Plasma C-reactive protein is not elevated in physically active postmenopausal women taking hormone replacement therapy

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Stauffer, Brian L., Greta L. Hoetzer, Derek T. Smith, and Christopher A. DeSouza. Plasma C-reactive protein is not elevated in physically active postmenopausal women taking hormone replacement therapy. J Appl Physiol 96: 143–148, 2004. Published August 29, 2003; 10.1152/japplphysiol.00360.2003.—We tested the hypothesis that hormone replacement therapy (HRT)-related increases in C-reactive protein (CRP) would either be blunted or absent in postmenopausal women who regularly perform endurance exercise. Plasma CRP is an independent predictor of future cardiovascular events in healthy men and women. Oral HRT increases plasma CRP concentrations in postmenopausal women. Regular aerobic exercise reduces the risk of cardiovascular events and is associated with lower CRP concentrations in adults. To date, no study has evaluated the influence of habitual physical activity on the elevation of CRP associated with HRT. Plasma CRP concentrations were measured in 114 postmenopausal women: 39 physically active (endurance trained) and 75 sedentary postmenopausal subjects. Sixty-five women were users of HRT (22 physically active and 43 sedentary), and 49 were nonusers of HRT (19 physically active and 32 sedentary). CRP levels were ~75% higher (P < 0.01) in the sedentary users vs. nonusers of HRT (1.9 ± 1.8 vs. 1.1 ± 1.0 mg/l). In contrast, there was no difference in CRP levels between the physically active users and nonusers of HRT (0.6 ± 0.4 vs. 0.4 ± 0.2 mg/l; P = 0.61). Regardless of HRT status, CRP concentrations were ~65% lower in the physically active compared with sedentary women. In conclusion, physically active postmenopausal women exhibit lower plasma CRP concentrations compared with sedentary controls. Importantly, the HRT-related elevation in plasma CRP levels observed in sedentary women is absent in women who engage in regular endurance exercise. These data suggest that habitual physical activity may prevent the elevation in CRP concentrations due to HRT.

exercise; inflammation; cytokine

VASCULAR INFLAMMATION has been suggested to be integrally linked with the development of atherosclerotic disease (36). C-reactive protein (CRP), a marker of systemic inflammation, is an independent predictor of cardiovascular events in healthy women (28, 29). Oral hormone replacement therapy (HRT; both estrogen alone as well as in combination with progesterone) has been shown to increase plasma CRP levels in postmenopausal women (10, 11, 30, 43). Although it is not yet possible to conclude that an increased CRP level from HRT use is an indicator of cardiovascular risk (23), elevations in plasma CRP concentrations are predictive of future adverse cardiovascular events in women regardless of the presence or absence of hormone therapy (27). Moreover, a recent study suggests that CRP is a stronger predictor of cardiovascular events than the low-density lipoprotein (LDL) cholesterol level in women (34). Several large prospective trials have reported an increased risk of cardiovascular events associated with HRT (estrogen combined with progesterone) (18, 24, 37). Although no causality has been established, it has been postulated that the initiation of HRT increases CRP levels and results in increased plaque instability and a propensity to thrombosis (10, 11, 27, 28). This hypothesis is strengthened by the recent immunohistochemical evidence of CRP incorporation in human coronary plaque (40, 45) and the potential causative role in atherosclerosis (8, 21, 22, 40, 41).

Given the association between CRP and cardiovascular events, there is increasing clinical interest in interventions that attenuate or prevent the rise in CRP. For example, use of inhibitors of hydroxymethylglutaryl-coenzyme A reductase ("statins") have been shown to decrease plasma CRP concentrations independent of cholesterol lowering (32, 33). Recently, Ridker et al. (31) postulated that statin therapy may be effective for primary prevention of cardiovascular events in a low-cholesterol, high-CRP population. A subsequent decision-analytic model estimated a 6-mo increase in life expectancy with statin use in individuals without overt hypercholesterolemia but with elevated CRP levels (5). Regular physical activity has been shown to favorably affect plasma CRP concentrations in young men training for a marathon (19). In addition, several cross-sectional studies reported a significant association between higher levels of physical activity and lower CRP concentrations in healthy middle-aged and older adults (1, 9, 15). To date, no study has evaluated the influence of habitual physical activity on the elevation of CRP associated with HRT. Given the clinical benefit associated with lower CRP levels observed in patients using statin therapy (31), it may be reasonable to hypothesize that attenuating or preventing the HRT-associated elevation in CRP levels with regular physical activity may result in comparable cardiovascular benefit. This is of particular importance because lifestyle modification remains the cornerstone of any intervention to reduce cardiovascular risk.

