Inflammatory Biomarkers, 
Hormone Replacement Therapy, 
and Incident Coronary Heart Disease 
Prospective Analysis From the 
Women's Health Initiative Observational Study

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Several studies indicate that oral postmenopausal hormone replacement therapy (HRT) leads to an increase in plasma C-reactive protein (CRP) levels, an observation that raises the possibility of an up-regulation of inflammation among women taking these agents. This issue is of clinical concern because CRP represents a potent independent risk marker for the development of cardiovascular events, and completed and ongoing randomized trials on the prevention of cardiovascular disease have reported an unexpected increase in rates of venous and arterial thrombotic events following initiation of HRT. It is unclear, however, whether the observed effects of HRT on CRP represent a generalized proinflammatory effect mediated through the upstream cytokine interleukin 6 (IL-6) or whether these effects are due to a secondary mechanism. For example, in the Postmenopausal Estrogen/Progestin Interventions trial, although CRP levels increased with HRT, levels of fibrinogen, E-selectin, and other acute-phase reactants did not. Furthermore, although it has been hypothesized that elevations in CRP are partly respon-

Context  Postmenopausal hormone replacement therapy (HRT) has been shown to elevate C-reactive protein (CRP) levels. Several inflammatory biomarkers, including CRP, are associated with increased cardiovascular risk. However, whether the effect of HRT on CRP represents a clinical hazard is unknown.

Objectives  To assess the association between baseline levels of CRP and interleukin 6 (IL-6) and incident coronary heart disease (CHD) and to examine the relationship between baseline use of HRT, CRP, and IL-6 levels as they relate to subsequent vascular risk.

Design, Setting, and Participants  Prospective, nested case-control study of postmenopausal women, forming part of the Women's Health Initiative, a large, nationwide, observational study. Among 75,343 women with no history of cardiovascular disease or cancer, 304 women who developed incident CHD were defined as cases and matched by age, smoking status, ethnicity, and follow-up time with 304 study participants who remained event free during a median observation period of 2.9 years.

Main Outcome Measure  Incidence of first myocardial infarction or death from CHD.

Results  Median baseline levels of CRP (0.33 vs 0.25 mg/dL; interquartile range [IQR], 0.14-0.71 vs 0.10-0.47; \( P < .001 \)) and IL-6 (1.81 vs 1.47 pg/mL; IQR, 1.05-2.15; \( P < .001 \)) were significantly higher among cases compared with controls. In matched analyses, the odds ratio (OR) for incident CHD in the highest vs lowest quartile was 2.3 for CRP (95% confidence interval [CI], 1.4-3.7; \( P \) for trend = .002) and 3.3 for IL-6 (95% CI, 2.0-5.5; \( P \) for trend < .001). After additional adjustment for lipid and nonlipid risk factors, both inflammatory markers were significantly associated with a 2-fold increase in odds for CHD events. As anticipated, current use of HRT was associated with significantly elevated median CRP levels. However, there was no association between HRT and IL-6. In analyses comparing individuals with comparable baseline levels of either CRP or IL-6, those taking or not taking HRT had similar ORs for CHD. In analyses stratified by HRT, we observed a positively graded relationship between plasma CRP levels and the OR for CHD among both users and nonusers of HRT across the full spectrum of baseline CRP.

Conclusions  These prospective findings indicate that CRP and IL-6 independently predict vascular events among apparently healthy postmenopausal women and that HRT increases CRP. However, use or nonuse of HRT had less importance as a predictor of cardiovascular risk than did baseline levels of either CRP or IL-6.
sible for the hazards associated with HRT use, there are no clinical outcomes data addressing this issue.

We explored these issues in the Women’s Health Initiative Observational Study (WHI-OS), a prospective cohort of 75,343 initially healthy, postmenopausal women being followed up for the occurrence of first myocardial infarction (MI) or death from coronary heart disease (CHD). Using a nested case-control study design, we addressed whether baseline levels of CRP and IL-6 predict coronary risk among postmenopausal women, whether HRT use increased levels of IL-6 and CRP, and whether there was clinical evidence that HRT use affected vascular risk once these inflammatory effects were accounted for.

