Effects of Estrogen on Thrombosis and Inflammation

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Over the last 5 years, there have been remarkable findings regarding the effects of estrogen and estrogen agonists on the pathogenesis and prevention of cardiovascular disease in postmenopausal women. Notable among the many new developments are the following:

- New data on the expression of estrogen receptors in vascular tissue and the complex mechanisms through which the receptor-genome interaction is modulated;
- Information about the effects of estrogen on endothelial nitric oxide metabolism; and
- The discovery of new estrogenic compounds that have certain favorable cardiovascular effects without some of the potential risks of conventional estrogen therapy.

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The 6th International Graylyn Conference on Women's Health, held in Winston-Salem, NC, on October 12 and 13, 2000, brought together experts to review the current state of knowledge concerning the effects of estrogen on arterial thrombosis, venous thrombosis, and vascular inflammation. This article will summarize selected highlights of the symposium.

Inflammation and Thrombosis: Two Sides of the Same Coin? Drs. Russell Tracy, Mary Cushman

Both inflammatory and thrombotic reactions are "emergency" response systems that react to major insult or injury. However, they also play important homeostatic roles even when no major insult exists. Markers of activation of both the inflammatory and thrombosis systems can be detected at low levels even in apparently healthy, nonstressed individuals. A growing body of evidence suggests that variations in levels of these markers (eg, C-reactive protein [CRP], fibrinogen, D-dimer, or the plasmin-antiplasmin complex [PAP]) reflect

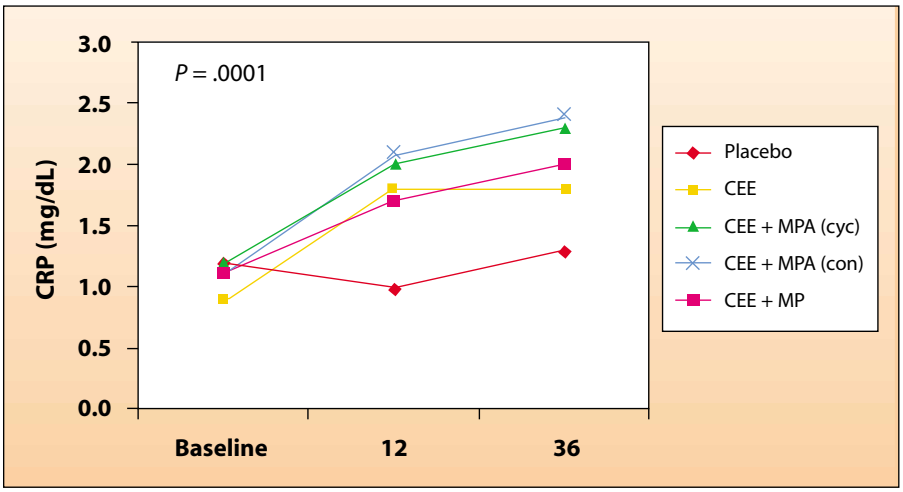

Figure 1. *Levels of C-reactive protein (mg/dL) in the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial after 12 months and 36 months of treatment. Treatment groups indicated in the legend: Placebo, no treatment; CEE, conjugated equine estrogens 0.625 mg/day; CEE + MPA (cyc), CEE plus cyclic medroxyprogesterone acetate (MPA) 10 mg/day, days 1–12 monthly; CEE + MPA (con), CEE plus continuous MPA 2.5 mg/day; CEE + MP, CEE plus continuous micronized progesterone 200 mg/day, days 1–12 monthly. P value is significant for differences between placebo group and each active treatment arm. Reproduced from Cushman et al,[5] with permission from the publisher. ©1999 Lippincott Williams & Williams.*

clinically significant differences in the state of vascular health and are closely related to each other.

Fibrinogen is a good example of a marker that is related to both thrombosis and inflammation. Elevated levels of fibrinogen were associated with subsequent ischemic heart disease in the Northwick Park Heart Study,[1] a cohort study of 1511 white men aged 40 to 64 years. A similar association was subsequently found in the Caerphilly and Speedwell Collaborative Heart Disease Studies.[2] Mild increases in interleukin-6 (IL-6) have been linked to increases in fibrinogen as well as monocyte tissue factor expression and levels of the coagulation factors V, VIII, and IX in normal individuals. IL-6 upregulates acute-phase proteins, including fibrinogen, as a normal homeostatic process. In one recent cohort study,[3] IL-6 levels were correlated with smoking, visceral fat, diabetes mellitus, and insulin sensitivity, and thus served as another marker of cardiovascular risk.

