Serum Levels of C-reactive Protein Are Associated with Obesity, Weight Gain, and Hormone Replacement Therapy in Healthy Postmenopausal Women

Emma Barinas-Mitchell,1 Mary Cushman,2 Elaine N. Meilahn,1 Russell P. Tracy,3 and Lewis H. Kuller1

The authors evaluated the cross-sectional and prospective associations between the serum concentration of C-reactive protein and measures of obesity and fat distribution, hormone replacement therapy (HRT) use, and serum sex hormones in postmenopausal women from the Healthy Women Study (Allegheny County, Pennsylvania, 1998). The authors tested the hypothesis that C-reactive protein levels would be higher among HRT users and among women with greater body mass index, waist circumference, or visceral fat. There were 207 women in the study who were ≥8 years postmenopausal (101 HRT users and 106 HRT nonusers). The median levels of C-reactive protein were 3.01 mg/liter in HRT users compared with 1.74 mg/liter in nonusers (p = 0.002). C-reactive protein levels were strongly positively correlated with measures of body size, fatness, fat distribution, and weight gain among HRT users and nonusers. C-reactive protein was also positively correlated with serum estrone levels (r = 0.38) among HRT nonusers. The highest level of C-reactive protein was found among HRT users in the highest quartile of visceral fat (4.29 mg/liter) compared with women not on HRT and in the lowest quartile of visceral fat (0.96 mg/liter). The use of HRT and measures of overall body fatness are important correlates of C-reactive protein among postmenopausal women.

High normal values of C-reactive protein, indicative of low-grade inflammation, have been associated with increased risk of future cardiovascular events in men and women (1–10). The association between C-reactive protein and increased risk of myocardial infarction and stroke appears to be independent of traditional cardiovascular disease risk factors (1, 11, 12) even among women with low risk, as defined by conventional risk factors (9). Concentrations of C-reactive protein have been related to established cardiovascular disease risk factors in middle-aged and older men and women (8, 13–16). Important positive correlates of C-reactive protein include age, smoking, obesity, markers of fibrinolytic activity, total cholesterol, triglycerides, glucose, and apolipoprotein B, and an important negative correlate is high density lipoprotein cholesterol (13, 14, 17, 18).

Cross-sectional associations between hormone replacement therapy (HRT) use and markers of inflammation were evaluated in women aged 65 years and older from the Cardiovascular Health Study (19). This study found that women taking unopposed estrogen had 59 percent higher mean C-reactive protein levels as compared with nonusers, yet fibrinogen and plasminogen activator inhibitor-1 antigen levels were lower in HRT users. We suggest that these rather contradictory results may implicate a differential effect of HRT use on transcriptional control, clearance, or cytokine regulation of these proteins. Furthermore, a recent report from the Postmenopausal Estrogen/Progestin Interventions clinical trial indicated that levels of C-reactive protein increased with HRT use (estrogen alone and in combination with progestin) during a 3-year follow-up (15). In a small clinical study, the short-term (12-week) effect of unopposed estradiol and sequentially combined HRT was to significantly increase levels of C-reactive protein (20). To our knowledge no studies have examined the association between endogenous sex hormones and levels of C-reactive protein in women.

The cross-sectional association between serum concentrations of C-reactive protein and measures of obesity and fat distribution, HRT use, and serum sex hormones was evaluated in postmenopausal women from the Healthy Women Study. In the same study population, we evaluated premenopausal determinants of C-reactive protein levels measured during the eighth year postmenopause. In a subset of the study population, changes in C-reactive protein levels during the postmenopausal period were examined.
MATERIALS AND METHODS

The Healthy Women Study is a longitudinal population-based study of changes in cardiovascular disease risk factors during the peri- to the postmenopause (21, 22). In the Healthy Women Study, 541 premenopausal women living in Allegheny County, Pennsylvania, were recruited from 1983 to 1984 from driver’s license lists. Study eligibility criteria included the following: age 42–50 years; menstrual bleeding within the last 3 months; no surgically induced menopause; not on hormone replacement therapy; diastolic blood pressure less than 100 mmHg; and no medications known to influence the biologic risk factors under study (e.g., lipid lowering, insulin, thyroid, antihypertensive, and psychotropic medications). A detailed description of population recruitment and characteristics has been published (23).

