

Hemostatic Factors and Estrogen during the Menopausal Transition

MaryFran R. Sowers, Karen A. Matthews, Mary Jannausch, John F. Randolph, Daniel McConnell, Kim Sutton-Tyrrell, Roderick Little, Bill Lasley, and Richard Pasternak

Departments of Epidemiology and Biostatistics, University of Michigan School of Public Health (M.R.S., M.J., J.F.R., D.M., R.L.), Ann Arbor, Michigan 48104; University of Pittsburgh (K.A.M., K.S.-T.), Pittsburgh, Pennsylvania 15260; University of California (B.L.), Davis, California 95616; and Merck & Co., Inc. (R.P.), Rahway, New Jersey 08889

Background: It has been speculated that gender differences in cardiovascular disease (CVD) mortality can be attributed to the effects of estrogens on inflammation and hemostatic marker profiles. Therefore, we evaluated endogenous hormone concentrations, menopause transition stages, and adoption of exogenous hormone use in relation to hemostatic and inflammation marker concentrations in women.

Methods: Longitudinally, we studied 3302 participants from the Study of Women's Health Across the Nation, aged 42–52 yr at baseline and self-identified as African-American (28%), Caucasian (47%), Chinese (8%), Hispanic (8%), or Japanese (9%). Serum samples from baseline and years 2001, 2003, and 2005 were assayed for estradiol and FSH. Hormone concentrations were related to CVD markers, including fibrinogen, factor VII-c, plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator, and human serum C-reactive protein (hsCRP).

Results: Lower estradiol levels were associated with higher levels of

PAI-1 and tissue plasminogen activator, but there were no significant relationships with fibrinogen, factor VII-c, or hsCRP. Higher FSH concentrations were associated with higher PAI-1 and factor VII levels, but lower fibrinogen and hsCRP levels. Transitions from premenopause and early perimenopause to postmenopause were not associated with significant differences in levels of hemostatic factors. The hsCRP concentrations were approximately 25% higher, and the PAI-1 concentrations approximately 20% lower among women who initiated hormone therapy, compared with nonusers.

Summary: Endogenous estrogens may reduce CVD risk via modulation of fibrinolytic factors, but not coagulation or inflammatory markers. Notably, conclusions derived from studies of exogenous hormones and CVD risk may not parallel or explain the effects of endogenous hormones or perimenopausal hormone changes on CVD risk. (*J Clin Endocrinol Metab* 90: 5942–5948, 2005)

THE FAILURE OF recent hormone replacement trials to demonstrate the expected protective effect for cardiovascular disease (CVD) among women (1, 2) has motivated a more intensive effort to understand why women have a relative advantage compared with men with respect to CVD mortality in the age range of 35–64 yr (3). Hemostatic factors became major candidates for alternative hypotheses, based in part on the presence of specific, high-affinity estrogen receptors on vessel walls (4). Both *in vivo* and *in vitro* studies suggest that endogenous estrogen affects vascular tone and inhibits the remodeling associated with vascular injury. Cell culture studies indicate that estrogen directly affects the proliferation of endothelial and vascular smooth muscle cells (5, 6) in a manner that is dose dependent and related to an interaction with androgens (5).

Studies of exogenous hormone use in postmenopausal women showed that estrogen replacement therapy lowered plasminogen activator inhibitor-1 (PAI-1) plasma levels, leading to speculation that a cardioprotective effect of es-

trogen replacement therapy could be expressed through maintenance of a more favorable fibrinolytic balance (7). Two reports indicated that premenopausal women had lower PAI-1 concentrations than postmenopausal women (8, 9), with the inference that differences in PAI-1 concentrations were related to the difference in estrogen concentrations in pre- vs. postmenopausal women. In the baseline examination of the Study of Women's Health Across the Nation (SWAN), a prospective study of 3302 women transitioning the menopause, the free estradiol index was weakly, but significantly, correlated with PAI-1 concentrations (10).

We related hemostatic and inflammation factors to the endogenous hormones, estradiol (E2) and serum FSH concentrations observed over a 5-yr period and to menopause status in women transitioning the menopause. The hemostatic markers included the fibrinolytic factors PAI-1 and tissue plasminogen activator (tPA), the coagulation factors fibrinogen and factor VII-c, and the acute phase protein, human serum C-reactive protein (hsCRP). We hypothesized that lower E2, higher FSH, and transition to postmenopause would be associated with higher levels of fibrinolytic factors and hsCRP.

Subjects and Methods

Population

SWAN is a prospective, multiethnic, multidisciplinary study of the natural history of the menopausal transition that is being conducted in

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Abbreviations: BMI, Body mass index; CRP, C-reactive protein; CV, coefficient of variation; CVD, cardiovascular disease; E2, estradiol; hsCRP, human serum CRP; HT, hormone therapy; PAI-1, plasminogen activator inhibitor-1; SI, Systeme International; tPA, tissue plasminogen activator.

