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Authors

Langer, Robert D
Pradhan, Aruna D
Lewis, Cora E
et al.

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Baseline associations between postmenopausal hormone therapy and inflammatory, haemostatic, and lipid biomarkers of coronary heart disease

The Women's Health Initiative Observational Study

Robert D. Langer[1], Aruna D. Pradhan[2], Cora E. Lewis[3], JoAnn E. Manson[4], Jacques E. Rossouw[5], Susan L. Hendrix[6], Andrea Z. LaCroix[7], Paul M. Ridker[4]

[1]Department of Family and Preventive Medicine, University of California, San Diego, La Jolla, California, USA
[2]Brigham and Women's Hospital, Department of Cardiology, Boston, Massachusetts, USA
[3] Division of Preventive Medicine, University of Alabama at Birmingham, Medical Towers, Birmingham, Alabama, USA
[4]Department of Medicine, Brigham and Women's Hospital, Division of Preventive Medicine, Boston, Massachusetts, USA
[5]National Heart Lung and Blood Institute, Rockledge Center, Bethesda, Maryland, USA
[6]Wayne State University, Hutzel Women's Hospital, Detroit, Michigan, USA
[7]Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

Summary

Clinical trials of postmenopausal hormone therapy (PHT) have found an early increase in cardiovascular events, and have not demonstrated the reduction in coronary heart disease (CHD) predicted from changes in conventional risk factors or found in observational studies, suggesting that PHT may increase coronary risk through other pathways. We compared baseline levels of C-reactive protein (CRP), interleukin-6 (IL-6), sICAM-1, tissue plasminogen activator antigen (tPA-antigen), D-dimer, homocysteine, triglycerides, total-, HDL- and LDL- cholesterol in 304 cases with incident CHD and 304 controls, according to self-reported use of PHT. Subjects were selected from the 75,343 participants in the WHI Observational Study without baseline cardiovascular disease or cancer. PHT was associated with higher CRP, HDL and triglycerides, and lower tPA-antigen and homocysteine. CRP was highest in users of unopposed conjugated equine estrogen. Levels of IL-6, sICAM-1, D-dimer and total cholesterol did not differ between PHT users and non-users. Transdermal estrogen users had low levels of D-dimer and CRP. Among users of estrogen plus progestin (EP), CRP, IL-6, tPA-antigen, D-dimer, total cholesterol and triglycerides were higher in women with incident coronary events than controls. Estrogen alone (E) controls shared only the tPA-antigen association, but had higher HDL and lower LDL than E cases. In non-users CRP, tPA-antigen and D-dimer were associated with incident CHD. In summary, risk markers differed by PHT category. Some associations differed between women with and without incident CHD, especially for EP, where inflammatory and thrombotic markers were higher in cases. These associations remain speculative pending confirmation in randomized trials.

Keywords

Postmenopausal hormone therapy, estrogens, coronary heart disease, inflammatory factors, haemostatic factors

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Introduction

The findings of an early increase in coronary events and no cardiovascular benefit after about 5 years of treatment in the Women's Health Initiative trial of Estrogen plus Progestin (WHI E+P trial) (1), which enrolled women in the age range 50–79 (mean 63) years with otherwise average cardiovascular risk, and a similar result in the Heart and Estrogen Replacement Study (HERS) (2) which enrolled women with known coronary disease, have not substantiated the expectation from observational studies that postmenopausal hormone therapy (PHT) reduces the risk of coronary heart disease (CHD). Most of the events occurred relatively soon after treatment was initiated. The WHI CEE-alone trial also found no benefit for CHD, but without a dif-

Correspondence to:
Robert D. Langer
Department of Family and Preventive Medicine
University of California, San Diego
9500 Gilman Drive, MC 0978
La Jolla, CA 92093–0978, USA
Tel.: + 1 858 622–5781, Fax: + 1 858 622–5777
E-mail: rdlanger@ucsd.edu

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ference in early events (3). The lack of atheroma reduction in several secondary prevention trials including the Estrogen Replacement and Atherosclerosis (ERA) (4) study, the Well-Hart trial (5) and the WAVE trial (6) have also created doubts regarding the hypothesized underlying mechanisms of CHD protection from hormone therapy.

The expectation of benefit, derived from observational data that largely represented the use of unopposed estrogen in recently postmenopausal women, was also supported by clinical trials such as the Postmenopausal Estrogen Progestin Interventions (PEPI) study that demonstrated improvements in certain conventional cardiovascular risk factors, including LDL- cholesterol and HDL-cholesterol (7), the latter of which may be of particular importance in women (8, 9). The WHI E+P trial, HERS and Well-Hart trials found modest improvements in lipids but these changes were not associated with protection from CHD events (1, 2, 5).

An unexpected finding from both the WHI E+P and HERS trials was an excess of events in the active treatment group that was greatest in the first year when coronary events were increased by 78% and 52%, respectively. This excess declined sharply in the second year in both studies, and there was no substantial early harm in the CEE-alone arm of the WHI (1, 3). The early hazard in the HERS secondary prevention trial was attributed in part to the possibility that women who had substantial atheroma when PHT was initiated were at risk for early plaque rupture. The WHI E+P trial, conducted in an average risk population with a mean age of 63, also found an early excess of events that was unrelated to known CHD when treatment was begun, although a proportion of women in this age range would be expected to have sub-clinical complicated plaques and the hazard increased directly with time since menopause (1, 11, 12).