After eligibility determination, all study participants were evaluated for a baseline examination in the morning after a 12-hour fast. This baseline examination included collection of blood samples for measurement of serum lipids and plasma fasting and 2-hour postload (75 g of glucose) glucose and insulin levels. Blood pressure and anthropometric measurements were made. A health-related behavior questionnaire was administered that assessed current cigarette smoking status, alcohol consumption, and level of physical activity, as well as a questionnaire that assessed the gynecologic and medical history, including weight at age 20, 30, and 40 years. Menses and the use of HRT were monitored monthly using postcards and telephone contact. After 12 consecutive months of amenorrhea and/or taking HRT (study definition of menopause), study participants were seen for an initial postmenopausal examination and thereafter at 2, 5, and 8 years after menopause (23). An oral glucose tolerance test was not administered during the eighth year postmenopausal clinic visit; therefore, 2-hour glucose and insulin levels are not available for this visit. For this report, baseline (premenopausal) and eighth year postmenopausal examination data were used. As of July 1, 1998 (the date blood specimens were pulled and then shipped for measurement of C-reactive protein), 298 women had completed their eighth postmenopausal clinic visit (55 percent of the cohort), 72 had follow-up by mail only, 114 had not reached their eighth year postmenopause, 51 had dropped out (or were lost to follow-up), and seven were deceased. Baseline characteristics (low density lipoprotein cholesterol, high density lipoprotein cholesterol, body mass index, education, age, cigarette smoking, fasting glucose, and triglycerides) were similar for women who had dropped out compared with those remaining in the study. Baseline systolic blood pressure and diastolic blood pressure were higher among women who had dropped out of the study.

Study population

Available serum specimens, collected at the eighth year postmenopausal clinic visit, from women who had an abdominal computed x-ray tomography scan or electron beam computed tomography scan done, as of July 1, 1998, were pulled for C-reactive protein analysis (n = 218). Of these 218 women, one did not have data on eighth year postmenopausal HRT use. There were 111 HRT users and 106 nonusers. Among the HRT users, 14 took estrogen alone, 87 took combined estrogen and progestin, one took progestin alone, and nine took other HRT preparations (four took vaginal creams and five took androgen and estrogen combinations). For the present analysis, the latter two groups were excluded, resulting in 101 HRT users and 106 nonusers for our study population (n = 207). The eighth year postmenopausal characteristics of women who had C-reactive protein measured were similar to those of all Healthy Women Study participants who attended their eighth postmenopausal clinic visit with two exceptions. Women with no measure of C-reactive protein available had higher low density lipoprotein cholesterol levels (p = 0.04) and consumed less alcohol (p = 0.004). The eighth year postmenopausal clinic visit was a mean of 11.5 (standard deviation, 1.7) years after the baseline examination.

As a subanalysis, 50 of the 207 women had C-reactive protein measured again from stored serum specimens collected during a visit after the eighth year postmenopausal visit (mean, 2.7 years; range, 1–5 years after the eighth year postmenopausal visit).