**METHODS**

**Study Population**

As described elsewhere, the WHI has clinical trial and observational study components. The latter component is an ongoing, nationwide, prospective cohort study of postmenopausal women of diverse races and ethnicities and is designed to examine the association between clinical, socioeconomic, behavioral, and dietary risk factors and the subsequent incidence of several health outcomes, including MI. Between 1994 and 1998, the WHI-OS enrolled 93,724 women aged 50 to 79 years at 40 clinical centers throughout the United States.

Recruitment strategies for the WHI were complex, with allocation of eligible participants to each of 3 clinical trial components and the large observational study. Participants were recruited from areas surrounding clinical centers in 24 states and the District of Columbia. Enrollment of racial/ethnic minority groups in proportion to the US population was a priority, although the groups were not a probability sample. Women ineligible or unwilling to participate in the clinical trial were invited to participate in the observational study. A total of 37,309 women completed the initial screening data form. Of these, 25% were either ineligible or unwilling to enroll in the clinical trial and were enrolled in the observational study. Women were eligible to participate in the observational study if they were postmenopausal, unlikely to change residence or die within 3 years, and not enrolled in the WHI or any other clinical trial. Among participants ineligible for the WHI-OS, 76.6% were excluded because of lack of interest or signed consent.

Among WHI-OS participants, 75,343 had no history of cardiovascular disease or cancer. At baseline, women completed screening and enrollment questionnaires and underwent a physical examination and fasting blood specimen collection. Blood was processed for long-term storage at −70°C. The study was reviewed and approved by human subjects review committees at each participating institution, and signed informed consent was obtained from all women enrolled.

**Baseline Clinical Variables**

After eligibility determination, participants underwent initial screening visits during which personal information, medical history, and medication and vitamin use were reviewed and anthropometric measurements, blood pressure, and fasting blood specimens were obtained. Blood pressure was measured with a mercury sphygmomanometer after subjects had been seated for 5 minutes. Two measurements were recorded and averaged. Fasting was defined as no food or beverage intake except water in the 12-hour period before blood collection. A health-related personal habits questionnaire was completed to assess smoking status, alcohol consumption, and physical activity level.

Ethnicity was identified as white not of Hispanic origin, African American, Hispanic, American Indian or Alaskan Native, Asian or Pacific Islander, or unknown (none of the above). History of hypertension was defined as self-reported history of treated or untreated diagnosed high blood pressure. If self-report of diagnosed hypertension was missing (n=22), hypertension was coded for subjects with a measured baseline systolic blood pressure of 140 mm Hg or higher or a diastolic blood pressure of 90 mm Hg or higher. History of diabetes was defined as self-report of diagnosed diabetes mellitus. Family history of premature coronary artery disease was defined by self-report of MI in a first-degree male relative before 55 years of age or first-degree female relative before 65 years of age. Unknown family history was coded for those participants unsure of family history of MI or age at presentation (n=33). Smoking status (nonsmoker, 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 the number of weekly episodes of strenuous recreational physical activity. Alcohol consumption was computed from a food frequency questionnaire.

Hormone replacement therapy status was classified as never, past, or current use of unopposed estrogen or estrogen with progestin from pills or patches. Most current HRT users were undergoing treatment with conventional doses of conjugated equine estrogens with or without medroxyprogesterone acetate. Specifically, 82% of current estrogen users were taking oral conjugated equine estrogens, and 74% of these users were treated with a dose of 6.25 mg/d. Among current users of estrogen with progestin, 70% were taking oral conjugated equine estrogens, and most (75%) were treated with a daily dose of 6.25 mg. Eighty-seven percent of current users of estrogen with progestin were undergoing treatment with medroxyprogesterone acetate, 2.5 mg/d (39%), 5.0 mg/d (18%), 10 mg/d (21%), or unknown (2%).

**Follow-up and Outcome Ascertainment**

As of February 2000, the median duration of follow-up 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 MI. Confirmation of self-reported nonfa-
tal MI was based on medical record re-
view with documentation of new chest
pain syndromes accompanied by char-
acteristic evolution of electrocardio-
graphic changes or clear evidence of
myocyte damage as evidenced by el-
evated creatine kinase–MB or tropo-
nin values. Deaths caused by coronary
disease were confirmed on the basis of
death certificates, autopsy reports, cir-
cumstances of death, electrocardio-
gram, laboratory test results, and re-
ports from all relevant procedures. We
included cases of sudden cardiac death
in which death occurred within 1 hour of
symptom onset in the absence of
other potentially lethal noncardiac dis-
ease processes.