The observation of an early excess in CHD events in both average and high risk women, despite changes in some conventional risk factors which should convey protection, suggests that this early risk may involve pathways besides those associated with the conventional markers currently assessed in routine screening. Candidate mechanisms meeting these criteria include haemostatic and inflammatory processes, and changes in oxidative markers like homocysteine (13).

Pharmacologic interventions including PHT may modify the risks of coronary artery disease ascribed to tPA (14–18). These risks may be further modified by progestins used in combined hormone preparations. Results from several clinical trials suggest that PHT is associated with increases in pro-thrombotic effects (19–24). Data regarding associations between D-dimer and PHT are limited, primarily due to small sample size or relatively short duration of treatment (20, 22, 25, 26). These investigations found non-significant but graded downward trends in plasma D-dimer levels with increasing duration of PHT use, suggesting reduced fibrinolytic activity.

Menopause and estrogen status have a variety of effects on inflammatory markers (16, 27, 31), including increases in serum C-reactive protein (CRP) levels and decreases in fibrinogen and Intereukin-6 (IL-6) (28), underscoring the complexity of the inflammatory mechanisms affected by PHT use. Soluble intercellular adhesion molecule type 1 (sICAM-1) was associated with an increased risk of cardiovascular disease in women (13), although information on its relation to PHT use is sparse. Furthermore, PHT may modulate the positive relationship between obesity and inflammatory cytokine production (30).

Both estrogen alone, and estrogen plus progestin preparations have been associated with diminished plasma homocysteine levels, particularly in those with the highest baseline levels (27, 33, 34). However, small numbers of postmenopausal women and short duration of treatment limit the utility of these studies, and there is currently no evidence for the expected reductions in cardiovascular events.

In summary, there is a clear need to better understand the associations between PHT and inflammatory, haemostatic and oxidative markers of CHD risk. The Women's Health Initiative, a large prospective study of post-menopausal women, includes a non-intervention component (the WHI Observational Study). Data collected as part of a case-control study in this population provided the opportunity to compare levels of a variety of risk factors at the baseline visit, according to current PHT use, in women with incident CHD after the baseline examination, and matched controls without incident coronary events.

Methods

Study population

The Women's Health Initiative (WHI) is a large program including both a prospective Observational Study (WHI-OS) and three overlapping clinical trials. WHI-OS participants are not receiving interventions in the study, and their use of treatments generally available to U.S. women was unrestricted (35).

The objectives and design of the WHI-OS have been described elsewhere (35). Briefly, the WHI-OS is an ongoing nationwide prospective cohort study of post-menopausal women with a racial/ethnic distribution reflecting that of U.S. women of the same age. It is designed to examine the associations between a variety of clinical, socioeconomic, behavioral, and dietary risk factors and the incidence of several health outcomes including CHD. Between 1994 and 1998, the WHI-OS enrolled 93,724 women aged 50–79 at forty clinical centers throughout the United States. Women were eligible if they were postmenopausal, unlikely to change residence or die within three years, not enrolled in the WHI Clinical Trial, and not participating in any other clinical trial. At baseline, women completed questionnaires assessing personal and family health history, health-related behaviors, and social/environmental factors. Basic physical measurements were taken and they provided fasting plasma and serum for future use. Fasting was defined as no food or beverage intake except water in the 12-hour period prior to blood collection. Blood samples were processed and placed in long-term storage at –70º C. The study was approved by Institutional Review Boards at each participating institution, and informed consent was provided by all women enrolled.

The population for this cross-sectional survey was comprised of both cases and controls derived from a prospective nested case-control study of the relationship between markers of inflammation and thrombosis and incident coronary disease. We studied cases and controls selected from the 75,343 participants who were free of cardiovascular disease and cancer at baseline.

Case subjects were WHI-OS participants who developed a first coronary event after baseline. Control subjects were selected from women who remained free of coronary disease. Controls were 1:1 matched to cases by age (within 1 year), smoking status (non-smoker, former smoker, or current smoker), ethnicity (Non-Hispanic White, Black, Hispanic, American Indian/Alaskan Native, Asian/Pacific Islander, or not otherwise specified), and follow-up time (6 month intervals). Women with any of the following conditions at baseline were excluded: angina, congestive heart failure, myocardial infarction, coronary angioplasty or bypass surgery, stroke and cancer (except non-melanoma skin-cancer). As of February 2000, 315 cases-control pairs who met these criteria were identified for analysis. Eleven case-control pairs were eliminated due to inadequate blood specimen at time of laboratory analysis, leaving 304 cases matched to 304 controls.