Laboratory assays and measurements

The serum concentration of C-reactive protein was measured in fasting blood specimens by colorimetric competitive immunoassay (C-reactive protein antibodies and antigens from Calbiochem, La Jolla, California) with a coefficient of variation of 4.8 percent (24). Serum lipid (total and high density lipoprotein cholesterol and triglycerides), plasma glucose, insulin and fibrinogen, and serum endogenous hormone (estrone, estradiol, testosterone, and androstenedione) levels were measured with standard assays as previously described (21, 25–27). The Friedewald equation was used to estimate low density lipoprotein cholesterol. Current cigarette smoking was reported as yes/no and number of cigarettes per day, alcohol consumption as grams of alcohol per day, and physical activity level as kilocalories per week. Body mass index was calculated using weight in kilograms divided by height in meters squared, and the waist/hip ratio was calculated as the waist girth (measured at the smallest circumference) divided by the hip girth (measured at the largest circumference). Weight gain from age 20 was calculated as the difference between the weight measured during the clinic visit and the self-reported weight at age 20. Visceral adipose tissue (cm²) was measured using computed x-ray tomography as described by Ferland et al. (28) using a model 9800-CT scanner (General Electric, Milwaukee, Wisconsin) and commercial computed tomography software (GE Medical Systems, Milwaukee, Wisconsin) with high inter- and intrascan reproducibility (29). The detailed methods for measurement of visceral adipose tissue used in the Healthy Women Study have been published (30). Data on visceral adipose tissue were available for 191 (92.3 percent of study population) women. The percent total body fat was measured via dual-energy x-ray absorptiometry using a model QDR-2000 DXA (Hologic, Waltham, Massachusetts); data on this measure were available for 161 (77.8 percent of the study population) women.
Statistical analysis

Data were analyzed using Statistical Analysis System software on a Windows operating system (Release 6.12: SAS Institute, Inc., Cary, North Carolina). Data are presented as the mean (standard deviation) for normally distributed variables, as percentages for categorical variables, or as the median [interquartile range] for nonnormal continuous variables. Comparisons of means between two groups were performed using the unpaired t test for normally distributed variables and the Mann-Whitney test for nonnormal variables. The χ2 and Fisher’s exact test, when appropriate, were used to test differences in proportions. Because of the skewness of the C-reactive protein data, between-group comparisons of C-reactive protein levels were presented as the median [interquartile range]. Analysis of variance was used to test the univariate difference in log-transformed values of C-reactive protein across risk factor levels, and analysis of covariance was used to examine associations between log-transformed values of C-reactive protein and HRT use, adjusting for potential confounding covariates. Nonparametric correlation coefficients (Spearman’s rho) were calculated between C-reactive protein levels and other continuous measures. Multiple linear regression was used to assess associations between C-reactive protein (log transformed) and measures of obesity and weight gain, adjusting for HRT use and other univariately associated covariates. For multiple linear regression (backward selection), the p value for adding a variable was set at 0.05 and for removing variables at 0.10. This analysis was also performed separately by eighth year postmenopausal HRT use. In a subgroup of women (n = 50) with two measures of C-reactive protein 1–5 years apart (mean, 2.7 years), Pearson’s correlation coefficients of the two measures of C-reactive protein were calculated.

RESULTS

Mean and median C-reactive protein levels were 3.16 (standard deviation, 3.53) mg/liter and 2.21 [interquartile range, 1.02–3.73] mg/liter, respectively, for the study population (207 women). Eighth year postmenopausal characteristics of Healthy Women Study participants by HRT use are shown in table 1. Significantly (p < 0.01) more HRT users had greater than a high school education and fewer were smokers compared with HRT nonusers. HRT users and nonusers were similar with respect to age, race, alcohol intake, body mass index, levels of physical activity, waist/hip ratio, blood pressure, total and low density lipoprotein cholesterol, fasting glucose, and fibrinogen (fibrinogen levels were available for 72.5 percent (150 women) of the study population). HRT users had significantly higher triglyceride and high density lipoprotein cholesterol levels. Weight gain from age 20 and from the baseline visit did not significantly differ by HRT use (data on weight gain from age 20 were available for 175 women). Visceral adipose tissue did not differ by HRT use; however, the median subcutaneous adipose tissue was significantly (p = 0.01) lower in HRT users (319.9 [interquartile range, 259.8–386.5] cm2) compared with nonusers (346.1 [interquartile range, 291.9–464.5] cm2). The age at menopause did not differ by eighth year postmenopausal HRT use. As expected, more women on HRT reported having had a hysterectomy than did nonusers.