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community-based groups of women located in Boston; Chicago; the Detroit area; Los Angeles; Hudson County, NJ; Pittsburgh; and Oakland, CA. Recruitment was a two-stage process commencing with a 15-min cross-sectional survey among 16,065 women, aged 40–55 yr and living in the geographic area defined by the clinic sites. This initial stage served as the sampling frame for the second recruitment stage, which led to the enrollment of 3302 menstruating women, aged 42–52 yr. Enrolled women were not using exogenous hormone preparations that could affect ovarian function in the 3 months before enrollment, had at least one menstrual period in the 3 months before enrollment, and self-identified with the site's designated race/ethnic group. These criteria precluded enrollment of postmenopausal women or premenopausal women using oral contraceptives or hormone replacement to the longitudinal cohort.

Women who were Caucasian or a member of a designated race/ethnic group were enrolled at each site, including African-American women at Boston, Chicago, the Detroit area, and Pittsburgh as well as Japanese, Chinese, and Hispanic women at Los Angeles, Oakland, and Hudson County, NJ, respectively. Additional information has been published about the eligibility criteria, sampling frames, and participant characteristics (11). Data were collected via protocols reviewed and endorsed by an appropriate institutional review board at each site. This report is based on data from the four annual examinations at which hemostatic factors were measured (baseline and follow-up year 2001, 2003, and 2005 examinations).

Measures

Menopausal status was based on self-report of decreased predictability in the time between menses in the previous 3 months (early perimenopausal), no decreased predictability in the same time period (premenopausal), no menses for 3–11 months (late perimenopausal), or no menses for 12 or more months (menopausal). Height (centimeters) and weight (kilograms) were measured with stadiometers and calibrated scales and were used to calculate body mass index [BMI; weight (kilograms)/height (meters)²]. Waist was measured at the narrowest part of the torso (centimeters).

Assays

At baseline, blood was drawn during d 2–5 of the follicular phase of the menstrual cycle and after fasting. In follow-up examinations, blood draws became increasingly less like to occur in the d 2–5 follicular phase window because menstrual bleeding became increasingly unpredictable in women approaching the last menstrual period (64% at follow-up year 2001, 48% at yr 2003, and 27% at yr 2005). Samples for hormone assays were kept at room temperature for 30–60 min and then refrigerated for 30–60 min. Other samples were refrigerated for up to 2 h, spun, separated, frozen at –20 C (or lower), and sent on dry ice to either the CLIA-certified CLASS laboratory at the University of Michigan (for assay of E2, testosterone, SHBG, FSH, or TSH) or the Medical Research Laboratories (for assay of fibrinogen, hsCRP, tPA, PAI-1, and factor VII-c). Throughout the study, the MRL Laboratory participated in the certification by the National Heart, Lung, and Blood Institute (12).

tPA was measured in plasma using a double antibody in an ELISA (IMUBIND tPA ELISA, American Diagnostica, Greenwich, CT). The assay uses human single-chain tPA as a standard calibrated against an international standard (National Institute for Biological Standards and Control, Hertfordshire, UK). Monthly interassay coefficients of variation (CVs) were 4.7–8.7% and 3.8–7.8% at mean concentrations of 5.6 and 11 ng/dl, respectively.

Plasma PAI-1 was measured with a sandwich procedure using a solid phase monoclonal antibody and an enzyme-labeled goat second antiserum (IMUBIND plasma PAI-1 ELISA, American Diagnostica). The monthly interassay CVs were 5–9% and 4–9% at mean concentrations of 7 and 22.5 ng/dl, respectively.

Fibrinogen was measured in frozen citrated plasma on an MLA ELECTRA 1400C (Medical Laboratory Automation, Inc., Mt. Vernon, NY) using a clot-based turbidometric detection system. The monthly interassay CVs were 2.3–3.5% and 2.6–3.6% at mean concentrations of 250 and 140 mg/dl, respectively.

Factor VII-c activity was measured in frozen citrated plasma on the MLA ELECTRA 1400C (Medical Laboratory Instrumentation, Inc.) using

a turbidometric detection system and factor VII-deficient plasma (George King Bio-Medical, Overland Park, KS) in preparation of the standard curve. The monthly interassay CVs were approximately 7.8%, 5%, and 4% for mean activities of 8%, 45%, and 99%, respectively.

hsCRP was quantified using an ultrasensitive rate immunonephelometric method (hsCRP on BN 100, Dade-Behring, Marburg, Germany). The method is based on monitoring light scattering during agglutination of CRP to polystyrene particles coated with monoclonal antibodies to CRP. The sensitivity of the assay (lowest detectable concentration) was 0.03 mg/dl. The CVs at CRP concentrations of 0.05 and 2.2 mg/dl were 10–12% and 5–7%, respectively.