Baseline clinical variables

At the clinic visit height was measured to the nearest 0.1 centimeter and weight to the nearest 0.1 kilogram while participants were dressed in indoor clothes with shoes removed. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. Blood pressure was measured using a conventional mercury sphygmomanometer after the subject had been seated and resting for five minutes. Two measurements each were taken for systolic and diastolic pressure; these were averaged for use in statistical analyses.

History and exposure data were reported on self-administered forms. Race/ethnicity was self-identified as Non-Hispanic White, Black, Hispanic, American Indian/Alaskan Native, Asian/Pacific Islander, or other. Hypertension was defined as history of treated or untreated high blood pressure or measured baseline systolic blood pressure 140 mmHg or diastolic blood pressure 90 mmHg. History of diabetes was defined as self-report of diagnosed diabetes mellitus. A positive family history of premature coronary artery disease was defined by self-report of myocardial infarction in a first-degree male relative prior to the age of 55 or first-degree female relative prior to the age of 65. Unknown family history was coded for those participants unsure of family history of MI or age at presentation. Smoking status (non-smoker, former smoker, or current smoker) was determined from lifetime smoking of at least 100 cigarettes, current daily cigarette smoking, and self-report of smoking cessation. Physical activity was quantified by episodes per week of strenuous recreational physical activity. Alcohol consumption was computed from a food frequency questionnaire and categorized according to number of alcoholic beverages per week. History of hysterectomy was determined by self-report.

Classification of use of postmenopausal hormone replacement therapy

A detailed baseline questionnaire was used to characterize exposure to hormone therapy at several levels. PHT use was first classified as never, past, or current use of pills or patches. It was then classified as estrogen alone (E) or estrogen plus progestin (EP). The three categories of current use thus created: non-user, E, and EP were used for the primary comparisons. Subgroups within these categories were also evaluated. For women who used EP, the regimen was classified according to days a progestin was used per month as follows: less than 5 days per month (ineffective), 5 to 12 days per month (cyclic), and 13 or more days per month (continuous). For women who used either E or EP, estrogen type was categorized as conjugated equine estrogen (CEE) or non-CEE estrogen (which was mostly 17-β estradiol). Women who used an unspecified estrogen (N = 5) were excluded from these subgroup analyses. Among current users, duration of current treatment was calculated based upon self-reported duration of the most recent PHT regimen.

Follow-up and ascertainment of first coronary events

As of February 2000, median duration of follow-up for the cohort was 2.9 years. At that time, 2.5% of subjects had withdrawn or were otherwise lost to follow-up. Participants are sent annual medical update forms to report the occurrence of any hospitalization and a wide variety of outcomes including CHD. Confirmation of self-reported non-fatal CHD was based upon medical record review with documentation of new chest pain accompanied by characteristic evolution of electrocardiographic changes or clear evidence of myocyte damage as demonstrated by elevated CK-MB or troponin values. Deaths due to coronary disease were confirmed on the basis of autopsy reports, circumstances of death, electrocardiogram, laboratory test results, and reports from all relevant procedures. In addition, we included cases of sudden cardiac death in which death occurred within one hour of symptom onset in the absence of other potentially lethal non-cardiac disease processes.

Laboratory procedures

Baseline fasting plasma samples were thawed and assayed for high sensitivity C-reactive protein (CRP), interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), tissue plasminogen activator antigen (tPA-antigen), D-dimer, homocysteine, triglycerides, total-, HDL-, and LDL- cholesterol. CRP was measured by a high-sensitivity method on the Hitachi 911 analyzer using reagents from Denka Seiken (Niigata, Japan). IL-6 was measured by a commercially available ELISA (R & D Systems, Minneapolis, MN). sICAM-1 was measured by ELISA (R&D Systems, Minneapolis, MN). tPA-antigen and D-dimer were determined by ELISA (American Diagnostica, Greenwich, CT). Total cholesterol, HDL cholesterol, directly obtained LDL cholesterol levels, and triglycerides were measured on a Hitachi 911 analyzer with reagents from Roche Diagnostics (Indianapolis, IN) and Genzyme Corporation (Cambridge, MA). Homocysteine was measured by a microparticle enzyme immunoassay on the IMx analyzer (Abbott Laboratory, Abbott Park, IL). Samples were analyzed in randomly ordered case-control pairs so as to minimize systematic bias and interassay variation. The coefficients of variation for CRP, IL-6, sICAM-1, tPA-antigen, D-dimer and homocysteine derived from a 5% sample of simultaneously analyzed blinded quality control specimens were 3.3%, 10.1%, 7.4%, 9.9%, 19.9%, and 1.5%, respectively.

Statistical analysis

We used the student's *t* test for two-group comparisons, and analysis of variance (ANOVA) for comparisons of more than two groups, to evaluate differences in means. We used the χ^2 statistic

to evaluate differences in proportions. Spearman correlation coefficients were calculated to assess univariate associations between biomarkers. We used one-way ANOVA to test differences in means of each biomarker in subgroups of women defined by PHT status for all participants, and separately for cases and controls. As the distributions of CRP, IL-6, tPA-antigen, D-dimer, homocysteine and triglycerides were skewed, these variables were natural log-transformed to meet the assumptions of parametric tests, and differences in natural log transformed means were assessed. Medians (inter-quartile range) and geometric means are presented in tables. Multivariate-adjusted mean biomarker levels were computed and subgroup differences tested using the generalized linear models procedure in SAS. Adjusted models included case-control status and variables that differed significantly between categories of PHT use: age (linear), body-mass index (quadratic), history of diabetes, and hysterectomy status. In this exploratory analysis, a two-sided p-value of less than 0.05 was considered statistically significant.

