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...
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 75343 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 93724 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 minorities in proportion to the US population of women aged 50 to 79 years 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 373092 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, 75343 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 progesterin, 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 progesterin were undergoing treatment with medroxyprogesterone acetate, 2.5 mg/d (59%), 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-
tual MI was based on medical record review with documentation of new chest pain syndromes accompanied by characteristic evolution of electrocardiographic changes or clear evidence of myocardial damage as evidenced by elevated creatine kinase-MB or troponin values. Deaths caused by coronary disease were confirmed on the basis of death certificates, autopsy reports, circumstances of death, electrocardiogram, laboratory test results, and reports 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 disease processes.

**Nested Case-Control Study Design**

We used a prospective, nested case-control approach in which case subjects were WHI-OS participants who were free of cardiovascular disease or cancer at study entry and subsequently developed a first MI during follow-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, ethnicity, and follow-up time (±6 months). Exclusion criteria were a baseline history of angina, congestive heart failure, MI, coronary revascularization, stroke, or cancer (except nonmelanoma skin cancer). As of February 2000, 315 case-control pairs who met these criteria were identified. Eleven case-control pairs were eliminated because 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 commercially 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 measured with reagents from Roche Diagnostics (Indianapolis, Ind) and Genzyme Corporation (Cambridge, Mass). Samples were analyzed in randomly ordered case-control pairs to minimize systematic bias and interassay variation. The coefficients of variation for CRP, IL-6, total cholesterol, HDL-C, LDL-C, and triglycerides derived from a 5% sample of simultaneously analyzed blinded quality-control specimens were 3.8%, 10.1%, 1.8%, 2.5%, 6.5%, and 3.0%, respectively.

**Statistical Analysis**

We used the t test to evaluate differences in means and the χ² statistic to evaluate differences in proportions. Because 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% confidence intervals (CIs) after the population 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 models, which accounted for matching variables, and were additionally adjusted for body mass index, history of diabetes, history of hypertension, family history of premature coronary artery disease, exercise 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 covariates of interest.

To assess for effect modification by obesity, we determined the OR for incident CHD in subgroups of women defined by the upper tertile cut point of body mass index among control subjects (27.8 kg/m²) and low, intermediate, and high tertiles of the inflammatory 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 biomarkers, and the OR for CHD, median values of CRP and IL-6 were determined for cases and controls according to HRT status at baseline. Differences in medians were tested by using the Wilcoxon rank sum test. We then divided the study population into 6 groups according to HRT status (nonusers vs current users) and low, intermediate, and high levels of each biomarker according to tertile cut points of the respective control distributions and derived subgroup-specific ORs by conditional logistic regression analysis. We estimated the relationship of CRP with subsequent coronary risk along the full spectrum of plasma values stratified by baseline HRT status by using generalized additive logistic regression analysis performed in SPLUS. 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 sensitive to the influence of outliers, we excluded from analysis those individuals 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 underlying 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. 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 regression 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 calculated 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-
4.2; \( P = .03 \)) and 2.0 (95% CI, 1.0-4.0; \( P \) for trend = .03), respectively. In models adjusting for education and income level, we found similar results among women providing these socioeconomic data (234 case-control pairs); the fully adjusted ORs in the highest vs lowest quartile of CRP and IL-6 were 2.2 (95% CI, 1.0-4.8; \( P = .04 \)) and 1.8 (95% CI, 0.8-3.9; \( P = .14 \)), respectively.

Because prior studies have shown that obesity may be strongly associated with subclinical inflammation\(^{16-18} \) and is an important determinant of CHD risk in women,\(^{19} \) we assessed the consistency of risk relationships among normal weight and overweight women by dividing the study population into 6 groups according to the upper tertile cut point of body mass index among controls (27.8 kg/m\(^2\)) and low, intermediate, and high ter-
tiles of the inflammatory biomarkers (FIGURE 1). In this analysis, higher baseline plasma levels of CRP and IL-6 appear to be associated with a stepwise increase in odds among both low and high body mass index strata.

To evaluate the influence of HRT on markers of inflammation and coronary risk, we first computed the median values of CRP and IL-6 among current users and nonusers and assessed differences according to treatment and case status (TABLE 4). Plasma concentrations of CRP and IL-6 were higher among cases than among controls within each category of HRT use. Although IL-6 levels were similar if not slightly lower for current HRT users compared with nonusers when cases

### 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>Lp(a), mg/dL‡</td>
<td>32.2 (65.2)</td>
<td>27.1 (50.9)</td>
<td>.013</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; Lp(a), lipoprotein(a); 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; Lp(a), 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.

### Table 2. 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>.002</td>
</tr>
<tr>
<td>2</td>
<td>1.5 (0.9-2.4)</td>
<td>.30</td>
</tr>
<tr>
<td>3</td>
<td>1.3 (0.8-2.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>4</td>
<td>2.3 (1.4-3.7)</td>
<td>.001</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.

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

### 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>&lt;.001</td>
</tr>
<tr>
<td>2</td>
<td>2.0 (1.2-3.4)</td>
<td>.01</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>.001</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 4). 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 among current users and nonusers evalu-

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

<table>
<thead>
<tr>
<th>C-Reactive Protein, Median (Interquartile Range), mg/dL</th>
<th>Interleukin 6, Median (Interquartile Range), pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
<td><strong>Controls</strong></td>
</tr>
<tr>
<td>Nonusers: 0.27 (0.11-0.62)</td>
<td>0.20 (0.08-0.40)</td>
</tr>
<tr>
<td>Current users: 0.42 (0.21-0.78)</td>
<td>0.34 (0.15-0.55)</td>
</tr>
<tr>
<td><strong>P Value</strong></td>
<td><strong>Value</strong></td>
</tr>
<tr>
<td>.003</td>
<td>1.83 (1.32-2.76)</td>
</tr>
<tr>
<td>0.01</td>
<td>1.68 (1.23-2.74)</td>
</tr>
</tbody>
</table>

The odds ratio for coronary heart disease was adjusted according to body mass index (<27.8 vs ≥27.8 kg/m²) and tertiles of C-reactive protein (A) and interleukin 6 (B) controlled for matching variables (age, ethnicity, smoking, and follow-up time) and was additionally adjusted for total/high-density lipoprotein cholesterol ratio, body mass index, hypertension, diabetes, and family history of premature coronary artery disease. The horizontal line indicates the 1.0 reference mark.

**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 graded 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 undergoing 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. In prior work, CRP levels within the low-normal range have consistently been correlated with coronary risk among healthy, middle-aged men and women and among patients with stable angina pectoris, acute coronary ischemia, or a history of MI. Similar associations between baseline elevations of IL-6 with incident vascular events and cardiovascular mortality 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.

The findings that CRP and IL-6 predict incident CHD among a large, ethnically diverse co-

![Figure 1. Adjusted Odds Ratio for Coronary Heart Disease](image-url)
Inflammatory Biomarkers, HRT, and CHD

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></th>
<th>OR (95% CI by Biomarker Tertile</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>Current users</td>
<td>1.0</td>
<td>2.4 (0.9-6.6)</td>
<td>2.4 (0.9-6.5)</td>
</tr>
<tr>
<td></td>
<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>Current users</td>
<td>1.0</td>
<td>1.4 (0.6-3.0)</td>
<td>2.3 (1.0-5.0)</td>
</tr>
<tr>
<td></td>
<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](image)

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.


dividuals. In this regard, a consistent finding has been elevated CRP levels concomitant with either oral unopposed or combined estrogen-progestin 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-

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menopausal women, a 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. That use or non-use 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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