Median C-reactive protein levels by HRT status and potential confounding variables are presented in table 2. Median C-reactive protein levels were 3.01 mg/liter for women taking HRT versus 1.74 mg/liter for those not taking HRT, a difference which was statistically significant. Among HRT users, users of estrogen alone and combined estrogen and progestin did not differ in C-reactive protein levels. The median concentration of C-reactive protein was 2.33 [interquartile range, 0.97–4.44] mg/liter in estrogen alone and 3.03 [interquartile range, 1.42–4.26] mg/liter in combined estrogen and progestin users (p = 0.97). Results similar to those presented in table 2 were obtained when 11 women with C-reactive protein levels of >10 mg/liter were excluded.

The difference in C-reactive protein levels by HRT use remained significant after adjusting for all the covariates in table 2 in addition to body mass index. In this model, only HRT use (p < 0.001) and higher body mass index (p < 0.001) were associated with higher levels of C-reactive protein (overall adjusted R2 = 0.27). The difference in levels of C-reactive protein by HRT use adjusting for some measure of obesity/fat was maintained regardless of what measure was used (i.e., body mass index, waist/hip ratio, visceral or subcutaneous fat, and weight gain from age 20; data not shown).

Measures of central adiposity (waist/hip ratio, waist girth, visceral fat) and overall adiposity (body mass index, subcutaneous fat, weight, percent body fat) were correlated (ranging from weakly to strongly) with C-reactive protein levels (table 3). As indicated in figure 1, C-reactive protein increased linearly across quartiles of visceral adipose tissue (p < 0.001). Similar results were obtained for association between C-reactive protein and body mass index and waist/hip ratio (data not shown). Associations between measures of adiposity and C-reactive protein appeared to be stronger among women who did not report use of HRT. The body mass index measured at the baseline visit was positively correlated with eighth year postmenopause C-reactive protein levels in both HRT users (r = 0.28, p = 0.005) and nonusers (r = 0.57, p < 0.001). Waist circumference was not measured at the baseline visit.

Weight change from age 20 was more strongly correlated with C-reactive protein levels among HRT nonusers (table 3). Regardless of HRT use, women who gained weight from age 20 had significantly higher levels of C-reactive protein compared with women who lost weight or stayed the same (data not shown). Analysis of covariance resulted in HRT use (p = 0.002) and weight gain from age 20 (p = 0.02) being associated with levels of C-reactive protein (weight gain and body mass index were entered into the model as continuous variables).

Among HRT users and nonusers, C-reactive protein levels were significantly and positively associated with triglyceride and fibrinogen levels and negatively associated with levels of high density lipoprotein cholesterol (data in appendix table 1). Furthermore, among nonusers
C-reactive Protein in Postmenopausal Women

Results of multiple regression analysis revealed that among HRT users, significant positive correlates of C-reactive protein levels were fibrinogen (partial $R^2 = 0.25$, $p < 0.05$) and visceral fat (partial $R^2 = 0.12$, $p < 0.01$). Among HRT nonusers, significant positive correlates of C-reactive protein levels were fibrinogen (partial $R^2 = 0.31$, $p < 0.001$), serum estrone (partial $R^2 = 0.12$, $p = 0.005$), and visceral fat (partial $R^2 = 0.25$, $p < 0.001$), after entering the same variables as in the previous model (adjusted $R^2 = 0.61$). Weight change was a significant determinant of C-reactive protein levels in models that included body mass index but not visceral fat (data not shown).

There were 50 women with serum C-reactive protein measured at eighth year postmenopause and a mean of 2.7 years later. The levels of C-reactive protein were very highly correlated ($r = 0.78$, $p < 0.001$) between these two measures. The median C-reactive protein levels were 2.35 (interquartile range, 1.09–4.65) mg/liter and 1.98 (interquartile range, 0.96–3.03) mg/liter for the first and second measures of C-reactive protein, respectively.