Results

Of the 608 women in the study population, 222 (36.5%) reported that they were taking some form of PHT when they entered the study (Table 1). Nearly twice as many women were taking E (N=146), as were taking EP (N=76). Hormone users, particularly users of EP, were younger than non-users and had lower mean BMI. Nearly 10 percent of women who were not taking PHT reported a history of diabetes compared with 3.4 and 2.6 percent among women on E and EP, respectively. Since the data in Table 1 are organized by hormone use category rather than case-control status, percentages of cases and controls are provided for each category. Non-users were more likely to have had an incident coronary event (54.4 percent) than users of E (42.5 percent) or EP (42.1 percent). Among women on E, 93 percent had undergone hysterectomy and the median duration of estrogen use was 16.5 years. The duration of use was considerably less (median 6.0 years) in women taking EP, 96 percent of whom had their uterus. About two-thirds of the women on EP were using both the estrogen and progestin daily (continuous regimen), 26 percent reported being on a cyclical regimen, and 7 percent were on unknown or ineffective regimens. There were no differences in racial/ethnic composition, history of hypertension, family history of premature CHD, smoking status, physical activity, or alcohol consumption, according to PHT use category.

Correlations between risk markers

Correlations among the inflammatory, haemostatic, oxidative and lipid markers are shown in Table 2. As expected, CRP levels were strongly correlated with IL-6 ($r = 0.52$, $P < 0.001$). In general, modest statistically significant correlations were observed between the inflammatory and haemostatic factors. With the exception of CRP, there were also significant correlations between the inflammatory and haemostatic markers and homocysteine. HDL (inverse) and triglycerides, but not LDL and total cholesterol, were significantly correlated with CRP, IL-6, sICAM-1 and tPA-antigen, although the magnitude of these correlations was modest.

Differences in risk factor levels by PHT use category

Table 3 displays baseline medians (inter-quartile range) and adjusted geometric means (to permit statistical comparisons) for each biomarker according to PHT. CRP was increased in PHT users compared to non-users and highest in women on E; this difference remained after adjustment. Homocysteine levels were uniformly lower in E and EP users compared with non-users.

Table 1: Baseline characteristics of the study population according to postmenopausal hormone therapy use.

Characteristic	Non-Users (n=386, 63.5%)	Estrogen Alone (n=146, 24.0%)	Estrogen plus Progestin (n=76, 12.5%)
Mean age, years (SD)	69.9 (6.1)	68.6 (7.2)†	65.6 (6.6)§
Mean body-mass index , kg/m^2 (SD)	28.0 (5.7)	26.2 (4.9)‡	25.8 (4.6)‡
Race, %			
African American	7.0	5.5	4.0
Hispanic	1.6	2.1	1.3
Non-Hispanic White	86.8	85.6	89.5
Asian/Pacific Islander	2.1	4.8	4.0
American Indian/Alaskan Native	0.5	1.4	0.0
Other	2.1	0.7	1.3
History of hypertension, %	47.2	48.0	35.5
History of high cholesterol requiring treatment, %	16.8	12.3	9.2
History of diabetes, %	9.8	3.4†	2.6†
Family history of premature coronary disease, %			
No	74.1	74.0	79.0
Yes	19.7	21.9	17.1
Unknown	6.2	4.1	4.0
Smoking status, %			
Never	51.8	46.6	44.7
Past	39.4	44.5	46.1
Current	8.8	8.9	9.2
Frequency of strenuous recreational physical activity, %			
None	79.6	70.8	73.3
1–2 episodes per week	9.1	12.5	8.0
3 episodes per week	6.7	12.5	12.0
4 episodes per week	4.6	4.2	6.7
Alcohol consumption, %			
Nondrinker or past drinker	35.8	26.9	18.4
< 1 per month	12.7	16.6	14.5
< 1 per week	16.6	20.0	18.4
1–6 per week	22.3	21.4	30.3
7 per week	12.7	15.2	18.4
Self-Reported History of RA or SLE	4.9	9.6†	7.9
Self Reported History of Any Arthritis	57.4	57.2	56.6
Baseline Use of Anti-Inflammatory Medications and Statins:			
Use of NSAIDs	19.7	21.2	23.7
Use of Corticosteroids	1.6	5.5†	1.3
Use of COX-2 Inhibitors	0	0	0
Statins	8.6	8.2	5.3
History of Hysterectomy, %	31.7	93.2§	4.0§
Median duration of Current Regimen Use, years	NA	16.5¥	6.0¥
Estrogen plus Progestin Regimen, % (n)			
Ineffective			1.3 (1)
Continuous	NA	NA	67.1 (51)
Cyclic			26.3 (20)
Unknown			5.3 (4)
Case:Control Composition, %			
Cases	54.4	42.5†	42.1†
Controls	45.6	57.5	57.9

* Missing Data: Body Mass Index 6; Physical Activity 16; Alcohol Consumption 1, History of Hysterectomy 1.
P-values are for comparisons between each subgroup of users versus non-users. † p<0.05, ‡ p<0.01, ‡ p<0.005, § p<0.001. ¥ p < 0.001 versus each other.