The role of CRP as a marker for cardiovascular risk has also received much attention, and it may be a surrogate for activity in the entire IL-6/inflammatory cascade. In an observational study, the Cardiovascular Heart Study (CHS), CRP was associated with incident heart disease events, especially in participants with subclinical disease at baseline.[4] In the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial, all four hormone treatments were associated with a large increase in CRP levels (Figure 1) and a decrease in E-selectin levels,[5] the combination of which would be expected to have a neutral effect on inflammation. Fibrinogen was decreased in all treatment groups compared to placebo; however, there were no differences in fibrinogen among the four active arms.[5,6] Hormone replacement therapy (HRT) use was associated with 59% higher mean CRP levels, 50% lower levels of plasminogen activator inhibitor-1 (PAI-1), and modest increases in fibrinogen and increases in Factor VIIc.[7] Lower levels of antithrombin and higher levels of protein C, both potentially prothrombotic changes, were also found in HRT users in this cohort.[8] However, in the Insulin Resistance and Atherosclerosis Study (IRAS), there was an inverse relationship between CRP and insulin sensitivity,[9] yet there was no association between CRP levels and smoking or underlying atherosclerosis in that cohort. The inconsistent correlations with CRP across several cohort studies may be due to variations in the prevalence of other illnesses or inflammatory challenges from cohort to cohort. CRP may be a sign not of a specific disease but of a series of challenges to homeostasis, as is seen in diabetes or other chronic illnesses.

Markers of activation of the coagulation and fibrinolytic cascades also appear to be informative metrics of vascular health. Data from CHS demonstrated a link between rates of fatal and nonfatal myocardial infarction (MI) and levels of PAP and D-dimer, but only in the oldest members of the cohort.[5] In factor analysis from CHS,[10] there were correlations among fibrinogen, D-dimer, CRP, and Factor VIIc, suggesting that these factors represent a cluster related to inflammation and possibly to risk for disease. Fibrinogen, IL-6, and possibly CRP may contribute to the pathogenesis of atherosclerosis: when atherosclerosis is progressing, levels of the markers of process (eg, D-dimer, PAP) increase; in turn, these changes pro-

Fibrinogen is a good example of a marker that is related to both thrombosis and inflammation.

mote expression of IL-6 and the downstream acute-phase reactants.

Arterial and Venous Thrombosis: The Same Side of Two Different Coins? Dr. Kenneth Bauer

Several markers of activation of the coagulation cascade can be considered in studies of thrombosis. These include Factor$_{1,2}$ ($F_{1,2}$), Factors Xa, VIIa, and IXa, and fibrinopeptide. Studies employing these markers have provided important information regarding hemostatic system function both under normal conditions and in response to pathogenic stimuli.

Data suggest that markers of process show evidence of a prothrombotic state that may be relevant to both arterial and venous thrombosis. One illustration is $F_{1,2}$ in the Northwick Park study. In the Northwick Park Heart Study II, researchers asked whether the frequency of acute MI in a cohort of nearly 3000 healthy men was determined by the balance between basal coagulation system activity and fibrinolytic system function. While $F_{1,2}$ levels increase over time as a function of aging, they were not linked to increased risk for clinical cardiovascular events.[11] These and other data suggest that hemostatic markers may be associated with risk but do not add to other markers' utility in terms of predicting first events.

The situation may be different in people who have already had an MI. Preliminary findings from the Northwick Park cohort indicate that compared to healthy men, those who had suffered an MI had higher $F_{1,2}$ levels and that these levels remain elevated persistently. $F_{1,2}$ levels were also higher in cohort members with the prothrombin 20210A and Factor V Leiden mutations,[12] both of which are predictive of risk for venous thromboembolic events (VTE). Earlier work found that estrogen replacement also raises $F_{1,2}$ levels,[13,14] which may account for the link between HRT and VTEs in observational studies and clinical trials.[15–18]

The transition from the prethrombotic state to the thrombotic state is still poorly understood. Although assays for components such as $F_{1,2}$ can denote the presence of a "biochemical" hypercoagulable state before overt thrombotic phenomena appear, clinical utility in the risk assessment for venous or arterial