### TABLE 1. Characteristics of participants by hormone replacement therapy status, eighth year postmenopausal visit, Healthy Women Study, Allegheny County, Pennsylvania, 1998†

<table>
<thead>
<tr>
<th>Sociodemographic and health behavior</th>
<th>HRT‡ users ($n = 101$)</th>
<th>HRT nonusers ($n = 106$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.5 (2.1)</td>
<td>59.3 (1.8)</td>
</tr>
<tr>
<td>Race (% White)</td>
<td>93 [93.0]</td>
<td>98 [92.9]†</td>
</tr>
<tr>
<td>Education &gt; high school (%)</td>
<td>80 [79.2]</td>
<td>66 [62.3]**</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>8 [7.9]</td>
<td>23 [21.9]**</td>
</tr>
<tr>
<td>Alcohol intake (g/day)</td>
<td>2.7 [0.7–7.2]</td>
<td>2.3 [0–5.0]</td>
</tr>
<tr>
<td>Physical activity (kcal/week)</td>
<td>1,516 (1,840–2,603)</td>
<td>1,626 (791–2,347)</td>
</tr>
</tbody>
</table>

**Anthropometric**

- Body mass index (kg/m²): 26.8 (4.5) vs 28.0 (6.1)
- Waist/hip ratio: 0.79 (0.07) vs 0.79 (0.09)
- Visceral fat tissue (cm²): 126.8 (91.9–155.9) vs 121.1 (90.0–170.3)
- Percent body fat: 41.0 (7.2) vs 43.0 (7.7)
- Weight change from age 20 (kg): 13.5 (7.4–19.7) vs 14.1 (6.8–23.4)
- Weight change from baseline visit (kg): 4.3 [0.9–9.0] vs 5.1 [1.2–9.1]

**Biologic and clinical**

- SBP‡ (mmHg): 121.9 (16.8) vs 121.5 (18.3)
- DBP‡ (mmHg): 71.8 (9.3) vs 72.1 (7.9)
- Pulse pressure (mmHg): 68.3 (8.0) vs 69.3 (8.9)
- Total cholesterol (mg/dl): 213.7 (28.1) vs 214.9 (42.7)
- LDL‡ cholesterol (mg/dl): 122.5 (26.3) vs 130.7 (39.0)
- HDL‡ cholesterol (mg/dl): 62.7 (54.2–75.5) vs 55.4 (45.8–74.1)*
- Triglycerides (mg/dl): 118 (84–161) vs 99 (72–130)**
- Fasting glucose (mg/dl): 90.0 (84.0–94.0) vs 90.0 (84.0–94.0)
- Fibrinogen (mg/dl): 62.7 {54.2–75.5} vs 55.4 {45.8–74.1}*
- Hysterectomy (%): 15 [14.9] vs 6 [5.7]*

* $p < 0.05$; ** $p < 0.01$.
† Data are presented as number [%], mean (standard deviation), and median (interquartile range).
‡ HRT, hormone replacement therapy; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein.
§ HRT users ($n = 92$) and HRT nonusers ($n = 99$).
¶ HRT users ($n = 84$) and HRT nonusers ($n = 91$).
# HRT users ($n = 69$) and HRT nonusers ($n = 81$).
Analysis of the use of medications revealed that of the total study population, 24 participants (11.6 percent) reported use of medication for treating hypertension. The serum concentration of C-reactive protein was statistically significantly higher ($p < 0.02$, $t$ test of log-transformed C-reactive protein values) among women taking medication (3.08 [interquartile range, 1.36–4.65] mg/liter) compared with those not reporting use of hypertension medication (2.14 [interquartile range, 0.97–3.58] mg/liter). No differences in C-reactive protein levels were found by use of lipid-lowering medication or antiinflammatory agents (data not shown).

DISCUSSION

The results of this study indicate that healthy postmenopausal women taking hormone replacement therapy have a significantly higher concentration of C-reactive protein than do women who are not currently taking HRT. Measures of central adiposity (waist/hip ratio, waist girth, visceral fat) and overall adiposity (body mass index, subcutaneous fat) were also significantly associated with C-reactive protein levels (data not shown).

