs (Refs 43,44). Therefore, circulating concentrations of E_2 might not accurately reflect biologically active concentrations of ligand at the cellular level. Estrone and estriol, through binding to ERs, affect cellular functions that contribute to vascular remodeling, including proliferation, matrix secretion and migration of VSMCs (Refs 44–46). Differential metabolism of estrogen at various locations throughout the vasculature (arteries, veins, capillaries or blood elements) would result in varying concentrations of specific biologically active ligands at each anatomical site. Therefore, net ER activation will be determined, not only by the number of ERs, but also by the level of ligand–receptor binding. The type and route of administration of various estrogens for hormone replacement therapy will influence the overall activation of ERs in various cells of the vascular wall. For example, conjugated equine estrogen, which is used clinically for estrogen replacement therapy, comprises ~50% estrone sulfate, which is given orally, hydrolyzed to estrone and converted to E_2 by tissues. Oral administration of estrogens carries a greater risk of venous thrombosis than does transdermal application, probably as a result of 'first-pass' metabolism by the liver with oral administration. However, the effects of differential circulating metabolites with either preparation on vascular responses have not been studied extensively. Further studies are also needed to determine effects of estrogen metabolites on veins, capillaries and blood elements.

Estrogen also undergoes hydroxylation to 2-hydroxyestrone, a catechol estrogen. Although circulating concentrations of this compound are low, it can bind to catechol *O*-methyltransferase, the enzyme that inactivates catecholamines, such as dopamine and norepinephrine[47]. Thus, estrogen might indirectly affect adrenergic mechanisms by altering the bioavailability of norepinephrine[48]. It remains to be determined whether genetic differences in enzymes

Table 1. Binding affinity of various compounds for ERα and ERβ[a,b]

Compound	RBA[c] ERα	RBA[c] ERβ	K_i (nM)[d] ERα	K_i (nM)[d] ERβ
E_2	100	100	0.13	0.12
Tamoxifen	7	6	3.40	2.50
Estrone	60	37	0.30	0.40
17α-estradiol	58	11	0.20	1.20
Estriol	14	21	1.40	0.70
Estrone-3-sulfate	<1	<1		
Genistein	5	36	2.60	0.30

[a]Abbreviations: E_2, 17β-estradiol; ER, estrogen receptor; RBA, receptor binding affinity.
[b]Adapted from Ref. 41.
[c]RBA of each competitor was calculated as the ratio E_2 : competitor concentration ratio required to reduce the specific radioligand binding by 50% (ratio of IC_{50} values). RBA value for E_2 was arbitrarily set at 100. The IC_{50} of E_2 was 0.21 nM for ERα and 0.13 nM for ERβ.
[d]The Cheng-Prusoff formula was used to calculate the K_i of the various competitors.

associated with metabolism of estrogen are related to the development of some vasospastic diseases that are more prevalent in women (e.g. Raynaud's disease), or related to the development of venous thrombosis.

Phytoestrogens are the second naturally occurring type of ER ligand; several groups exist, but isoflavones (e.g. genistein) have the greatest estrogen-like potency. Soy contains the highest concentration of phytoestrogens, of which isoflavones predominate. Genistein binds to ERβ with higher affinity than it does to ERα (Table 1). Higher consumption of soy products might contribute to the lower incidence of CVD in Asian than in Western countries[49]. However, in experimental studies, the effectiveness of phytoestrogens in reducing atherosclerotic lesions is controversial[50]. Additional studies are needed to define effects of phytoestrogens on smooth muscle and endothelial components of arteries and veins.

Synthetic compounds such as SERMs comprise the third class of ER ligand. Tamoxifen and raloxifene are SERMS approved for clinical use in humans for the prevention of breast cancer and osteoporosis[51]. Little is known regarding long-term cardiovascular effects of these treatments. Raloxifene increases the risk of venous thrombosis, as does estrogen[52]. Cardiovascular effects other than venous thrombosis have been less studied. Tamoxifen and raloxifene acutely relax rabbit coronary arteries by an ER-dependent mechanism, which might require production of NO (Refs 53,54). However, raloxifene did not reduce atherosclerosis in experimental animals fed a high cholesterol diet[55]. Two ongoing clinical trials will provide additional information on the cardiovascular effects of both of these SERMs: cardiovascular events will be recorded as secondary outcomes of the STAR study (study of tamoxifen and raloxifene in prevention of breast cancer) and as primary outcomes in the RUTH trial (raloxifene use for the heart)[56,57]. Little is known about effects of SERMs on venous tissue, the specificity of SERM activation on intracellular pathways in either endothelial cells or VSMCs, or their direct effects on blood elements.