**Nested Case-Control Study Design**

We used a prospective, nested case-
control approach in which case sub-
jects were WHI-OS participants who
were free of cardiovascular disease or
cancer at study entry and subse-
quently developed a first MI during fol-
low-up. Controls were selected from
women who did not experience an MI.
Controls were 1:1 matched to cases by
age (±2 years), smoking status, ethnic-
ity, and follow-up time (±6 months).
Exclusion criteria were a baseline his-
tory of angina, congestive heart fail-
ure, MI, coronary revascularization,
stroke, or cancer (except nonmela-
noma skin cancer). As of February
2000, 315 case-control pairs who met
these criteria were identified. Eleven
case-control pairs were eliminated be-
cause of inadequate blood specimens.
Given our sample size of 304 case-
control pairs and using the median cut
point for controls to define exposure,
we estimated our power to detect an
odds ratio (OR) of 1.6, 1.8, and 2.0 to
be 82%, 95%, and 99%, respectively,
for incident cardiovascular events (α = .05).

**Laboratory Procedures**

Baseline plasma samples were thawed
and assayed for CRP, IL-6, and lipids.
C-reactive protein was measured with a
high-sensitivity assay by using reagents
from Denka Seiken (Niigata, Japan).
Interleukin 6 was measured by a com-
mercially available enzyme-linked
immunosorbent assay (R & D Systems,
Minneapolis, Minn). Total cholesterol,
high-density lipoprotein cholesterol
(HDL–C), directly obtained low-
density lipoprotein cholesterol (LDL-
C), and triglyceride levels were mea-
sured with reagents from Roche Diagnostics (Indianapolis, Ind) and Gen-
zyme Corporation (Cambridge, Mass).
Samples were analyzed in randomly
ordered case-control pairs to minimize
systematic bias and interassay varia-
tion. The coefficients of variation for
CRP, IL-6, total cholesterol, HDL–C,
LDL–C, and triglycerides derived from
a 5% sample of simultaneously ana-
lyzed blinded quality-control speci-
mens were 3.8%, 10.1%, 1.8%, 2.5%,
6.5%, and 3.0%, respectively.

**Statistical Analysis**

We used the t test to evaluate differ-
ences in means and the χ² statistic to
evaluate differences in proportions. Be-
cause the distributions of CRP and IL-6
are skewed, differences in medians were
tested by using the Wilcoxon rank sum
test. Conditional logistic regression was
used to estimate ORs and 95% confi-
dence intervals (CIs) after the popula-
tion was divided into groups according
to the quartile cut points for the control
distribution of each biomarker. Tests for
linear trends were computed by using an
ordinal variable for biomarker quartiles.
Multivariate ORs were estimated from
conditional logistic regression mod-
els, which accounted for matching vari-
ables, and were additionally adjusted for
body mass index, history of diabetes, his-
tory of hypertension, family history of
premature coronary artery disease, ex-
ercise frequency, alcohol consumption,
use of HRT, and total cholesterol to
HDL–C ratio. Adjusted models were
based on case-control pairs for whom complete data were available on all co-
variates of interest.

To assess for effect modification by
obesity, we determined the OR for in-
cident CHD in subgroups of women de-
finied by the upper tertile cut point of
body mass index among control sub-
jects (27.8 kg/m²) and low, interme-
iate, and high tertiles of the inflamma-
tory biomarkers. Conditional logistic
regression was used to obtain ORs in
each of these 6 groups.