TABLE 2. Levels of C-reactive protein (mg/liter) by use of hormone replacement therapy and variables measured at the eighth year postmenopausal visit, Healthy Women Study, Allegheny County, Pennsylvania, 1998*  

<table>
<thead>
<tr>
<th>Covariate</th>
<th>HRT§ users ($n = 101$)</th>
<th>HRT nonusers ($n = 106$)</th>
<th>Main effects $p$ value†,‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone use†</td>
<td>3.01 [1.31–4.31]</td>
<td>1.74 [0.95–2.73]</td>
<td>0.002</td>
</tr>
<tr>
<td>Current smokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3.63 [2.23–4.84]</td>
<td>2.06 [0.82–2.65]</td>
<td>0.018</td>
</tr>
<tr>
<td>No</td>
<td>2.99 [1.27–4.31]</td>
<td>1.73 [0.94–2.88]</td>
<td>0.438</td>
</tr>
<tr>
<td>Alcohol intake &gt; 3 g/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.54 [0.95–3.88]</td>
<td>1.04 [0.72–2.48]</td>
<td>0.001</td>
</tr>
<tr>
<td>No</td>
<td>3.33 [1.79–4.44]</td>
<td>2.02 [1.11–2.76]</td>
<td>0.032</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whites</td>
<td>2.66 [1.29–4.19]</td>
<td>1.71 [0.92–2.65]</td>
<td>0.045</td>
</tr>
<tr>
<td>Blacks</td>
<td>4.74 [3.43–13.90]</td>
<td>2.71 [1.68–7.50]</td>
<td>0.004</td>
</tr>
<tr>
<td>Education &gt; high school</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3.07 [1.28–4.35]</td>
<td>1.66 [0.82–2.73]</td>
<td>0.007</td>
</tr>
<tr>
<td>No</td>
<td>2.66 [1.39–4.10]</td>
<td>2.14 [1.01–2.94]</td>
<td>0.332</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4.09 [2.09–8.30]</td>
<td>1.41 [0.81–2.22]</td>
<td>0.04</td>
</tr>
<tr>
<td>No</td>
<td>3.07 [1.24–4.34]</td>
<td>1.73 [0.95–2.65]</td>
<td>0.857</td>
</tr>
</tbody>
</table>

* Data are presented as median (interquartile range).  
† $p$ value based on analysis of covariance of log-transformed C-reactive protein values.  
‡ None of the interactions was statistically significant.  
§ HRT, hormone replacement therapy.  
¶ Analysis of covariance showed that log-transformed C-reactive protein values differed significantly by HRT use ($p < 0.001$) after adjusting for body mass index (continuous variable) in addition to the variables in the table; adjusted geometric mean of C-reactive protein among HRT users (2.87 mg/liter) and HRT nonusers (1.76 mg/liter).

TABLE 3. Correlation between serum concentration of C-reactive protein and variables related to body composition measured at the eighth year postmenopausal visit by use of hormone replacement therapy, Healthy Women Study, Allegheny County, Pennsylvania, 1998†  

<table>
<thead>
<tr>
<th>Covariate</th>
<th>HRT§ users ($n = 101$)</th>
<th>HRT nonusers ($n = 106$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>0.20*</td>
<td>0.54**</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.33**</td>
<td>0.58**</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.18</td>
<td>0.44**</td>
</tr>
<tr>
<td>Waist girth</td>
<td>0.28**</td>
<td>0.05**</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>0.27**</td>
<td>0.55**</td>
</tr>
<tr>
<td>Subcutaneous fat</td>
<td>0.33**</td>
<td>0.46**</td>
</tr>
<tr>
<td>Percent body fat§</td>
<td>0.45**</td>
<td>0.48**</td>
</tr>
<tr>
<td>Weight change from age 20¶</td>
<td>0.33**</td>
<td>0.52**</td>
</tr>
<tr>
<td>Weight change from baseline visit</td>
<td>0.25*</td>
<td>0.21*</td>
</tr>
</tbody>
</table>