Mechanisms of action

Estrogen alters cellular function by two mechanisms: one that does not require gene transcription (non-genomic effects) and one that does (genomic effects). These effects probably do not occur in isolation but sequentially. As estrogen diffuses into a cell or binds to a surface receptor, a series of biochemical events is initiated, including changes in enzyme activity, activation of ion channels or changes in HSPs (Refs 58–63). Some evidence supports the notion that the non-genomic effects of estrogen are mediated by ERα (Refs 58,64). However, in a man deficient in ERα, sublingual administration of estrogen produced a rapid vasodilatation of the brachial artery[65]. This suggests that additional mechanisms might contribute to a rapid non-genomic effect of estrogen. Regardless of the mechanism, these changes determine the biochemical background upon which the genomic actions or gene transcription could be expressed. Binding of estrogen to the receptor in the cytosol produced a conformational change that might involve the dissociation of HSPs. At the nucleus, ERs dimerize, forming either a homodimer of homologous ERα or ERβ units or a heterodimer comprising an ERα and an ERβ unit. These receptors then bind to estrogen-response elements on a gene, where transcription is activated[42]. In most cells, additional proteins called coregulatory proteins modulate the binding of the estrogen–receptor complex to a gene. Conformations of the estrogen–receptor complex dictate subsequent binding of coregulators. It is this interaction between the coregulatory factors and the ER dimer that might vary from cell to cell and thus provide cell specificity of transcriptional events. Genomic effects might dictate long-term remodeling of the vasculature under the influence of estrogen[11,13,14,66]. For a control system, these complementary actions (rapid non-genomic effects and long-term genomic effects) might represent regulation of both threshold and gain of the input/output response.

Both genomic and non-genomic effects of estrogen might account for beneficial effect of estrogen on lipids. Estrogen modulates hepatic expression of apoprotein genes and upregulation of hepatic low-density lipoprotein (LDL) receptors[39,67]. In addition, antioxidant properties of the molecule prevent oxidation of LDL, thereby increasing bioavailability of substances such as NO (Ref. 68).

Sex and vascular responses to estrogen

ERs are present in the vasculature of male and female animals, and some effects of estrogen seem to be independent of sex. For example, venous thrombosis occurs in both men and women given estrogen therapies[6–8,69,70]. As mentioned, acute sublingual administration of estrogen dilated brachial arteries of a man deficient in ERα (Ref. 65). Long-term administration of estrogen to males in the absence of testosterone (male to female transsexuals) improves cardiovascular function[71]. However, administration of estrogen to gonadally intact male rats did not reduce a hyperplastic response to arterial injury[11]. This suggests that testosterone might influence estrogenic effects on the vasculature, and a greater understanding of these interactions is needed to develop cardioprotective SERMs.

Conclusion and future directions

Observational and epidemiological studies indicate that the incidence of CVD is decreased with estrogen replacement in postmenopausal women without existing CVD, but is associated with a small increase in the risk of venous thrombosis in healthy women and men, and increases risk of MI in women with previous disease. Although ERs have been found in all components of the vascular wall, it is unclear how the distribution of receptors affects differential responses