Serum FSH concentrations were measured with a two-site chemiluminometric immunoassay with CVs of 12.0% and 6.0%. SHBG was a *de novo* two-site chemiluminescent assay with CVs of 9.9% and 6.1%. Serum E2 concentrations were measured with a modified, off-line ACS:180 (E₂-6) immunoassay with CVs of 10.6% and 6.4%. Total E2 was indexed to SHBG (free estradiol index = 100 × total estradiol/272.11 × SHBG) to estimate nonbound E2 activity.

Statistical methods

Variables for fasting status, time of day of blood draw, and day of blood draw (d 2–5 of the early follicular phase) were eligible for inclusion in models because tPA, PAI-1, and E2 had significant diurnal variation, and tPA, PAI-1, E2, and BMI values were higher in women whose blood was drawn at times other than d 2–5 of the menstrual cycle. A variable for site and ethnicity was included in all models to account for sampling design.

Data from women treated with anticoagulants (*n* = 17) were excluded from analyses for the particular examination in which women reported using anticoagulants. Data from women who reported using aspirin were retained, because the reason for aspirin use could not be explicitly discerned, and there was no difference in the hemostatic factor values among women who reported aspirin use compared with those who did not. When hormone therapy (HT) use was reported at postbaseline examinations, the data were segregated into an HT stratum. Data from women with hysterectomy were censored at the time of its report.

Continuous variables, other than age, were log transformed to satisfy model assumptions including normally distributed residuals. Consistency of associations was evaluated including and excluding those women with TSH values outside the euthyroid range of 0.5–5.0 mIU/ml.

Data were first described with cross-sectional analyses and are reported in Tables 1 and 2. Subsequently, longitudinal analyses were used to describe the associations of hormones and menopause status variables with hemostatic factor concentrations using linear mixed models (SAS Proc Mixed, SAS Institute, Inc., Cary, NC) and accounting for the autocorrelation of repeated measures (Tables 3 and 4). The longitudinal linear mixed models included age (as a time varying covariate) and menopause status or reproductive hormones in relation to the hemostatic factors. These variables were treated as main effects (or combined with age as an interaction term) to describe change with time. Models were fit separately for each of the hemostatic factors. Waist circumference and BMI were evaluated in models as time-varying covariates. Self-reported race/ethnicity, site, and baseline smoking behavior were entered into models as single-time covariates. Data from longitudinal models were incorporated into bar graphs to facilitate the interpretation of the β coefficients for menopause status (Fig. 1) and a range of representative values of FSH or E2 (Figs. 2 and 3); additional effects of hormone × age interaction, when statistically significant, were presented, with age held constant at 50 yr.

Model fit was assessed using residual analyses and the Akaike criteria, as appropriate. Log transformations were returned to appropriate units using the technique of Duan (13). *P* < 0.01 was considered statistically significant.

Results

Data are reported in Systeme Internationale (SI) units. At baseline, the study sample included 1545 (47% of total) Caucasian women, 927 (28% of total) African-American women, 285 (9% of total) Japanese women, 279 (9% of total) Hispanic women, and 250 (8% of total) Chinese women.

TABLE 1. Cross-sectional measures of hemostatic factors, reproductive hormones, and body size over a 5-yr period