Table 1
Summary of Estrogen's Effects on Markers of Thrombosis

Selected Citations	Marker	Positive Effects
Cushman et al,[7] Herrington et al,[24] van Baal et al,[25] Andersen et al,[30] Gottsater et al[33]	Fibrinogen	↓
Caine et al,[13] Kroon et al,[14] Nabulsi et al,[19] Herrington et al,[24] Scarabin et al,[32] Gottsater et al[33]	Antithrombin III	↓
Caine et al,[13] Høibraaten et al,[28] Winkler et al.[31]	Protein S	↓
Cushman et al,[7] Koh et al,[22] Walsh et al,[26] de Valk-de Roo et al,[21] Herrington et al,[24] Andersen et al,[30] Scarabin et al[32]	PAI-1	↓
Shahar et al,[20] Herrington et al,[24] Andersen et al,[30] Scarabin et al[32]	tPA	↑
Selected Citations	**Marker**	**Negative/ Neutral Effects**
Cushman et al,[7] Kroon et al,[14] Nabulsi et al,[19] Høibraaten et al,[29] Andersen et al[30]	Factor VII	↑
Nabulsi et al,[19] Høibraaten et al,[28] Høibraaten et al[29]	Protein C	↑
Caine et al,[13] Kroon et al,[14] de Valk-de Roo et al,[21] Winkler et al[31]	$F_{1,2}$	↑
Kroon et al,[14] Høibraaten et al,[28] Høibraaten et al[29]	TAT	↑
Van Baal et al[25]	Thrombomodulin	↓

As mentioned in the text, these studies differ considerably in aims, design, duration, and types and doses of hormone replacement therapy used. For example, oral conjugated equine estrogen was used in references 5, 13, 19, 21, 22, and 26. Oral estradiol was used in references 25, 29–32, and 33. Transdermal estradiol was used in references 14, 22, 28, and 32. Type of estrogen was not reported in reference 7 or 20.

PAI-1, plasminogen activator inhibitor-1; TAT, thrombin antithrombin complex; tPA, tissue plasminogen activator.

thrombotic events has not yet been demonstrated.

Coagulation/Fibrinolytic Factors: Dr. Karin Schenck-Gustafsson

Numerous studies have been published examining the effects of HRT on markers of arterial thrombosis. There are limitations with these data, as some studies have been only observational[7,19,20]; some have included only women with hysterectomies[14,21]; some are short-term[13,14,22–25]; and the type of HRT, dose, method of administration, and use or exclusion of a concomitant progestin have differed widely.[26–32] Despite these factors, some generalizations can be made about the effects of estrogen (see Table 1), with the caveat that they are not necessarily consistent from study to study.

In addition, the durability of estrogen-associated changes in markers of arterial thrombosis is not well established. For example, one study showed detrimental short-term changes in Factor VII levels that improved over 12 months in women taking estradiol valerate.[33] There is still a great deal of uncertainty in this area, and a need for more data, particularly for different HRT regimens.

Venous Thrombosis: Drs. Elaine Meilahn, Frits Rosendaal

There is some question whether risk of VTEs has been overestimated in oral contraceptive (OC) users, or if VTEs tend to be recognized preferentially in this population.[34] Third-generation OC formulations have not been in use long enough in Europe to draw firm conclusions about their relative safety profile. However, they increase activated protein C levels relative to nonusers, suggesting a procoagulatory effect. It appears that OC users with thrombophilic disorders are indeed at a higher risk for VTEs regardless of the agents used; furthermore, the higher risk remains even after OC use is discontinued.[35] In women already at high risk for VTEs (see Table 2), OCs may be the "final push" that brings about an adverse event.

Table 2
Risks for Venous Thromboembolic Events (VTE) Associated with Oral Contraceptive (OC) Use Versus Nonusers

	VTE Risk
Overall risk	3.8
3rd generation OCs	6.0
2nd generation OCs	2.2
Factor V Leiden carriers	34.7
Increased Factor VIII and Factor V Leiden	10.3
< 6-month use in women with thrombophilia	18.5

Data from Bloemenkamp et al[34,35,59] and Vandenbroucke et al.[60]