To evaluate the relationship between
baseline use of HRT, inflammatory bio-
markers, and the OR for CHD, median
values of CRP and IL-6 were deter-
mained for cases and controls accord-
ing to HRT status at baseline. Differ-
ences in medians were tested by using
the Wilcoxon rank sum test. We then
divided the study population into 6
groups according to HRT status (non-
users vs current users) and low, inter-
mediate, and high levels of each bio-
marker according to tertile cut points
of the respective control distributions
and derived subgroup-specific ORs by
conditional logistic regression analy-
isis. We estimated the relationship of
CRP with subsequent coronary risk
along the full spectrum of plasma val-
ues stratified by baseline HRT status by
using generalized additive logistic
regression analysis performed in SPLUS.13
This procedure provides a
graphical representation of the OR for
CHD associated with increasing levels
of the inflammatory biomarker on a
continuous scale after adjustment for
matching factors and other clinical risk
factors. Because this technique is sen-
sitive to the influence of outliers, we
excluded from analysis those individu-
als with CRP levels in the lowest 2.5%
and above a value of 1.5 mg/dL, a level
considered to be indicative of an under-
lying clinically relevant inflammatory
condition. The estimated curves were
derived by using locally weighted
regression splines with window spans
chosen by optimization of Akaike’s
information criteria.13 The reference
level is the median CRP for controls in
the respective HRT strata. To improve
symmetry of plasma CRP levels over the
range of prediction, baseline values were
log-transformed for entry into regres-
sion models and back-transformed for
ease of interpretation of graphs. SPLUS
was used for graphical displays and SAS
for all other analyses (SAS Institute Inc,
Cary, NC). All CIs are 2-tailed and cal-
culated at the .05 level.
RESULTS

Baseline characteristics are shown in Table 1. Case subjects had a higher prevalence of traditional cardiovascular risk factors than controls. Among study participants, 36.5% reported current use of HRT; most were long-term users who had been undergoing treatment for more than 4 years. Although duration of treatment appeared to be somewhat shorter among cases, this difference did not attain statistical significance. In addition, there were no differences in the proportion of women taking unopposed estrogen vs combined estrogen plus progestin formulations. Baseline rates of aspirin, statins, or other lipid-lowering medication use were not significantly different among groups.

Baseline levels of CRP and IL-6 were higher among cases than controls for CRP (0.33 vs 0.25 mg/dL; \( P < .001 \)) and IL-6 (1.81 vs 1.47 pg/mL; \( P < .001 \)) (Table 1). Women experiencing MI were also more likely to have higher plasma levels of total cholesterol, LDL-C, and triglycerides and a higher total cholesterol to HDL-C ratio, whereas levels of HDL-C were significantly lower among women with subsequent events. In analyses matched for age, smoking, ethnicity, and follow-up time (Table 2 and Table 3), increasing levels of both biomarkers were associated with increased CHD risk; the ORs for women in the highest quartile vs lowest quartile were 2.3 (95% CI, 1.4-3.7; \( P \) for trend = .002) for CRP and 3.3 (95% CI, 2.0-5.5; \( P \) for trend < .001) for IL-6. Adjustment for the ratio of total cholesterol to HDL-C attenuated these risks only slightly. In fully adjusted models that additionally controlled for other conventional cardiovascular risk factors, the odds of CHD among women with the highest levels of either CRP or IL-6 remained 2-fold greater than for women in the lowest quartile. Additional control for baseline use of aspirin or statins did not materially alter our results. In fully adjusted models, including baseline aspirin and statin use, the ORs in the highest vs lowest quartile of CRP and IL-6 were 2.1 (95% CI, 1.1-
TABLE 1. Baseline Clinical Characteristics and Biochemical Parameters* (cont)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women Developing CHD (Cases) (n = 304)</th>
<th>Women Free of CHD (Controls) (n = 304)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid values, mean (SD), mg/dL‡</td>
<td>Total cholesterol 228.7 (40.2)</td>
<td>222.5 (36.5)</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>LDL-C 130.8 (33.0)</td>
<td>123.5 (32.9)</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>HDL-C 58.1 (15.6)</td>
<td>64.1 (17.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>172.8 (94.9)</td>
<td>155.3 (85.9)</td>
<td>.02</td>
</tr>
<tr>
<td>Ratio of total cholesterol to HDL-C, median (SEM)</td>
<td>4.2 (1.3)</td>
<td>3.7 (1.1)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Because of rounding, not all percentages total to 100. CHD indicates coronary heart disease; HRT, hormone replacement therapy; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol. Missing data: body mass index, 4 cases, 2 controls; physical activity, 6 cases, 10 controls; alcohol consumption, 1 case; total cholesterol, 2 controls; LDL-C, 1 case, 1 control; HDL-C, 2 controls; triglycerides, 1 control; and total cholesterol–HDL-C ratio, 2 controls.