Table 2: Spearman correlation coefficients of inflammatory and haemostatic markers for coronary heart disease.

	CRP	IL-6	sICAM-1	TPA-antigen	D-dimer	HCY	TC	LDL	HDL	TG
CRP	---	0.52§	0.14§	0.19§	0.28§	0.06	0.05	0.02	–0.20§	0.32§
IL-6		---	0.24§	0.36§	0.32§	0.22§	0.00	0.03	–0.30§	0.22§
sICAM-1			---	0.19§	0.08†	0.11‡	0.06	0.07	–0.17§	0.10†
TPA-antigen				---	0.09†	0.19§	0.03	0.09†	–0.33§	0.20§
D-dimer					---	0.17§	0.02	–0.01	–0.05	0.05
HCY						---	0.01	0.07	–0.07	–0.05
TC							---	0.74§	0.06	0.32§
LDL								---	–0.22§	0.17§
HDL									---	–0.47§
TG										---

† p<0.05, ‡ p<0.01, § p<0.001
CRP = C-reactive protein; TC = Total cholesterol; IL-6 = Interleukin-6; LDL = Low density lipoprotein cholesterol; HCY = Homocysteine; HDL = High density lipoprotein cholesterol; sICAM-1 = Soluble intercellular adhesion molecule type;-1; TG = Triglycerides, TPA-antigen = Tissue plasminogen activator-antigen

Table 3: Median and geometric mean baseline values of biomarkers according to postmenopausal hormone therapy use.

Characteristic	Non-Users (n=386, 63.5%)	E (n=146, 24.0%)	EP (n=76, 12.5%)
C-reactive protein (mg/dl)			
Median (IQR)	0.23 (0.10 – 0.52)	0.39 (0.20 – 0.74)	0.29 (0.12 – 0.54)
Adjusted Geometric Mean[1] (SE)	0.22 (0.01)	0.38 (0.03) §	0.27 (0.03)
Adjusted Geometric Mean[2] (SE)	0.21 (0.01)	0.38 (0.04) §	0.33 (0.04) §
Interleukin-6 (pg/ml)			
Median (IQR)	1.75 (1.21 – 2.47)	1.60 (1.17 – 2.62)	1.44 (0.94 – 2.04)
Adjusted Geometric Mean[1] (SE)	1.80 (0.06)	1.81 (0.09)	1.54 (0.11) †
Adjusted Geometric Mean[2] (SE)	1.75 (0.05)	1.82 (0.10)	1.74 (0.12)
sICAM-1 (ng/ml)			
Median (IQR)	276.2 (243.2 – 320.6)	274.7 (242.7 – 320.6)	249.7 (222.6 – 292.0)
Adjusted Geometric Mean[1] (SE)	278.2 (4.0)	283.1 (6.6)	256.9 (8.3) †
Adjusted Geometric Mean[2] (SE)	277.0 (4.2)	286.8 (8.0)	261.3 (9.1)
Tissue Plasminogen Activator (ng/ml)			
Median (IQR)	9.0 (6.3 – 12.5)	6.7 (4.4 – 8.9)	7.0 (4.2 – 9.9)
Adjusted Geometric Mean[1] (SE)	8.5 (0.24)	6.4 (0.29) §	6.7 (0.43) §
Adjusted Geometric Mean[2] (SE)	8.4 (0.23)	6.4 (0.33) §	7.4 (0.48)
D-dimer (ng/ml)			
Median (IQR)	24.8 (15.5 – 42.8)	25.8 (16.1 – 45.3)	25.7 (14.9 – 46.3)
Adjusted Geometric Mean[1] (SE)	25.5 (1.1)	26.4 (1.9)	24.9 (2.5)
Adjusted Geometric Mean[2] (SE)	24.4 (1.1)	27.5 (2.3)	27.9 (3.0)
Homocysteine (uM)			
Median (IQR)	8.4 (6.8 – 10.2)	7.5 (6.3 – 8.9)	7.3 (6.4 – 8.6)
Adjusted Geometric Mean[1] (SE)	8.6 (0.14)	7.6 (0.20) §	7.6 (0.28) ‡
Adjusted Geometric Mean[2] (SE)	8.5 (0.14)	7.8 (0.24) †	7.8 (0.31)
Total Cholesterol (mg/dL)			
Median (IQR)	223.9 (200.9 – 250.5)	222.6 (193.6 – 246.8)	227.5 (204.5 – 249.3)
Adjusted Geometric Mean[1] (SE)	223.0 (1.9)	220.1 (3.1)	224.2 (4.4)
Adjusted Geometric Mean[2] (SE)	223.4 (2.0)	218.0 (3.6)	227.5 (4.7)
LDL Cholesterol (mg/dL)			
Median (IQR)	128.8 (111.1 – 149.3)	113.0 (98.3 – 139.3)	125.6 (108.6 – 146.2)
Adjusted Geometric Mean[1] (SE)	125.9 (1.7)	114.2 (2.6) §	123.8 (3.9)
Adjusted Geometric Mean[2] (SE)	126.1 (1.8)	112.8 (3.0) §	126.1 (4.2)
HDL Cholesterol (mg/dL)			
Median (IQR)	55.7 (46.7 – 68.5)	66.5 (53.4 – 76.9)	60.2 (53.1 – 73.3)
Adjusted Geometric Mean[1] (SE)	56.7 (0.7)	63.7 (1.4) §	61.9 (1.9) ‡
Adjusted Geometric Mean[2] (SE)	57.1 (0.8)	62.7 (1.5) ‡	61.4 (1.9) †
Triglycerides (mg/dL)			
Median (IQR)	136.5 (94.9 – 188.5)	169.0 (126.1 – 213.2)	145.6 (113.8 – 209.3)
Adjusted Geometric Mean[1] (SE)	136.8 (3.4)	166.1 (6.7) §	149.7 (8.3)
Adjusted Geometric Mean[2] (SE)	135.2 (3.4)	166.1 (7.7) §	159.3 (9.2) ‡