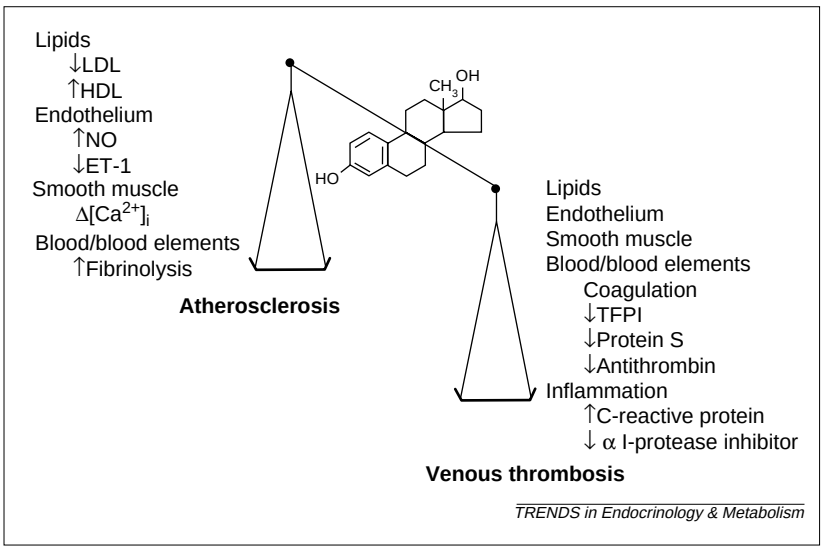

Fig. 4. Factors contributing to the apparent paradox of estrogen replacement being protective against arterial vascular disease while simultaneously increasing the risk of venous thrombosis. Abbreviations: ET-1 endothelin 1; HDL, high-density lipoprotein; I-protease, alpha 1-protease; LDL, low-density lipoprotein; NO, nitric oxide; TFPI, tissue factor pathway inhibitor; $\Delta[Ca^{2+}]_i$, changes in intracellular Ca^{2+} concentrations.

in arteries and veins. When estrogen binds to a receptor, both rapid non-genomic and long-term genomic effects occur. Although the genomic effects should be related to the distribution of ERs, some of the non-genomic actions might not be, especially if they are related to antioxidant effects or enzyme or ion channel interactions that do not require receptors. Most of the evidence suggests that estrogen provides protection to the arterial system through several actions: (1) changing the lipid profile, causing a decrease in LDL and an increase in high-density lipoprotein; and (2) changing the production of endothelium-derived factors, such as increasing NO and decreasing ET-1. These effects would act to decrease platelet aggregation, cause vasodilatation and decrease proliferation of the underlying smooth muscle in response to injury. At the level of the arterial smooth muscle, estrogen decreases intracellular Ca^{2+}, which would also result in vasodilatation and decreases in proliferative responses[60]. However, it is unclear whether these effects occur in veins (Fig. 4). Some experimental data indicate that changes in the lipid profile do not affect venous function[72]. Responses of venous endothelium to changes in the physical or chemical environment might not be the same as in the arterial system, because blood flow-induced shear stress and partial O_2 pressure (PO_2) are not the same in the two systems. For example, non-genomic antioxidant effects of estrogen might not be as important in veins as in arteries, given that the PO_2 is lower in venous than in arterial blood. In addition, endothelium-derived factors in veins might not be produced in the same proportions as in the arterial system[73].

The harmful effects of estrogen on the venous wall are not known. Effects of estrogen on the coagulation cascade in relation to venous endothelium are also unknown. At present, except for factor prothrombin variant[74], there are no reliable biological markers to identify a woman at risk for developing venous thrombosis when she considers estrogen replacement. Markers of inflammation such as CRP have been considered to be predictive of atherosclerotic disease[34,36]. However, the relevance of similar markers for venous disease in women remains to be determined[41]. Understanding interactions between coagulation, inflammation and the vascular wall might provide insight not only into mechanisms of venous thrombosis, but also into mechanisms associated with the occurrence of adverse cardiovascular events reported in the HERS trial[9,10].