†Matched on age, ethnicity, smoking, and length of follow-up.

‡To convert cholesterol values to mmol/L, multiply by 0.0259. To convert triglycerides to mmol/L, multiply by 0.0113.

Crude and Adjusted Odds Ratios for Coronary Heart Disease According to Baseline Plasma Concentration of C-Reactive Protein

<table>
<thead>
<tr>
<th>Quartile of C-Reactive Protein</th>
<th>Odds ratio (95% CI)</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>.525</td>
</tr>
<tr>
<td>2</td>
<td>1.5 (0.9-2.4)</td>
<td>.145</td>
</tr>
<tr>
<td>3</td>
<td>1.3 (0.8-2.1)</td>
<td>.02</td>
</tr>
<tr>
<td>4</td>
<td>2.3 (1.4-3.7)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Adjusted for TC:HDL-C ratio (n = 302)

<table>
<thead>
<tr>
<th>Odds ratio (95% CI)</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.10</td>
</tr>
<tr>
<td>2</td>
<td>.30</td>
</tr>
</tbody>
</table>

Adjusted for all risk factors (n = 280)

<table>
<thead>
<tr>
<th>Odds ratio (95% CI)</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.20</td>
</tr>
<tr>
<td>2</td>
<td>.60</td>
</tr>
</tbody>
</table>

Adjusted for TC:HDL-C ratio (n = 302)

<table>
<thead>
<tr>
<th>Odds ratio (95% CI)</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.04</td>
</tr>
<tr>
<td>2</td>
<td>.04</td>
</tr>
</tbody>
</table>

Adjusted for all risk factors (n = 280)

<table>
<thead>
<tr>
<th>Odds ratio (95% CI)</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.11</td>
</tr>
<tr>
<td>2</td>
<td>.10</td>
</tr>
</tbody>
</table>

*CI indicates confidence interval; TC:HDL-C, total cholesterol to high-density lipoprotein cholesterol ratio; n, number of case-control pairs included in the analysis.

†Matched on age, ethnicity, smoking, and length of follow-up.

‡Matched on age, ethnicity, smoking, and length of follow-up and controlled for TC:HDL ratio, body mass index, history of hypertension, family history of premature coronary artery disease, diabetes, exercise frequency, alcohol consumption, and value of hormone replacement therapy.

Table 3. Crude and Adjusted Odds Ratios of Coronary Heart Disease According to Baseline Plasma Concentration of Interleukin 6*  

<table>
<thead>
<tr>
<th>Quartile of Interleukin 6</th>
<th>Odds ratio (95% CI)</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>.104</td>
</tr>
<tr>
<td>2</td>
<td>2.0 (1.2-3.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>3</td>
<td>2.2 (1.3-3.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>4</td>
<td>3.3 (2.0-5.5)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Adjusted for TC:HDL-C ratio (n = 302)

<table>
<thead>
<tr>
<th>Odds ratio (95% CI)</th>
<th>P Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.01</td>
</tr>
<tr>
<td>2</td>
<td>.04</td>
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</table>

Adjusted for all risk factors (n = 280)

<table>
<thead>
<tr>
<th>Odds ratio (95% CI)</th>
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<tr>
<td>1</td>
<td>.11</td>
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<tr>
<td>2</td>
<td>.10</td>
</tr>
</tbody>
</table>

*See Table 2 for footnote explanations.
and controls were examined separately, CRP levels were higher in current users. The CRP values were 55% higher in current users of HRT compared with nonusers in cases (\(P = .001\)) and 70% higher in HRT users vs nonusers among controls (\(P < .001\)).

To further assess the clinical significance of these findings, we divided the study population into 6 groups according to HRT status and tertiles of each biomarker (TABLE 3). In matched analyses simultaneously adjusted for conventional coronary risk factors, we found that although increasing levels of CRP and IL-6 were independently associated with a graded increase in risk of CHD among current users and nonusers, the OR appeared equivalent for current users within each category of low, medium, and high biomarker levels. To further examine this issue, we constructed response curves between baseline CRP and the adjusted OR for CHD associated with a graded increase in risk of CHD among current users and nonusers within analyses stratified by underlying levels of each inflammatory biomarker. Thus, at least in these observational data, use or nonuse of HRT had less importance in terms of subsequent cardiovascular risk than baseline levels of either CRP or IL-6.