HT – Hormone Therapy; E – estrogen alone; EP – estrogen plus progestin.
P-values are shown for comparisons between subgroups of users versus non-users: † p<0.05, ‡ p<0.01, ‡ p<0.005, §p<0.001
(1) Adjusted for case status.(2) Adjusted for age, body-mass index, diabetes, history of hysterectomy, and case status.

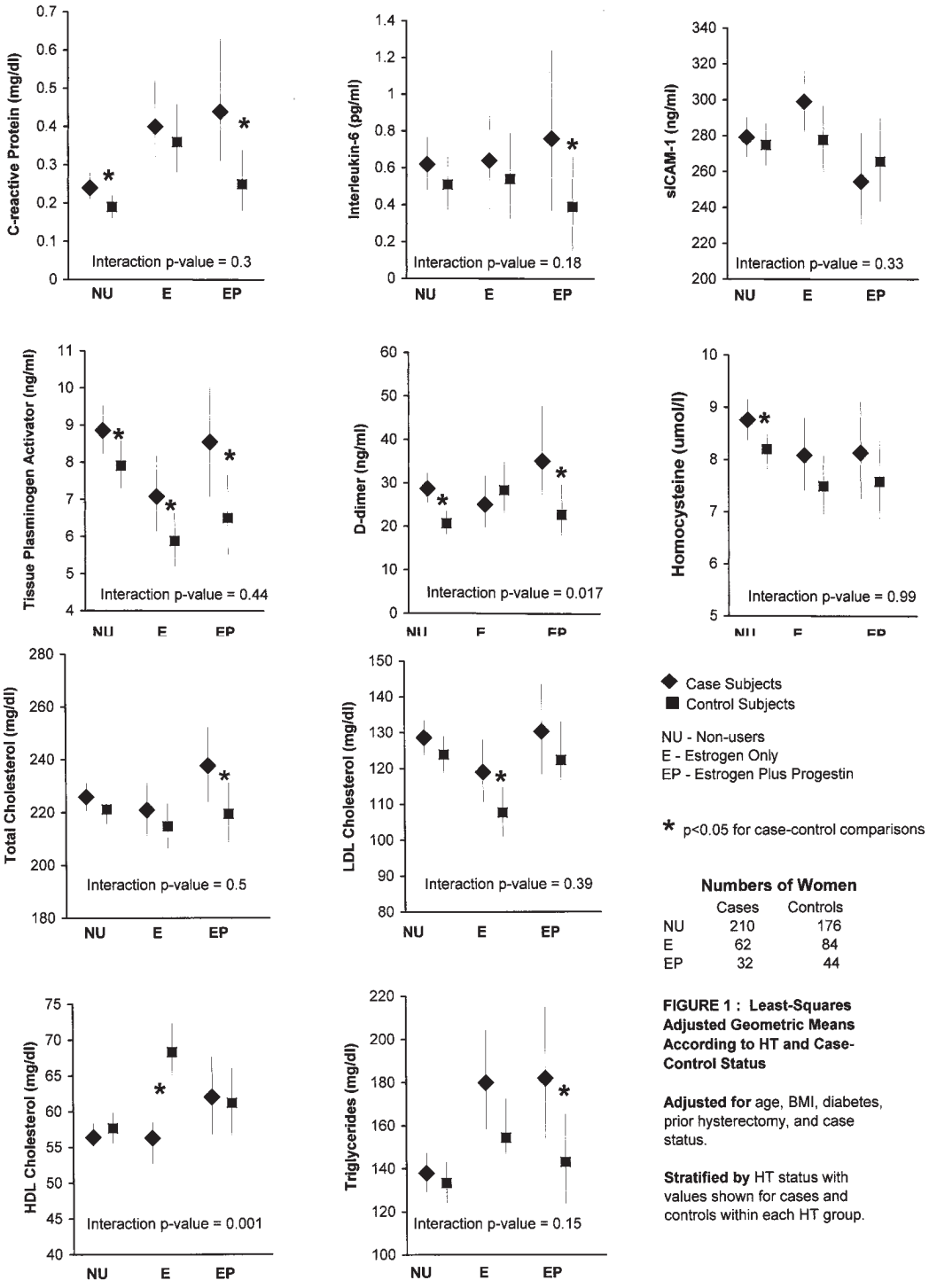

Figure 1: Least. squares adjusted geometric means for risk factors according to HT use and case-control status.