References

1 American Heart Association (1998) *Heart and Stroke Statistical Update*, American Heart Association
2 Varas-Lorenzo, C. *et al.* (2000) Hormone replacement therapy and incidence of acute myocardial infarction. A population-based nested case-control study. *Circulation* 101, 2572–2578
3 Barrett-Connor, E. and Bush, T.L. (1991) Estrogen and coronary heart disease in women. *J. Am. Med. Assoc.* 265, 1861–1867
4 Kannel, W.B. *et al.* (1976) Menopause and risk of cardiovascular disease: the Framingham study. *Ann. Intern. Med.* 85, 447–452
5 Rossouw, J.E. (1999) Hormone replacement therapy and cardiovascular disease. *Curr. Opin. Lipidology* 10, 429–434
6 Varas-Lorenzo, C. *et al.* (1998) Hormone replacement therapy and the risk of hospitalization for venous thromboembolism: a population-based study in southern Europe. *Am. J. Epidemiol.* 147, 387–390
7 van Kesteren, P.J.M. *et al.* (1997) Mortality and morbidity in transsexual subjects treated with cross-sex hormones. *Clin. Endocrinol.* 47, 337–342
8 Perez Gutthann, S. *et al.* (1997) Hormone replacement therapy and risk of venous thromboembolism: population based case-control study. *Br. Med. J.* 314, 796–800
9 Hulley, S. *et al.* (1998) Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *J. Am. Med. Assoc.* 280, 605–613
10 Grady, D. *et al.* (2000) Postmenopausal hormone therapy increases risk for venous thromboembolic disease. The heart and estrogen/progestin replacement study. *Ann. Intern. Med.* 132, 689–696
11 Chen, S-J. *et al.* (1996) Estrogen reduces myointimal proliferation after balloon injury of rat carotid artery. *Circulation* 93, 577–584
12 Li, G. *et al.* (1999) Estrogen inhibits vascular smooth muscle cell-dependent adventitial fibroblast migration *in vitro*. *Circulation* 100, 1639–1645
13 Iafrati, M.D. *et al.* (1997) Estrogen inhibits the vascular injury response in estrogen receptor α-deficient mice. *Nat. Med.* 3, 545–548
14 Karas, R.H. *et al.* (1999) Estrogen inhibits the vascular injury response in estrogen receptor beta-deficient female mice. *Proc. Natl. Acad. Sci. U. S. A.* 96, 15133–15136
15 Virchow, R.L.K. (1856) Phlogose und Thrombose im Gefäßsystem. In *Gesammelte Abhandlungen zur wisenschaftlichen Medizin*, Von Meidinger Sohn, Frankfurt
16 Bhagyalakshmi, A. and Frangos, J.A. (1989) Mechanism of shear-induced prostacyclin production in endothelial cells. *Biochem. Biophys. Res. Commun.* 158, 31–37
17 Miller, V.M. and Barber, D.A. (1996) Modulation of endothelium-derived nitric oxide in canine femoral veins. *Am. J. Physiol.* 271, H668–H673
18 Miller, V.M. and Michener, S.R. (1995) Modulation of contractions to and receptors for endothelins in canine veins. *Am. J. Physiol.* 268, H345–H350
19 Sevitt, S. (1978) Pathology and pathogenesis of deep vein thrombi. In *Venous Problems* (Bergan, J.J. and Yao, J.S.T., eds), pp. 257–279, Year Book Medical Publishers
20 Smith, D.F. and Toft, D.O. (1993) Steroid receptors and their associated proteins. *Mol. Endocrinol.* 7, 4–11
21 Kim-Schulze, S. *et al.* (1996) Expression of an estrogen receptor by human coronary artery and umbilical vein endothelial cells. *Circulation* 94, 1402–1407
22 Lindner, V. *et al.* (1998) Increased expression of estrogen receptor-beta mRNA in male blood vessels after vascular injury. *Circ. Res.* 83, 224–229

23 Losordo, D.W. *et al.* (1994) Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries of premenopausal women. *Circulation* 89, 1501–1510

24 Hodges, Y.K. *et al.* (2000) Estrogen receptors α and β. Prevalence of estrogen receptor β mRNA in human vascular smooth muscle and transcriptional effects. *Circulation* 101, 1792–1798

25 Sudhir, K. *et al.* (1997) Premature coronary artery disease associated with a disruptive mutation in the estrogen receptor gene in a man. *Circulation* 96, 3774–3777

26 Khetawat, G. *et al.* (2000) Human megakaryocytes and platelets contain the estrogen receptor β and androgen receptor (AR): testosterone regulates AR expression. *Blood* 95, 2289–2296

27 Okada, M. *et al.* (1997) Effects of 17 beta-estradiol and progesterone on migration of human monocytic THP-1 cells stimulated by minimally oxidized low-density lipoprotein *in vitro*. *Cardiovasc. Res.* 34, 529–535

28 Garcia-Duran, M. *et al.* (1999) Estrogen stimulates neuronal nitric oxide synthase protein expression in human neutrophils. *Circ. Res.* 85, 1020–1026

29 Bar, J. *et al.* (1993) The effect of estrogen replacement therapy on platelet aggregation and adenosine triphosphate release in postmenopausal women. *Obstet. Gynecol.* 81, 261–264

30 Ben-Hur, H. *et al.* (1995) Menopause is associated with a significant increase in blood monocyte number and a relative decrease in the expression of estrogen receptors in human peripheral monocytes. *Am. J. Reprod. Immunol.* 34, 363–369