	Baseline	Yr 2001	Yr 2003	Yr 2005
Time since base visit (yr)	0	1.03 ± 0.17	3.06 ± 0.19	5.05 ± 0.20
Age (yr)	46.4 ± 2.7	47.5 ± 2.7	49.5 ± 2.7	51.6 ± 3.4
Reproductive hormones				
FSH (IU/liter)				
In 2–5 ^a	17.0 ± 11.1	19.1 ± 12.4	20.1 ± 13.1	17.5 ± 13.6
Not in 2–5	20.5 ± 21.2	27.1 ± 28.1	45.7 ± 47.4	52.9 ± 34.1
Using HT		25.4 ± 8.6	36.3 ± 33.4	40.0 ± 32.7
E2 (pmol/liter) ^b				
In 2–5	198.2 ± 145.4	178.0 ± 128.1	156.0 ± 117.5	159.3 ± 153.1
Not in 2–5	234.6 ± 236.0	214.4 ± 215.5	129.9 ± 132.9	94.0 ± 80.0
Using HT		194.6 ± 175.8	165.6 ± 155.3	141.0 ± 140.6
Hemostatic factors				
CRP (nmol/liter)				
Using HT		94.4 ± 128.8	106.0 ± 104.6	110.8 ± 107.4
Not using HT	76.3 ± 104.6	69.80 ± 95.3	78.6 ± 141.8	79.5 ± 150.2
Fibrinogen (μmol/liter)				
Using HT		8.06 ± 1.78	7.90 ± 1.65	7.80 ± 1.74
Not using HT	8.42 ± 1.91	8.11 ± 1.76	8.09 ± 1.61	7.99 ± 1.70
PAI-1 (pmol/liter)				
Using HT		316 ± 352	324 ± 442	214 ± 302
Not using HT	414 ± 326	382 ± 292	436 ± 328	320 ± 202
tPA (pmol/liter) ^c				
Using HT		106.0 ± 52.9	94.7 ± 51.5	84.2 ± 41.5
Not using HT	101.5 ± 44.3	109.5 ± 51.5	105.0 ± 45.8	100.1 ± 47.2
Factor VIIc (%)				
Using HT		115.1 ± 27.3	128.8 ± 28.1	122.9 ± 29.7
Not using HT	115.8 ± 30.7	111.1 ± 28.3	118.4 ± 30.6	116.9 ± 31.4
Body size measures				
BMI (kg/m ²)	27.4 ± 6.5	27.3 ± 6.5	27.7 ± 6.6	28.0 ± 6.6
Waist (cm)	86.3 ± 15.2	86.6 ± 15.2	87.5 ± 15.4	88.7 ± 15.6
Hip (cm)	106.9 ± 14.1	107.0 ± 14.1	107.1 ± 14.1	107.6 ± 14.1
Waist/hip ratio	0.80 ± 0.07	0.81 ± 0.07	0.81 ± 0.07	0.82 ± 0.07

These are cross-sectional descriptions, and no *P* values are provided because no inferences are made. Values are the mean ± SD.

^a Designates blood collection on d 2–5 after menses.

^b There were three extreme tPA values excluded at yr 2001 and 2003, respectively, and extreme E2 values were excluded at baseline (*n* = 1), yr 2001 (*n* = 1), and yr 2005 (*n* = 2).

Table 1 shows the unadjusted cross-sectional mean values of the hemostatic factors and hormones (or their indices) at each of the four examinations at which CVD markers were assayed and according to the presence or absence of HT.

Factor VII-c levels were significantly lower in African-American women compared with Caucasian, Chinese, Hispanic, and Japanese women (data not shown).

Stages of menopausal status

At baseline, 54% of women were classified as being premenopausal, and the remaining 46% were classified as being in the early perimenopause (Table 2). Only 5% of women

remained classified as premenopausal at the fifth follow-up examination. As a condition of enrollment in the longitudinal cohort, women did not use HT at the baseline examination; however, by the fifth follow-up examination, 20% of women, cumulatively, had used HT.

When evaluated with longitudinal models, there were no statistically significant associations of hemostatic factor levels with menopause status as women transitioned from premenopause to the early postmenopause (Table 3 and Fig. 1). All data were adjusted for BMI, waist circumference, and smoking, the covariates that were independently associated with the hemostatic factors.

TABLE 2. The number and frequency (percentage) of SWAN enrollees according to menopause status at baseline and follow-up visits

	Baseline	Yr 2001	Yr 2003	Yr 2005
No.	3286 ^a	2747	2329	2171
Menopause status ^b				
Premenopausal	1758 (54)	693 (25)	262 (11)	100 (5)
Early perimenopausal	1496 (46)	1656 (60)	1217 (52)	813 (37)
Late perimenopausal	0	117 (4)	201 (9)	235 (11)
Postmenopausal	0	56 (2)	227 (10)	540 (25)
Surgical menopause	0	11 (<1)	28 (1)	50 (2)
HT use	0	202 (7)	393 (17)	441 (20)

^a Based on the number of women with cardiovascular measures, reproductive hormone concentrations, and physical measures, and excluding eight women taking anticoagulants.

^b Data excluded the pregnancy reported in yr 2001 and 2005. Women who could not be classified according to menopausal status were 32 at baseline, 11 at yr 2001, and two at yr 2003.

TABLE 3. Longitudinal models of hemostatic factors and menopausal status or HT use, adjusted for covariates

	log _e hsCRP		log _e Fibrinogen		log _e PAI-1		log _e tPA		log _e Factor VIIc	
	Estimate	P value	Estimate	P value	Estimate	P value	Estimate	P value	Estimate	P value
Intercept	-13.865		4.205		-6.914		-3.318		3.175	
Age (centered)	0.009	NS	-0.008	<0.0001	-0.0198	<0.0001	-0.002	NS	0.004	0.0004
HT use	0.219	<0.0001	-0.029	0.001	-0.236	<0.0001	-0.063	0.002	0.046	<0.0001
HT × age (centered)	0.019	NS	0.001	NS	-0.034	0.0003	-0.039	<0.0001	0.013	0.002
H × age (centered)							0.004	0.002	-0.002	0.004
Postmenopausal	-0.090	NS	0.005	NS	-0.078	NS	0.027	NS	0.022	NS
Late perimenopausal	-0.040	NS	0.005	NS	-0.040	NS	-0.021	NS	0.020	NS
Early perimenopausal	-0.007	NS	-0.006	NS	-0.022	NS	0.002	NS	-0.002	NS
Premenopausal	Referent		Referent		Referent		Referent		Referent	

All models are restricted to samples obtained in the fasting state and BMI, waist, smoking, site, and ethnicity covariates. Women taking anticoagulants or with surgical menopause were excluded. Age × status interactions (data not shown) were not significant except within HT users. Age² (centered) values were not statistically significant for any hemostatic factors. NS, Not significant.