There are only a few studies of VTE and estrogen in the literature[15–18,27,36,37]; of those, only two[18,27] were randomized clinical trials. HERS was the first such study to examine the effects of HRT on risk for coronary heart disease (CHD) events in women with heart disease (mean age 66.7 years) randomized to either HRT (0.625 mg oral conjugated estrogen and 2.5 mg medroxyprogesterone acetate daily; n = 1380) or placebo (n = 1383). After 4.1 years of follow-up, there was no difference in the primary outcome: 172 women in the HRT group and 176 women in the placebo group experienced an MI or fatal CHD event during the trial (relative hazard [RH] 0.99; 95% confidence interval [CI] 0.80-1.22).[38] Annual rates of VTE in HERS were substantially higher in the treatment group (HRT = 0.63% vs placebo 0.22%; RH 2.9; 95% CI 1.5-5.6; *P* = .002). As

Table 3
Relative Hazard (RH) Ratios for Venous Thromboembolic Events (VTE) in the HERS Trial, by Year

Year	RH for VTE	RH for Primary CHD Events*
4	1.5	0.67
3	2.4	0.87
2	4.1	1.00
1	3.3	1.52

*Defined as nonfatal myocardial infarction or coronary death.
CHD, coronary heart disease.
Data from Grady et al[18] and Hulley et al.[38]

was the case for cardiovascular events, the increased risk for VTE with HRT was greatest in the first year (RH = 3.3, $P < .05$)[38] (see Table 3).

A recently reported study, the Estrogen in Venous Thromboembolism Trial (EVTET) was a randomized, placebo-controlled clinical trial of the effects of oral estradiol 2 mg and norethisterone acetate 1 mg in postmenopausal Norwegian women with previously documented VTEs.[27] The primary outcome of the 2-year trial was VTE or pulmonary embolism. After publication of the HERS results, recruitment was discontinued, and a subsequent review by the study's monitoring board recommended termination of the trial. Eight of 71 women in the treatment group (11.3%) and 1 of 69 women in the placebo group (1.4%) developed a VTE. In the HRT group, all events occurred within 261 days of inclusion in the trial. The results suggest that women who have had a previous VTE have a greatly increased risk of recurrence if they take HRT.

Coronary Arterial Thrombosis: Dr. Bruce Psaty

In a review of 10 cohort studies, 3 angiographic studies, and 12 case-control studies, the odds ratio for MI was 0.70 for users of unopposed estrogen and 0.66 for users of estrogen plus progestin.[39] However, the risk rose to 0.99 in women using estrogen plus progestin in HERS,[38] a cohort with known CHD. The current challenges are to account for known confounders such as compliance bias and to uncover possible unmeasured confounders. Identification of susceptible subgroups who should not be receiving estrogen, for genetic or other reasons, may be the next most important inquiry to clarify the HERS results.

The findings of HERS and EVTET are supported by results from the Nurses' Health Study, in which short-term current HRT users had a multivariate-adjusted relative risk (RR) for major CHD events of 1.25, compared with never-users. After longer-term hormone use, however, the rate of second events was lower in current users than in never-users (RR = 0.38; P for trend = .002).[40]

Platelet Function: Dr. Pascal Goldschmidt

Platelet function plays a key role in unstable coronary syndromes, especially genetic mutations (specifically the Pl^{A2} polymorphism) that change the structure of the glycoprotein IIIa subunit. This change results in increased platelet responsiveness and recombinant cell adhesion. The Pl^{A2} polymorphism has been associated with coronary thrombosis[41] and it could be an important cause of sudden death in the young.[42] However, because there are 20 or more gene polymorphisms associated with MI, the Pl^{A2} polymorphism may play a role in concert with others. Platelets with this polymorphism are much more sensitive to estradiol: 1000 times more estradiol is required to equate effects in platelets of the $Pl^{A1,A1}$ variety versus those with the Pl^{A2} polymorphism (in both men and women), an effect that is estrogen receptor–dependent.[43] Aspirin had no additional effect on Pl^{A2} platelets, suggesting that in people with the polymorphism who take aspirin, adding estrogen would have no further effects on platelets. This may indicate that women with the Pl^{A2} polymorphism who take hormone replacement would not benefit from aspirin; indeed, 80% of women in HERS took aspirin, and those in the treatment group showed no benefits with respect to coronary events compared to controls. Methylation of the estrogen receptor may negate estrogen's protective effects, as has been shown in atherosclerotic smooth muscle cells in vitro.[44]