31 Yamada, K. *et al.* (1996) Physiological concentration of 17 beta-estradiol inhibits chemotaxis of human monocytes in response to monocyte chemotactic protein 1. *Artery* 22, 24–35

32 Li, N. *et al.* (2000) Platelet–leukocyte cross talk in whole blood. *Arterioscler. Thromb. Vasc. Biol.* 20, 2702–2708

33 Byrne, G.I. *et al.* (2000) Collaborative multidisciplinary workshop report: interface of lipid metabolism, atherosclerosis, and *Chlamydia* infection. *J. Infect. Dis.* 181, S490–S491

34 Lagrand, W.K. *et al.* (1999) C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation* 100, 96–102

35 Walsh, B.W. *et al.* (2000) The effects of hormone replacement therapy and raloxifene on C-reactive protein and homocysteine in healthy postmenopausal women: a randomized, controlled trial. *J. Clin. Endocrinol. Metab.* 85, 214–218

36 Torzewski, M. *et al.* (2000) C-reactive protein in the arterial intima. Role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. *Arterioscler. Thromb. Vasc. Biol.* 20, 2094–2099

37 Bilsel, A.S. *et al.* (2000) Long-term effect of 17 beta-estradiol and thrombin on tissue factor pathway inhibitor release from HUVEC. *Thromb. Res.* 99, 173–178

38 Scarabin, P.Y. *et al.* (1997) Effects of oral and transdermal estrogen/progesterone regimens on blood coagulation and fibrinolysis in postmenopausal women: a randomized controlled trial. *Arterioscler. Thromb. Vasc. Biol.* 17, 3071–3078

39 Walsh, B.W. *et al.* (1998) Effects of raloxifene on serum lipids and coagulation factors in healthy postmenopausal women. *J. Am. Med. Assoc.* 279, 1445–1451

40 Kon Koh, K. *et al.* (1999) Relation of fibrinolytic potentiation by estrogen to coagulation pathway activation in postmenopausal women. *Am. J. Cardiol.* 83, 466–469

41 Cushman, M. *et al.* (1999) Effect of postmenopausal hormones on inflammation-sensitive proteins. The postmenopausal estrogen/progestin interventions (PEPI) study. *Circulation* 100, 717–722

42 Kuiper, G.G. *et al.* (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* 138, 863–870

43 Bayard, F. *et al.* (1995) Oestrogen synthesis, oestrogen metabolism and functional oestrogen receptors in bovine aortic endothelial cells. *Ciba Found. Symp.* 191, 122–132

44 Kikuchi, N. *et al.* (2000) Atheroprotective effect of estriol and estrone sulfate on human vascular smooth muscle cells. *J. Steroid Biochem. Mol. Biol.* 72, 71–78

45 Dubey, R.K. *et al.* (1999) Estrogen and tamoxifen metabolites protect smooth muscle cell membrane phospholipids against peroxidation and inhibit cell growth. *Circ. Res.* 84, 229–239

46 Dubey, R.K. *et al.* (2000) Clinically used estrogens differentially inhibit human aortic smooth muscle cell growth and mitogen-activated protein kinase activity. *Arterioscler. Thromb. Vasc. Biol.* 20, 964–972

47 Kalsner, S. (1969) Steroid potentiation of responses to sympathomimetic amines in aortic strips. *Br. J. Pharmacol.* 36, 582–593

48 Hamlet, M.A. *et al.* (1980) Effects of estradiol on release and disposition of norepinephrine from nerve endings. *Am. J. Physiol.* 239, H450–H456

49 Adlercreutz, H. (1990) Western diet and Western disease: some hormonal and biochemical mechanisms and associations. *Scand. J. Clin. Lab. Invest.* 210, 3–23

50 Clarkson, T.B. (2000) Soy phytoestrogens: what will be their role in postmenopausal hormone replacement therapy? *Menopause* 7, 71–75

51 Plouffe, L. (2000) Selective estrogen receptor modulators (SERMs) in clinical practice. *J. Soc. Gynecol. Invest.* 7, S38–S46

52 Agnusdei, D. and Iori, N. (1999) Tolerability profile of SERMs. *J. Endocrinol. Invest.* 22, 641–645

53 Figtree, G.A. *et al.* (2000) Tamoxifen acutely relaxes coronary arteries by an endothelium, nitric oxide, and estrogen receptor-dependent mechanism. *J. Pharmacol. Exper. Ther.* 295, 519–523