Reproductive hormones and hemostatic factors

Endogenous E2 concentrations were negatively associated with the fibrinolytic markers, PAI-1 and tPA, and the association with PAI-1 became more positive over time as E2 concentrations declined ($P < 0.0001$; Table 4 and Fig. 2). There was no association of E2 with hsCRP, fibrinogen, or factor VII-c.

FSH concentrations were positively associated with PAI-1 and factor VII-c (Table 4). FSH was negatively associated with fibrinogen and hsCRP concentrations (Fig. 3).

HT use

HT use, initiated after the baseline examination, was associated with significant differences in hemostatic factors (Fig. 1). The degree of impact was typically greater with longer HT use, as estimated with the significant age × HT interaction term (Table 3). Notably, hsCRP concentrations were 25% higher among the HT users compared with those in premenopausal and early perimenopausal women. Fibrinogen concentrations were 3% lower. PAI-1 concentrations were 21% lower in women with HT use and declined 4% with each additional year of use. On the average, tPA concentrations were approximately 6% lower in women using HT and declined 3.5%/yr of HT use. Factor VII-c was increased among women using HT by 5% compared with levels in pre- and perimenopausal women and continued a 1% increase with each additional year of use.

Discussion

This study used three approaches to evaluate homeostasis and inflammation markers in relation to estrogen status, including endogenous hormone concentrations, menopausal

stages, and use of HT. Higher circulating endogenous E2 concentrations suppressed the fibrinolytic factors, PAI-1 and tPA. Additionally, levels of the fibrinolytic factors were somewhat lower among women who subsequently became HT users compared with those who remained non-HT users during the 5-yr study period. There was no indication that stages of the menopausal transition were significantly associated with the inflammation or hemostatic profiles; however, HT use was associated with higher hsCRP concentrations.

This is, to our knowledge, the first published longitudinal study of endogenous E2 concentrations in relation to the fibrinolytic markers, especially PAI-1, and the findings are consistent with the limited literature data. Cell culture systems have been used to demonstrate that 17β-estradiol inhibits the synthesis of PAI-1 in endothelial cells (14), and there have been reports of potential estrogen (and glucocorticoid-progesterone) response elements in genes coding for tPA and PAI-1 (15). The lower PAI-1 levels associated with subsequent HT use in this study are consistent with the lower PAI-1 concentrations previously reported (16–20). Scarabin *et al.* (21) reported significantly lower PAI-1 levels with oral, but not transdermal, E2 replacement therapy in healthy postmenopausal women. These lower levels may be related to increased clearance, an interpretation provided by studies of oral contraceptive preparations in which ethinyl E2 administration led to reductions in urinary plasminogen activator, tPA, and PAI-1 (22). Importantly, the magnitude of differences with HT use was greater than that of differences in endogenous hormone levels.

We observed no significant association of endogenous E2 concentrations with factor VII-c concentrations, nor did we identify a difference in women who transitioned to postmenopause status compared with the premenopausal state.

TABLE 4. Individual longitudinal models of endogenous E2 or FSH in relation to five hemostatic factors, adjusted for covariates

	log _e E2 β (P value)		log _e FSH β (P value)	
	Main effect	Interaction with age	Main effect	Interaction with age
log _e hsCRP	NS		-0.063 (<0.0001)	
log _e Fibrinogen	NS		-0.008 (<0.002)	0.003 (0.0001)
log _e Factor VIIc	NS		0.0167 (<0.0001)	0.004 (0.0002)
log _e t(PA)	-0.021 (<0.0001)	0.006 (<0.0001)	NS	
log _e PAI-1	-0.07 (<0.0001)	NS	0.037 (0.0005)	

Final reduced models after evaluation with age as the time marker, a quadratic term with age, interaction terms with age × hormone, and the quadratic terms, as well as covariates. NS, Not significant.