tPA-antigen was lowest in E users, and this level was significantly different from non-users. IL-6 and sICAM-1 were lower in users of EP, but not E, compared to non-users; but these differences were eliminated by adjustment in multivariate models. D-dimer levels did not differ between PHT users and non-users. HDL and triglycerides were higher in PHT users with the strongest association in users of E alone. LDL was lower in users of E, but not EP, compared with non-users. Total cholesterol did not differ between groups.

Risk marker levels by PHT use category and case-control status

We performed exploratory analyses of the interactions between these biomarkers at baseline, the form of PHT use, and case-con-

trol status following 2.9 years of follow-up using general linear models (Fig. 1). The numbers of cases and controls among non-PHT users, E users and EP users were, respectively, 210 and 176, 62 and 84, and 32 and 44. Interactions were present for D-dimer and HDL cholesterol. D-dimer levels were higher in cases than controls among non-users and users of EP, but did not differ in E users. The HDL interaction was related to a strong case-control difference in users of E with no difference in the other two use categories. tPA-antigen was higher in women who had an incident CHD event in all three PHT use categories. Cases who used EP also had significantly higher pre-event levels of CRP, IL-6, total cholesterol and triglycerides than women who also took EP but did not have a CHD event. In users of E, HDL was significantly lower and LDL higher in cases than controls; these lipids did not differ by case-control status in non-users or users of EP. There were significant case-control differences in CRP levels among non-users and users of EP. Among E users there was no difference in CRP level between cases and controls ($p = 0.50$). Homocysteine was significantly associated with CHD events only in non-users.

Risk marker levels by sub-type of PHT used

Approximately 75 percent of participants on PHT (168 of 222) used oral conjugated equine estrogen (CEE) in either E or EP regimens. Of these, 119 women took E and 49 took EP. Another 41 women (18%) used a different oral estrogen, mainly 17-β estradiol; of these 23 used E and 18 used EP. Eight women used transdermal estrogen.

Use of oral CEE alone or in combination was associated with the highest levels of CRP. An apparent lower level in users of CEE with a progestin compared to users of CEE alone was eliminated by adjustment for the other factors that differed between groups including age, BMI, diabetes, use of steroid medications and hysterectomy status. In fully adjusted models, CRP levels were significantly greater in users of any CEE regimen than in non-users ($p < 0.001$), while levels in users of non-CEE regimens did not differ from those in women who did not take PHT. Users of transdermal estrogen had CRP levels similar to non-users, and lower than uses of any other form of PHT (all $p \leq 0.04$). Levels of sICAM-1 were lower in women who used CEE plus a progestogen compared with users of CEE alone ($p = 0.03$). tPA-antigen levels were lower in women who used CEE alone ($p < 0.001$) or in a combination regimen ($p = 0.04$) compared with non-users. D-dimer levels were significantly lower in users of transdermal estrogen compared with non-users and users of all other treatments. Homocysteine was lower only in users of CEE alone compared with non-users ($p = 0.01$).

Discussion

In this cross-sectional study, CRP, tPA-antigen and homocysteine levels differed in users of PHT compared with women who did not take PHT. CRP was increased in women on PHT, while tPA-antigen and homocysteine levels were lower in PHT users than in non-users. Lipid levels also differed for HDL and triglycerides, which were higher, and LDL which was lower, in PHT users. Some associations differed in users of EP compared to E alone, and in users of CEE compared with other estrogens. Exploratory analyses suggested case-control differences in some risk factors that were not evident in comparisons by PHT use category.

CRP levels were higher in women who took CEE compared to women who used other estrogens and non-users. Elevations of CRP associated with PHT use have been demonstrated in two other cohorts. Cushman et al. (29) found that this effect was modified by BMI such that CRP was higher with increasing BMI in EP users, but there was no interaction between BMI and CRP for users of E. The disappearance of a progestin-associated difference in CRP levels among users of CEE alone with adjustment for factors including BMI in our study is consistent with this. In the second cohort, baseline CRP levels in women without known disease were associated with subsequent cardiovascular events (31, 36), but an interaction between PHT use, CRP and cardiovascular events was not assessed. We found a positive association between CRP level and case status in both non-users and users of PHT in analyses that were adjusted for BMI, age, diabetes and hysterectomy. In our population CRP levels were higher in women who used CEE than in those taking other formulations, yet there was no case-control difference in CRP among women taking CEE. A small clinical trial of oral estradiol demonstrated an increase in CRP (37), while a brief clinical trial in 19 women suggested that this CRP increase may not occur with transdermal estradiol (38). Our findings for transdermal estrogen are consistent with the latter. The possibility that CEE and non-CEE PHT differ in the degree of CRP elevation is interesting and warrants further investigation given the possibility that CRP may predict coronary events (13, 36).