Estrogen, Lipoprotein Oxidation, and Initiation of Inflammation: Dr. Carol Banka

It is known that oxidized low-density lipoprotein (LDL) is present in atherosclerotic lesions[45] and that cellular modification of LDL involves lipid peroxidation.[46] Estrogen lowers LDL cholesterol; however, estrogen also protects high-density lipoprotein (HDL) from oxidation,[47] which in turn limits peroxidation and uptake of LDL. Yet animal studies have demonstrated[48,49] and epidemiologic observations have agreed[50] that the favorable effects of estrogen on atherosclerosis are to some degree independent of their effects on plasma cholesterol concentrations, leading some investigators (as detailed elsewhere in this report) to focus on the role of estrogen in inflammation. In one such experiment, ovariectomized LDL-knockout mice showed a sharp increase in monocytes and greater numbers of eosinophils versus normally cycling mice after 16 weeks of a high-fat diet,[51] even though plasma cholesterol levels in the two groups were comparable. Interestingly, it has been suggested that a common pathway may mediate oxidative stress, inflammation, and atherogenesis.[52]

Another focus of effort among cardiovascular scientists has been estrogen's effects on the vascular endothelium, also discussed in detail elsewhere in this report. One approach has been to use kallikrein activation as a way to examine inflammation of an endothelial origin. After 7 days of a high-fat diet, mice lacking the LDL receptor showed significantly greater kallikrein activation compared to wild-type mice. Future studies using this mouse

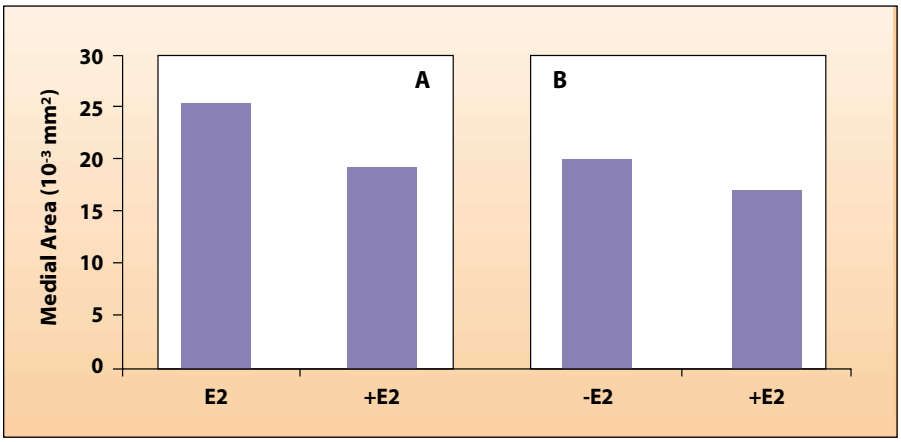

Figure 2. (A) *Vascular medial areas (mean) of injured carotid arteries in estrogen receptor-α (ER-α) knockout mice (n = 10–13) untreated (left bar) or treated (right bar) with estradiol.* **(B)** *Vascular medial areas of injured carotid arteries in estrogen receptor-β (ER-β) knockout mice untreated (left bar) or treated (right bar) with estradiol. *, P < .05 compared with both uninjured and injured estrogen-treated groups within the same genotype. ER-α data from Iafrati et al[53]; ER-β data from Karas et al.[54]*

model will assess the role of estrogen in upregulating kallikrein in the presence of hypercholesterolemia.

Observations in Estrogen Receptor–Deficient Mice: Dr. Michael Mendelsohn

Estrogen has been shown to inhibit the response to vascular injury (carotid de-endothelialization) in wild-type and ERα-deficient ovariectomized mice[53] as well as in ERβ-deficient mice,[54] suggesting that ERα and ERβ play redundant roles in mediating vascular response to estrogen (Figure 2). In ERβ-deficient mice, unlike ERα-deficient mice, estrogen enhances vasoconstriction in aortic rings, and it is hypothesized that ERβ is involved in vascular smooth muscle cell relaxation.[55] ERβ-deficient male mice have higher blood pressures than females and a greater incidence of hypertension. Early research suggests that inducible nitric oxide synthase expression in smooth muscle cells is mediated by ERβ and may play a role in the development of hypertension in these mice—a theory that, if borne out, has significant clinical implications for new and more effective treatments for hypertension.