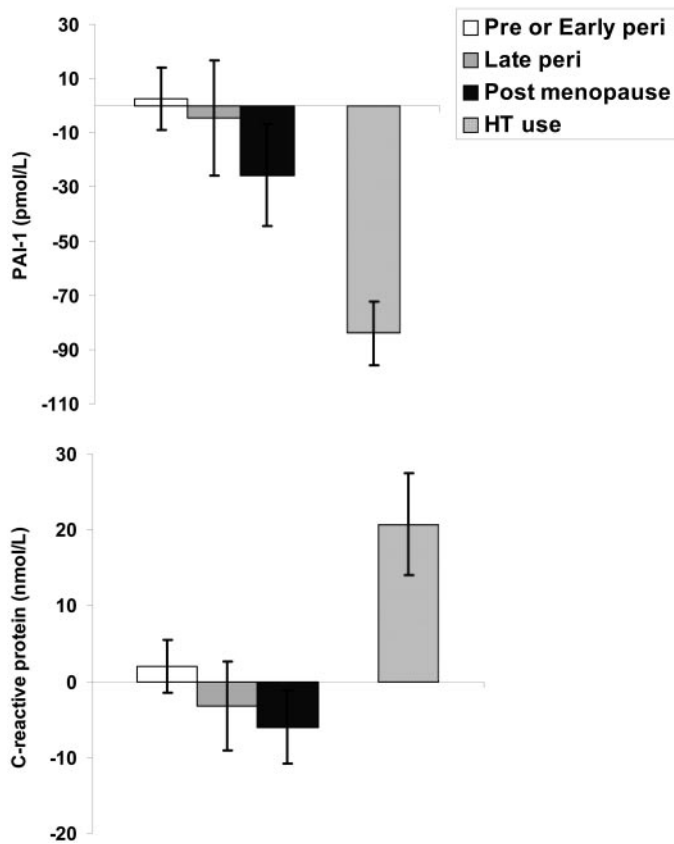


FIG. 1. Comparisons are based upon observations made at all follow-up visits; error bars are associated with the pairwise comparisons of means of each status group with premenopausal or early perimenopausal women. HLS, Mean differences in PAI-1 and hsCRP, according to menopause status or HT use from longitudinal models reported in Table 3.

This later observation is in contradiction to the findings of Scarabin *et al.* (23) and other studies (24–27) that show higher mean levels of factor VII-c and factor VII-a in postmenopausal women compared with premenopausal women. However, our longitudinal study compared the same women as they moved through the transitional states, which may lead to different conclusions than when comparing two different groups with differing menopausal states, who may have underlying intrinsic differences that account for the variation in factor VII-c values.

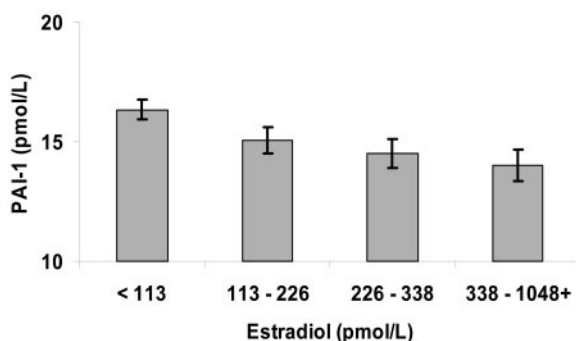


FIG. 2. E2 and PAI-1 from longitudinal models reported in Table 4 and in SI units.

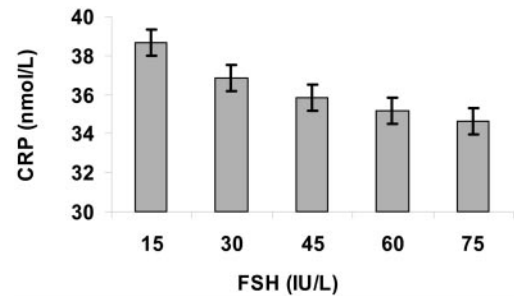


FIG. 3. FSH and hsCRP (in SI units) from longitudinal models (from Table 4).

Baseline hemostatic factor profiles of subsequent HT users differed slightly, but importantly, from the profiles of endogenous hormone levels. Thus, HT use may prove inappropriate as a model for endogenous hormone concentrations among women in midlife. Although adoption of HT use was associated with suppressed PAI-1 concentrations, hsCRP concentrations were markedly higher among those adopting HT use. Studies of both oral contraceptives and HT products have reported selective elevations of CRP (28, 29), suggesting that estrogens may have a direct effect on the liver and increase CRP levels. Other studies have noted that this elevated hsCRP response to HT may be limited to a specific set of acute phase reactants and may not occur with other acute phase proteins, such as serum amyloid A (30).

Higher FSH concentrations were associated with higher factor VII-c and tPA levels and were negatively associated with hsCRP and fibrinogen concentrations. Although there is little research to guide the interpretation of these findings, there are a number of possible explanations. First, these findings may reflect the aging process. Alternatively, FSH and IGF-I have been reported to induce the accumulation of low-density lipoprotein receptor mRNA in granulosa cells (31); hence, FSH has been shown to have the potential for genomic activity that could be associated with an inflammatory response. Thus, the FSH relationships may reflect an under-evaluated physiological response, but currently there is no ready explanation.