* $p < 0.05$; ** $p < 0.01$.  
† Data are presented as Spearman’s rho correlation coefficient.  
‡ HRT, hormone replacement therapy.  
§ HRT users ($n = 74$) and HRT nonusers ($n = 87$).  
¶ HRT users ($n = 84$) and HRT nonusers ($n = 91$).  
Analysis of the use of medications revealed that of the total study population, 24 participants (11.6 percent) reported use of medication for treating hypertension. The serum concentration of C-reactive protein was statistically significantly higher ($p = 0.02$, $t$ test of log-transformed C-reactive protein values) among women taking medication (3.08 [interquartile range, 1.36–4.65] mg/liter) compared with those not reporting use of hypertension medication (2.14 [interquartile range, 0.97–3.58] mg/liter). No differences in C-reactive protein levels were found by use of lipid-lowering medication or antiinflammatory agents (data not shown).
C-reactive Protein in Postmenopausal Women

FIGURE 1. Median C-reactive protein levels by visceral fat and eighth year postmenopausal hormone replacement therapy (HRT) use ($n = 191$), Healthy Women Study, Allegheny County, Pennsylvania, 1998. *, $p < 0.01$ for linear trend within hormone replacement therapy category.

Differences in adiposity and weight gain did not appear to account for differences in C-reactive protein by HRT use. The difference in levels of C-reactive protein by HRT use adjusting for some measure of obesity or adiposity was maintained regardless of which measure of adiposity was used (i.e., body mass index, waist/hip ratio, visceral or subcutaneous fat, weight gain from age 20).

In this sample of the Healthy Women Study population, HRT users appeared to be somewhat healthier than nonusers (more educated and fewer smoked), which is consistent with previously reported comparisons of future HRT users and nonusers from the Healthy Women Study (33). Women taking HRT had higher triglyceride and high density lipoprotein cholesterol levels and lower subcutaneous adipose tissue. With the exception of the higher triglyceride levels among HRT users, these findings are not consistent with the higher levels of C-reactive protein in this group, and adjusting for these factors did not change the relation between HRT use and C-reactive protein concentrations. In our population, past users of HRT had levels of C-reactive protein similar to those of never users and statistically significantly lower than those of current users, which suggests that HRT use may have short-term effects. This is consistent with a recent report of the short-term (12-week) effect of HRT (estrogen alone or estrogen plus progestin) on increasing C-reactive protein levels (20).

There were relatively few long-term cigarette smokers in this study. At the eighth year postmenopausal visit, only 15 percent of the women reported being current smokers. As shown in table 2, smokers had consistently higher levels of C-reactive protein than did nonsmokers in HRT users and nonusers, although not statistically significant. The relatively weak effect of cigarette smoking is probably related to the inverse association of smoking with measures of body mass (data not shown).

It has been hypothesized that adipose tissue may play a role in the regulation of serum concentrations of C-reactive protein via secretion of inflammatory cytokines (34), specifically tumor necrosis factor-$\alpha$ and interleukin-6 production (35, 36) and thus be an important determinant of low level, chronic, inflammatory states (17). Our data support this hypothesis as all measures of adiposity were positively correlated with C-reactive protein levels, including measures of visceral and subcutaneous adipose tissue. These findings are consistent with recently published data from the Third National Health and Nutrition Examination Survey, which found levels of C-reactive protein to be positively associated with body mass index and waist/hip ratio independent of known inflammatory diseases and other factors related to elevated C-reactive protein (37). Cushman et al. (19) proposed a hypothesis that in the setting of obesity, HRT may influence inflammation through adipocyte function. Moreover, increased fat also influences postmenopausal estrogen metabolism when the main source of estrone is aromatization in peripheral fat tissue, so that women with
more fat tend to have higher levels of estrone. This is consistent with our new findings of a positive moderate association between serum estrone and C-reactive protein levels and a weak association (but statistically significant) after adjusting for body mass index or visceral fat. Further research is needed to understand how endogenous and exogenous hormones affect the inflammatory process in women.