Estrogen Effects on Adhesion Molecules and Matrix Metalloproteinases in Postmenopausal Women: Dr. Richard O. Cannon III

It has been suggested that nitric oxide may have an anti-inflammatory effect via inhibiting the transport of NFκB into the nucleus. Although estrogen decreases levels of adhesion molecules, this effect is probably not solely due to its effects on nitric oxide. Estrogen can also function as an antioxidant, which may account for its ability to lower soluble levels of the endothelial adhesion molecule ICAM-1. Estrogen's decrease of PAI-1 and increase in D-dimer levels[22] may result in disinhibition of tissue plasminogen activator (tPA) and activation of plasmin and matrix metalloproteinases, ultimately promoting the digestion of matrix proteins in the vessel wall. This process could improve vessel distensibility in healthy women; however, in women with atherosclerotic plaque, greater vulnerability to rupture could result. This scenario could help to explain the results of the HERS trial. Preliminary studies using magnetic resonance imaging indicate that subtle degrees of vascular thickening, possibly the result of vascular inflammation, are associated with elevated serum markers of inflammation (IL-6, ICAM-1, VCAM-1, CRP, E-selectin), even in apparently healthy subjects.[56] Work is ongoing in a rabbit model to confirm that arterial thickening measured with magnetic resonance imaging is indeed the result of inflammation.

Estrogen appears to have mixed effects on markers of inflammation.

Transforming Growth Factor β1 and Hormone Replacement Therapy in Postmenopausal Women with Coronary Artery Disease: Dr. Ingrid Os

It has been hypothesized that transforming growth factor β1 ($TGF\beta_1$) can inhibit atherosclerosis by preserving endothelial function. Increases in $TGF\beta_1$ were observed in the Estrogen, Women and Atherosclerosis (EWA) trial in 118 women treated for 12 months with transdermal 17β-estradiol (with or without sequential MPA).[57] Eighty percent of these women were already taking a statin at entry into the study. Increases in $TGF\beta_1$ were apparent after 3 months of therapy and were greater in women with one-vessel disease compared to those with two- or three-vessel disease. Levels of ICAM-1 were inversely correlated with levels of $TGF\beta_1$. However, unlike previous studies in men, in EWA there was no association between levels of Lp(a)

and TGFβ_1, hinting at a gender difference in TGFβ_1 regulation. Other work has shown that the T allele polymorphism of TGFβ_1 is associated with a greater risk of MI in men but not in women,[58] suggesting a genetic as well as a sex difference in circulation of this protein.

Summary

In the aggregate, the current knowledge base concerning estrogen's effects on inflammation and thrombosis does not provide a clear unifying picture concerning the role of HRT for primary or secondary prevention of CHD. Although it is clear that CRP and other markers of activation of the inflammation and thrombosis cascades are related to each other and—to a greater or lesser degree—to risk for CHD, the effects of estrogen on these markers and subsequent CHD risk remain uncertain. Estrogen appears to have mixed effects on markers of inflammation, including increases in CRP, but decreases in other indicators of endothelial cell activation, including soluble levels of endothelial cell adhesion molecules and expression of TGF-β. With respect to thrombosis, estrogen also appears to have mixed effects, including activation of both coagulation and fibrinolysis. The effects of both HRT and oral contraceptives on increased rates of venous thrombosis are clear, whereas the impact on arterial events remains uncertain. The activation of plasmin and matrix metalloproteinases may not be benign if these proteases augment lysis of fibrous caps in vulnerable plaques. Clearly, more data are needed concerning estrogen action in inflammation and thrombosis and the clinical consequences with respect to cardiovascular diseases. ■

[Estrogen's] impact on arterial events remains uncertain.

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Main Points

- Inflammatory and thrombotic reactions are responses to major insult or injury, but they also play important homeostatic roles in the absence of major insult.
- Markers of activation of both the inflammatory and thrombosis systems can be detected at low levels even in apparently healthy, nonstressed individuals.
- Evidence suggests that variations in levels of markers such as C-reactive protein, fibrinogen, D-dimer, or the plasmin-antiplasmin complex reflect clinically significant differences in the state of vascular health and are closely related to each other.
- Hemostatic markers may be associated with risk but do not add to other markers' utility in terms of predicting first events.
- Third-generation oral contraceptive formulations increase activated protein C levels, suggesting a procoagulatory effect; oral contraceptive users with thrombophilic disorders may be at a higher risk for venous thromboembolic events, and the higher risk remains even after use is discontinued.
- Women who have had a previous venous thromboembolic event may have a greatly increased risk of recurrence if they take hormone replacement therapy.
- In the Nurses' Health Study, short-term current hormone replacement therapy users had a multivariate-adjusted relative risk for major coronary heart disease events of 1.25, compared with never-users, but after longer-term hormone use, the rate of second events was lower in current users than in never-users.

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