This study includes strengths and limitations. The study incorporates the direct measurement of endogenous hormones annually, assessment of menopause status annually, and adoption of exogenous hormone replacement use. This study involves a large sample being followed across time in the age range where the marked gender differences in atherosclerosis and coronary artery disease have been documented (3). Furthermore, members of the group were either premenopausal or early perimenopausal (based on menstrual bleeding definitions) and were not using HT at study onset, helping to establish the temporality of events. Despite these marked strengths, there are limitations. Obviously, the age and size of the population are insufficient to generate hard cardiovascular end points. Those outcomes will only become available after long-term follow-up of this cohort. It is recognized that the endogenous hormones described in this study are limited in their ability to represent the bioavailable fraction of the hormone that can potentially cross cell membranes and bind to nuclear steroid receptors (32),

and that annual specimen collection, even when timed to a day of the cycle window and a time of day, must be interpreted with caution given the cyclic and pulsatile nature of circulating endogenous hormones.

In summary, the roles of endogenous hormones and the hemostatic risk factors for heart disease in women have not been adequately evaluated. Most studies have not assessed endogenous estrogens in relation to hemostatic factors, but focused instead on oral contraceptive use, HT use, or cross-sectional comparisons of pre- vs. postmenopausal women. We found that lower endogenous E2 levels were associated with higher levels of PAI-1 and tPA, consistent with a mechanism of greater clearance of fibrinolytic factors with higher endogenous E2 levels. There were no significant associations of E2 and fibrinogen, factor VII-c, or hsCRP. Menopause status, defined by regularity and frequency of menstrual bleeding in the time interval between annual examinations, was not associated with hemostatic risk factors. Finally, HT use was not a good proxy for the associations of endogenous hormone concentrations and hemostatic factors among premenopausal women or transitioning women. Depending upon the formulation, HT may reduce CVD risk from the fibrinolytic component but increase risk in the inflammatory component (hsCRP).

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Address all correspondence and requests for reprints to: Dr. Mary-Fran R. Sowers, Department of Epidemiology, University of Michigan School of Public Health, 339 East Liberty Street, Suite 310, Ann Arbor, Michigan 48104. E-mail: mfsowers@umich.edu.

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References

1. Grady D, Wenger NK, Herrington D, Khan S, Furberg C, Hunninghake D, Vittinghoff E, Hulley S 2000 Postmenopausal hormone therapy increases risk for venous thromboembolic disease. The Heart and Estrogen/Progestin Replacement Study. *Ann Intern Med* 132:689–696
2. Writing Group for the Women's Health Initiative Investigators 2002 Risks

and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative Randomized Controlled Trial. *JAMA* 288:321–333

3. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A 1994 Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation* 90:583–612
4. Mendelsohn ME, Karas RH 1999 The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 340:1801–1812
5. Vargas R, Wroblewska B, Rego A, Hatch J, Ramwell PW 1996 Oestradiol inhibits smooth muscle cell proliferation of pig coronary artery. *Br J Pharmacol* 109:612–617
6. Farhat MY, Lavigne MC, Ramwell PW 1996 The vascular protective effects of estrogen. *FASEB J* 10:615–624
7. Teede HJ, McGrath BP, Smolich JJ, Malan E, Kotsopoulos D, Liang YL, Peverill RE 2000 Postmenopausal hormone replacement therapy increases coagulation activity and fibrinolysis. *Arterioscler Thromb Vasc Biol* 20:1404–1409
8. Gebara OC, Mittleman MA, Sutherland P, Lipinska I, Matheny T, Xu P, Welty FK, Wilson PW, Levy D, Muller JE, Tofler GH 1995 Association between increased estrogen status and increased fibrinolytic potential in the Framingham Offspring Study. *Circulation* 91:1952–1958
9. Grancha S, Estelles A, Tormo G, Falco C, Gilabert J, Espana F, Cano A, Segui R, Aznar J 1999 Plasminogen activator inhibitor-1 (PAI-1) promoter 4G/5G genotype and increased PAI-1 circulating levels in postmenopausal women with coronary artery disease. *Thromb Haemostasis* 81:516–521
10. Sowers MF, Derby C, Jannausch ML, Torrens JJ, Pasternak R 2003 Insulin resistance, hemostatic factors, and hormone interactions in pre- and perimenopausal women: SWAN. *J Clin Endocrinol Metab* 88:4904–4910
11. Sowers MF, Crawford S, Sternfeld B, Morgenstein D, Gold E, Greendale G, Evans D, Neer R, Matthews K, Sherman S, Lo A, Weiss G, Kelsey J 2000 Design, survey sampling and recruitment methods of SWAN: a multi-center, multi-ethnic, community-based cohort study of women and the menopausal transition. In: Lobo RA, Kelsey J, Marcus R, eds. *Menopause: biology and pathobiology*. New York: Academic Press; 175–188
12. Myers GL, Cooper GR, Winn CL, Smith SJ 1989 The Centers for Disease Control-National Heart, Lung and Blood Institute lipid standardization program. An approach to accurate and precise lipid measurements. *Clin Lab Med* 9:105–135
13. Duan N 1983 Smearing estimate: a nonparametric retransformation method. *J Am Stat Assoc* 78:605–610
14. Seeger H, Wallwiener D, Mueck AO 2001 Lipid-independent effects of an estrogen-statin combination: inhibition of expression of adhesion molecules and plasminogen activator inhibitor-1 in human endothelial cell cultures. *Climacteric* 4:209–214
15. Kooistra T, Bosma PJ, Jespersen J, Klufft C 1990 Studies on the mechanism of action of oral contraceptives with regard to fibrinolytic variables. *Am J Obstet Gynecol* 163:404–413
16. Nabulsi AA, Folsom AR, White A, Patsch W, Heiss G, Wu KK, Szklo M 1993 Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. The Atherosclerosis Risk in Communities Study Investigators. *N Engl J Med* 328:1069–1075
17. The Writing Group for the PEPI Trial 1995 Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. *JAMA* 273:199–208
18. Grodstein F, Stampfer MJ, Manson JE, Colditz GA, Willett WC, Rosner B, Speizer FE, Hennekens CH 1996 Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. *N Engl J Med* 335:453–461
19. Gilabert J, Estelles A, Cano A, Espana F, Barrachina R, Grancha S, Aznar J, Tortajada M 1995 The effect of estrogen replacement therapy with or without progestogen on the fibrinolytic system and coagulation inhibitors in postmenopausal status. *Am J Obstet Gynecol* 173:1849–1854
20. Meade TW 1994 Haemostatic function and arterial disease. *Br Med Bull* 50:755–775
21. Scarabin PY, Alhenc-Gelas M, Plu-Bureau G, Taisne P, Agher R, Aiach M 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
22. Klufft C 1993 Fibrinolysis risk markers for cardiovascular disease. *Gynecol Endocrinol* 7(Suppl):45–53
23. Scarabin PY, Vissac AM, Kirzin JM, Bourgeat P, Amiral J, Agher R, Guize L 1996 Population correlates of coagulation factor VII. Importance of age, sex, and menopausal status as determinants of activated factor VII. *Arterioscler Thromb Vasc Biol* 16:1170–1176
24. Balleisen L, Bailey J, Epping PH, Schulte H, Van de Loo J 1985 Epidemiological study on factor VII, factor VIII and fibrinogen in an industrial population, I: baseline data on the relation to age, gender, body weight, smoking, alcohol, pill using and menopause. *Thromb Haemostasis* 54:475–479

25. Folsom AR, Wu KK, Davis CE, Conlan MG, Sorlie PD, Szklo M 1991 Population correlates of plasma fibrinogen and factor VII, putative cardiovascular risk factors. *Atherosclerosis* 91:191–205
26. Meade TW, Imeson JD, Haines AP, Stirling Y, Thompson SG 1986 Menopausal status and haemostatic variables. *Lancet* 1:22–24
27. Scarabin PY, Bonithon-Kopp C, Bara L, Malmejac A, Guize L, Samama M 1990 Factor VII activation and menopausal status. *Thromb Res* 57:227–234
28. Kluff C, Gevers Leuvena JA, Helmerhorstb FM, Kransc HMJ 2002 Pro-inflammatory effects of oestrogens during use of oral contraceptives and hormone replacement treatment. *Vasc Pharmacol* 39:149–154
29. Cushman M, Legault C, Barrett-Connor E, Stefanick ML, Kessler C, Judd HL, Sakkinen PA, Tracy RP 1999 Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. *Circulation* 100:717–722
30. van Baal WM, Kenemans P, van der Mooren MJ, Kessel H, Emeis JJ, Stehouwer CD 1999 Increased C-reactive protein levels during short-term hormone replacement therapy in healthy postmenopausal women. *Thromb Haemost* 81:925–928
31. LaVoie HA, Garmey JC, Day RN, Veldhuis JD 1999 Concerted regulation of low density lipoprotein receptor gene expression by follicle-stimulating hormone and insulin-like growth factor I in porcine granulosa cells: promoter activation, messenger ribonucleic acid stability, and sterol feedback. *Endocrinology* 140:178–186
32. Pardridge WM 1986 Serum bioavailability of sex steroid hormones. *Clin Endocrinol Metab* 15:259–278

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