Plasma concentrations of CRP are a sensitive marker of underlying systemic inflammation and are largely regulated by IL-6–mediated hepatic biosynthesis, although IL-6–independent mechanisms have been described.20 In prior work, CRP levels within the low-normal range have consistently been correlated with coronary risk among healthy, middle-aged men and women4–10 and among patients with stable angina pectoris,21 acute coronary ischemia,22–24 or a history of MI.25 Similar associations between baseline elevations of IL-6 with incident vascular events26 and cardiovascular mortality27 have been documented among healthy individuals. Few of these studies, however, have evaluated the relationship between subclinical inflammation and the development of coronary disease among otherwise healthy postmenopausal women, among whom the effects of aging on cardiovascular risk may be exacerbated by hormonal changes accompanying menopause. Furthermore, several studies were confined to those with subclinical disease at baseline and limited by small numbers and the inability to adjust for other potential confounders.4–6,8 Thus, the findings that CRP and IL-6 predict incident CHD among a large, ethnically diverse co-

COMMENT

These data, derived from a large-scale cohort of initially healthy, postmenopausal, US women, demonstrate 3 major findings. First, baseline levels of CRP and IL-6 are independently associated with a 2-fold increase in the risk of developing CHD. Second, although long-term HRT use was associated with increased CRP levels, this effect was not seen for IL-6, suggesting that HRT use may not necessarily stimulate a generalized systemic inflammatory response. Third, the ORs for incident CHD were similar among HRT users and nonusers in analyses stratified by underlying levels of each inflammatory biomarker. Thus, at least in these observational data, use or nonuse of HRT had less importance in terms of subsequent cardiovascular risk than baseline levels of either CRP or IL-6.

Table 4. Median Levels of Inflammatory Biomarkers According to Baseline Use of Hormone Replacement Therapy

<table>
<thead>
<tr>
<th></th>
<th>Median (Interquartile Range), mg/dL</th>
<th>( P ) Value*</th>
<th>Median (Interquartile Range), pg/mL</th>
<th>( P ) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonusers</td>
<td>0.27 (0.11-0.62)</td>
<td>0.20 (0.08-0.40)</td>
<td>&lt;.001</td>
<td>1.83 (1.32-2.76)</td>
</tr>
<tr>
<td>Current users</td>
<td>0.42 (0.21-0.78)</td>
<td>0.34 (0.15-0.56)</td>
<td>.01</td>
<td>1.68 (1.23-2.74)</td>
</tr>
</tbody>
</table>

\( P \) value < .001 for difference between cases and controls within hormone replacement therapy category determined with the Wilcoxon rank sum test.

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We believe our findings regarding interrelations among CRP, IL-6, and HRT are also of clinical importance because postmenopausal HRT has until recently been viewed as a legitimate strategy for preventing or delaying the onset of a number of age-related conditions. Although nearly all analyses deriving from observational studies have found coronary risk reductions in association with HRT, clinical trials have failed to substantiate these findings and instead have suggested small net harm. Attempts to explain these findings have focused on intermediary atherothrombotic mechanisms that may be up-regulated in susceptible individuals. In this regard, a consistent finding has been elevated CRP levels concomitant with either oral unopposed or combined estrogen-progesterin therapy. Although the underlying mechanism of this effect is poorly understood, concurrent evaluation of the effect of HRT on other inflammation-sensitive biomarkers, such as fibrinogen, α1-acid glycoprotein, soluble E-selectin, homocysteine, and IL-6, have been discordant.