Other studies have shown both lower (28) and higher (39) levels of IL-6 in PHT users. There was no significant difference between PHT users and non-users in the present study. However, there was a significant case-control difference in IL-6 restricted to women taking EP. The lack of overall difference in IL-6 is also interesting in light of the differences found in CRP since IL-6 regulates CRP production. This observation suggests that CRP levels are influenced by other factors, some perhaps mediated by specific components of PHT.

We found no overall difference in D-dimer levels by PHT use category. Relatively brief clinical trials of PHT have found increased D-dimer levels (40–43), while studies in long-term users have generally not found differences or suggested a decline (25, 30). Our study reflects long-term use and is concordant with the latter. However, despite the overall lack of difference in D-dimer levels between PHT users and non-users in the present study, higher levels of D-dimer were associated with case status both in women who used EP and in non-users.

Short-term clinical trials of PHT have demonstrated reduced levels of sICAM-1 (44, 45). In the present study there were no overall differences between PHT use groups in levels of sICAM-1 although levels were low in women who used CEE with P. A categorical difference was masked by much higher levels in users of non-CEE with P. The reason for this discrepancy is not clear.

The accurate assessment of tPA and PAI-1 activity requires the use of non-standard anticoagulants in the collection process, broad-based epidemiologic studies like ours are unable to com-

prehensively evaluate these issues. Therefore we measured tPA-antigen as the closest surrogate. Population studies and clinical trials have demonstrated lower tPA-antigen levels in PHT users (23, 46, 47). We also found lower tPA-antigen levels in women taking PHT than in non-users, with no difference between users of E and EP. However, there was a significant case-control difference in tPA-antigen in both categories of PHT use which was supported by a non-significant test for interaction by PHT type.

Several clinical trials have shown that PHT reduces circulating homocysteine (33, 48, 49) although one suggested that this benefit declines with time and may be stronger with E than EP (32). Our results in long-term users suggest that this difference is similar in both subgroups of PHT and that it is sustained. Among non-users of PHT, homocysteine was significantly higher in cases than controls, and the trend was similar in the other two groups.

Our findings for lipid fractions largely mirror those of clinical trials that have evaluated CEE-based PHT including the PEPI trial which included CEE alone and three different CEE plus P groups (6). In PEPI, PHT increased HDL and triglycerides and decreased LDL but the HDL effect was smaller for CEE plus MPA than for CEE alone or CEE with micronized progesterone. Our findings differ from PEPI in that EP was not associated with lower LDL in the present analysis, although E was. This may be due to the relatively small number of women in the EP use group. As expected, cases had higher LDL levels in all PHT use groups but this effect was only significant in users of E. Higher HDL was associated with control status (no incident CHD) only in users of E. Both E and EP were associated with higher triglyceride levels and higher levels predicted case status in EP with a similar but non-significant trend in E users.

These results suggest that the progestin may modify some estrogen effects on inflammatory and haemostatic pathways. Taken together with other evidence that progestins negatively influence endothelial function (50) and classical risk factors such as lipids and glucose (7) these findings suggest a need for additional study of the role of progestins in the CHD risks found in recent clinical trials (1, 2) that were not confirmed with CEE-alone (3). The limited use of progestogens other than MPA in this population precludes the evaluation of differences between compounds on these pathways. Differences between progestogen compounds have been shown for some metabolic risk factors (7), so this possibility warrants evaluation.

Conclusion

Differences between PHT users and non-users were unfavorable for CRP and triglycerides, and favorable for tPA-antigen, homocysteine and HDL. Levels of IL-6, sICAM-1, D-dimer, total cholesterol and LDL were not different. The finding of substantially lower levels of sICAM-1 only in users of CEE with P is surprising, especially since levels were much higher in users of CEE alone, and users of non-CEE EP.

Exploratory analyses suggested that there were significant differences between cases and controls that did not conform to the relationships across PHT categories. Among EP users, 6 of the 10 markers differed by case-control status (inflammatory markers CRP and IL-6, thrombotic markers tPA-antigen and D-dimer, and lipid fractions total cholesterol and triglycerides), while for women who used E only 3 of the 10 markers differed between cases and controls (coagulation factor tPA-antigen, and lipid fractions HDL and LDL). It is intriguing that only one of the 10 factors, tPA-antigen, had the same case-control association for both E and EP. These case-control differences suggest that the pathways underlying CHD risk may not be the same for E and EP. This is consistent with recent clinical trial results showing no CHD harm for CEE-alone in contrast with the increased hazard found for EP. Since our data on pathways came from a non-experimental study we cannot infer causality. These and related markers should be explored in appropriate randomized trials.

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