Black participants had significantly higher C-reactive protein levels than did Whites, with Black HRT users having the highest levels (similar to findings from the Cardiovascular Health Study) (19). This difference persisted even after adjusting for body mass index or visceral adipose tissue. These racial differences must be interpreted with caution, given the small number of Black women in this population. Further studies examining racial differences in C-reactive protein levels are indicated.

Analysis of a subgroup of our study population with serum C-reactive protein measured a mean of 2.7 years apart revealed a very high, positive, and significant correlation between these two measures, which has positive implications for the reproducibility of measuring C-reactive protein. Moreover, even among women with C-reactive protein measured 4 years apart, the correlation was high ($r = 0.65$, $p = 0.02$, $n = 13$). These results indicate that women with high-normal levels are more likely to have high levels years later, which has potential implications in the identification of women at future high risk for cardiovascular events. This is clearly important in light of recent findings that higher levels of C-reactive protein predict future cardiovascular disease events in women (9, 38).

In conclusion, HRT is associated with higher serum levels of C-reactive protein among apparently healthy postmenopausal women, for reasons that remain unknown. Basic science research and clinical trials are needed to corroborate and explain the impact of HRT on inflammation. We were also able to show that HRT users with greater adiposity had the highest levels of C-reactive protein. Furthermore, a good deal of the variation in C-reactive protein levels could be explained by direct and indirect measures of adiposity and weight gain, especially among nonusers of HRT. This association between inflammation and fat may explain to some extent the obesity-related increase in risk of cardiovascular disease. The newly reported positive association between levels of C-reactive protein and serum estrone in nonusers of HRT may be mediated by obesity. The association of C-reactive protein and cardiovascular disease has not been proven to be part of a “causal pathway.” C-reactive protein may be only a marker of inflammation and the extent of obesity and of known increased production of proinflammatory cytokines, tumor necrosis factor-α, and interleukin-6, in obesity.

ACKNOWLEDGMENTS

This work was supported by grants from the National Heart, Lung, and Blood Institute (HL 07011 and HL 28266).

REFERENCES


APPENDIX

APPENDIX TABLE 1. Correlation† between serum concentration of C-reactive protein and other variables by use of hormone replacement therapy at the eighth year postmenopausal visit, Healthy Women Study, Allegheny County, Pennsylvania, 1998

<table>
<thead>
<tr>
<th>Variable</th>
<th>HRT‡ users (n = 101)</th>
<th>HRT nonusers (n = 106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.06</td>
<td>–0.07</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>–0.19</td>
<td>–0.24*</td>
</tr>
<tr>
<td>Physical activity</td>
<td>–0.10</td>
<td>–0.25**</td>
</tr>
<tr>
<td>SBP‡</td>
<td>0.19</td>
<td>0.15</td>
</tr>
<tr>
<td>DBP‡</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.002</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>–0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>–0.22*</td>
<td>–0.38**</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.43**</td>
<td>0.29**</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.18</td>
<td>0.12</td>
</tr>
<tr>
<td>Estradiol§</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Estrone§</td>
<td>0.38**</td>
<td></td>
</tr>
<tr>
<td>Testosterone¶</td>
<td>–0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>Androstenedione¶</td>
<td>–0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>0.18</td>
<td>0.25**</td>
</tr>
<tr>
<td>Fibrinogen#</td>
<td>0.39**</td>
<td>0.54**</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01.
† Spearman's rho correlation coefficients.
‡ HRT, hormone replacement therapy; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein.
§ HRT nonusers (n = 96).
¶ HRT users (n = 54) and HRT nonusers (n = 97).
# HRT users (n = 69) and HRT nonusers (n = 81).