Our study did not directly address the mechanisms underlying the increase in vascular risk associated with HRT. However, the results of our analysis suggest that although long-term estrogen replacement therapy is associated with increased CRP, HRT users appear to be at a risk similar to that of nonusers for any level of baseline CRP. In addition, we have shown that despite attenuation of case-control differences in plasma CRP among current users, CRP levels remain independently predictive of subsequent CHD events irrespective of HRT status at baseline. Thus, it would appear that the expressed level of CRP, rather than HRT, is a primary determinant of risk in these women. Our observation that IL-6 levels were not significantly higher among current HRT users suggests that if elevations in CRP levels are indicative of subclinical inflammation, these effects may be mediated through IL-6-independent pathways. Alternatively, our null data for IL-6 may imply that the HRT-related rise in CRP levels does not signal a more generalized proinflammatory state. Since most (94.9%) of the current HRT users in our study population were treated with oral estrogenic agents, it is possible that the observed increase in systemic CRP concentrations may be due to a so-called first pass effect of oral estrogens on hepatic protein synthesis, a hypothesis supported by the observation that transdermal delivery compared with oral estrogen preparations is not associated with elevated CRP. Such an effect may nonetheless alter vascular risk; tissue factor expression by monocytes in the basal state and on exposure to physiologic levels of CRP is blunted in monocytes retrieved from healthy postmenopausal women who are undergoing HRT as opposed to those who are not.

This investigation had important limitations. First, we relied on a single baseline blood sample and thus cannot account for variations in biomarker levels that occur over time. Although diurnal variation in plasma IL-6 may occur, our specimens were generally obtained in the morning or early afternoon. Nonetheless, random misclassification if present would tend to move our effect estimates for IL-6 toward the null. With regard to CRP, several longitudinal studies have found that plasma levels are stable during long-term follow-up, as long as measurements are not made within 2 weeks of an acute infection. Second, we did not adjust for changes in HRT status that may have occurred during the observation period. However, since most current users were undergoing long-term therapy, the influence of this factor is likely to be small. Third, our data are observational. Participants in the WHI-OS chose whether to undergo HRT and therefore are likely to differ from nonusers in ways that could affect CRP and IL-6 levels and the risk of developing CHD. Although a clear strength of the WHI-OS cohort is the uniformly high quality of covariate data regarding well-established risk factors, uncontrolled confounding cannot be excluded. In sum, these prospective data demonstrate that the inflammatory biomarkers CRP and IL-6 predict incident cardiovascular events in healthy post-

Table 5. Adjusted Odds Ratio (OR) for Coronary Heart Disease According to Baseline Use of Hormone Replacement Therapy and Tertiles of C-Reactive Protein and Interleukin 6

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein</td>
<td>1.0</td>
<td>2.4 (0.9-6.8)</td>
<td>2.4 (0.9-6.5)</td>
</tr>
<tr>
<td>Nonusers</td>
<td>2.6 (1.0-6.7)</td>
<td>2.8 (1.0-7.7)</td>
<td>3.3 (1.2-9.5)</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>1.0</td>
<td>1.4 (0.6-3.0)</td>
<td>2.3 (1.0-5.0)</td>
</tr>
<tr>
<td>Nonusers</td>
<td>1.5 (0.7-3.2)</td>
<td>2.2 (1.1-4.6)</td>
<td>2.4 (1.1-5.0)</td>
</tr>
</tbody>
</table>

*Controlled for matching variables (age, ethnicity, smoking, and follow-up time) and additionally adjusted for total cholesterol to high-density lipoprotein cholesterol ratio, body mass index, hypertension, diabetes, and family history of premature coronary artery disease. CI indicates confidence interval.
†Tertiles defined as low (<0.14 mg/dL), intermediate (0.14-0.38 mg/dL), and high (>0.38 mg/dL).
‡Tertiles defined as low (<1.20 pg/mL), intermediate (1.20-1.86 pg/mL), and high (>1.86 pg/mL).

Figure 2. Adjusted Odds Ratio for Coronary Heart Disease According to Baseline C-Reactive Protein Stratified by Hormone Replacement Therapy (HRT) Use

Estimated curves are adjusted for matching variables (age, ethnicity, smoking, and follow-up time), total/ high-density lipoprotein cholesterol ratio, body mass index, history of hypertension, diabetes, and family history of premature coronary heart disease. The horizontal line indicates the 1.0 reference mark.
menopausal women, an effect present among HRT users and nonusers. These issues are of particular interest, given recent findings that markers of inflammation such as CRP may be useful for targeting preventive therapies such as aspirin and statins.38 That use or nonuse of HRT had less importance than expressed CRP levels in terms of cardiovascular risk assessment also implies that diet, exercise, and smoking cessation are likely to remain the most important interventions for the primary prevention of vascular disease for some time to come.

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