54 Figtree, G.A. *et al.* (1999) Raloxifene acutely relaxes rabbit coronary arteries *in vitro* by an estrogen receptor-dependent and nitric oxide-dependent mechanism. *Circulation* 100, 1095–1101

55 Clarkson, T.B. *et al.* (1998) Lack of effect of raloxifene on coronary artery atherosclerosis of postmenopausal monkeys. *J. Clin. Endocrinol. Metab.* 83, 721–726

56 Vogel, V.C. (2001) Chemoprevention: reducing breast cancer risk. In *Management of Patients at High Risk for Breast Cancer* (Vogel, V.C., ed.), Blackwell Science

57 Barrett-Connor, E. *et al.* (1998) Hormone and nonhormone therapy for the maintenance of postmenopausal health: the need for randomized controlled trials of estrogen and raloxifene. *J. Womens Health* 7, 839–847

58 Chen, Z. *et al.* (1999) Estrogen receptor alpha mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J. Clin. Invest.* 103, 401–406

59 Russell, K.S. *et al.* (2000) Estrogen stimulates heat shock protein 90 binding to endothelial nitric oxide synthase in human vascular endothelial cells. Effects on calcium sensitivity and NO release. *J. Biol. Chem.* 275, 5026–5030

60 Prakash, Y.S. *et al.* (1999) Estrogen increases $[Ca^{2+}]$ efflux from female porcine coronary arterial smooth muscle. *Am. J. Physiol.* 45, H926–H934

61 Gratton, J.P. *et al.* (2000) Reconstitution of an endothelial nitric-oxide synthase (eNOS), hsp90, and caveolin-1 complex *in vitro*. Evidence that hsp90 facilitates calmodulin stimulated displacement of eNOS from caveolin-1. *J. Biol. Chem.* 275, 22268–22272

62 Shaul, P.W. (1999) Rapid activation of endothelial nitric oxide synthase by estrogen. *Steroids* 64, 28–34

63 Mendelsohn, M.E. (2000) Nongenomic, estrogen receptor-mediated activation of endothelial nitric oxide synthase. How does it work? What does it mean? *Circ. Res.* 87, 956–960

64 Russell, K.S. *et al.* (2000) Human vascular endothelial cells contain membrane binding site for estradiol, which mediate rapid intracellular signaling. *Proc. Natl. Acad. Sci. U. S. A.* 97, 5930–5935

65 Sudhir, K. *et al.* (1997) Endothelial dysfunction in a man with disruptive mutation in oestrogen-receptor gene. *Lancet* 349, 1146–1147

66 Oparil, S. *et al.* (1999) Estrogen attenuates the adventitial contribution to neointima formation in injured rat carotid arteries. *Cardiovasc. Res.* 44, 608–614

67 Parini, P. *et al.* (1997) Importance of estrogen receptors in hepatic LDL receptor regulation. *Arterioscler. Thromb. Vasc. Biol.* 17, 1800–1805

68 Begona Ruiz-Larrea, M. *et al.* (2000) Antioxidant activities of estrogens against aqueous and lipophilic radicals; differences between phenol and catechol estrogens. *Chem. Phys. Lipids* 105, 179–188

69 Daly, E. *et al.* (1996) Risk of venous thromboembolism in users of hormone replacement therapy. *Lancet* 348, 977–980

70 Jick, H. *et al.* (1996) Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens. *Lancet* 348, 981–983

71 New, G. *et al.* (1997) Long-term estrogen therapy improves vascular function in male to female transsexuals. *J. Am. Coll. Cardiol.* 29, 1437–1444

72 Komori, K. *et al.* (1989) Endothelium-dependent relaxation in response to aggregating platelets in porcine femoral veins and its modulation by diet. *Circulation* 80, 401–409

73 Barber, D. and Miller, V. (1998) Endothelium-dependent vasoconstrictors. In *Endothelial Cell Research* (Rubanyi, G., ed.), pp. 167–185, Harwood Academic Publishers

74 Psaty, B.M. *et al.* (2001) Hormone replacement therapy, prothrombotic mutations, and the risk of incident nonfatal myocardial infarction in postmenopausal women. *J. Am. Med. Assoc.* 285, 906–913