The present study was designed to test the hypothesis that HRT-related increases in CRP would either be blunted or absent in postmenopausal women who regularly perform endurance exercise. We used a cross-sectional study design, in which resting plasma CRP concentrations were measured in sedentary and habitually physically active postmenopausal women who were either taking or not taking HRT.

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MATERIALS AND METHODS

Subjects. One hundred fourteen healthy postmenopausal women ranging in age from 49 to 80 yr were recruited from the local community as a part of a vascular health evaluation: 39 physically active (endurance trained) women and 75 sedentary control subjects. The study population comprised primarily Caucasian (88%) and Hispanic (10%) women. The postmenopausal physically active women had been performing regular endurance exercise (distance running) for 17 ± 8 (SD) yr (range 6–26 yr), and they ran an average of 50 ± 13 km/wk (16–83 km/wk). The sedentary women had not participated in a regular exercise program for at least 1 yr before the start of the study. All of the women were at least 1 yr postmenopausal (11 ± 8 yr, 1–48 yr) as previously described (16, 38). Among the 114 postmenopausal women, 65 were users of HRT (22 physically active and 43 sedentary) and 49 were nonusers (17 physically active and 32 sedentary). All of the HRT users were taking an oral regimen of either unopposed estrogen (Premarin 0.3–2.5 mg/day) or conjugated estrogen in combination with medroxyprogesterone acetate (Premarin 0.625–1.0 mg/day; Provera, 2.5 mg/day) for at least 1 yr before the study (9 ± 9 yr; 1–48 yr). We observed no differences in plasma concentrations of CRP between the users of estrogen alone or estrogen combined with progesterone [consistent with prior studies (10, 30)]; therefore, the data were pooled and presented together. All subjects were free of overt disease as assessed by medical history, physical examination, and resting and exercise electrocardiograms. None of the subjects smoked or was taking medications other than hormone replacement (including aspirin, nonsteroidal anti-inflammatory medications, or statins). Before participation, all of the subjects had the research study and its potential risks and benefits explained fully before providing written informed consent according to the guidelines of the University of Colorado at Boulder.

Body composition. Body mass was measured to the nearest 0.1 kg by using a medical beam balance (Detecto, Webb City, MO). Percent body fat was determined by dual-energy X-ray absorptiometry (model DPX-IQ, Lunar Radiation, Madison, WI). Body mass index (BMI) was calculated as weight (in kg) divided by height (in m) squared.

Maximal oxygen consumption. To assess aerobic fitness, maximal oxygen consumption (VO$_2$max) was measured by using an online computer-assisted open-circuit spirometry during incremental exercise on a motorized treadmill as previously described (14). A true VO$_2$max was accepted when at least three of the following criteria were met: 1) a plateau in oxygen consumption with increasing work rate (<100 ml/min or <1.0 ml·kg$^{-1}$·min$^{-1}$), 2) respiratory exchange ratio at maximal exercise >1.10, 3) achievement of age-predicted maximal heart rate (220 − age), and 4) a rating of perceived exertion >17 (Borg scale) (9).

Metabolic measurements. Fasting plasma lipid and lipoprotein, glucose, and insulin concentrations were determined by using conventional methods by the clinical laboratory affiliated with the General Clinical Research Center as previously described (12).

Measurement of CRP and interleukin-6. Blood samples for the determination of circulating levels of CRP and interleukin-6 (IL-6) were collected in chilled EDTA tubes with minimal venostasis, after a 12-h overnight fast. In the endurance-trained subjects, blood samples for CRP determination were collected at least 24 h after the last bout of exercise to avoid the immediate (acute) effects of exercise while still representing their normal physiological state (i.e., habitually exercising) (20). Plasma concentrations of CRP and IL-6 were determined in duplicate by using commercially available highly sensitive monoclonal antibody-based ELISA assays (R&D Systems, Minneapolis, MN; ALPCO Diagnostics, Windham, NH). The intra- and interassay coefficients of variation for CRP were 7.8 and 3.0% and for IL-6 were 6.5 and 7.0%, respectively.

A standardized questionnaire designed to detect and document recent infection and/or inflammation (<2 wk) was administered before the phlebotomies. Subjects with a history of recent infection and/or inflammation did not receive a phlebotomy to avoid confounding effects from potential acute infection- and/or inflammation-associated changes in CRP concentration (27).

Statistical analysis. Because of the skewed distribution of plasma CRP concentrations, the data were log-transformed to satisfy the basic assumptions for parametric testing. Differences between the physically active and sedentary groups and users and nonusers of HRT for all selected variables were determined by a multivariate ANOVA (training status × HRT status). We observed no significant interaction effect for any variable of interest. As a result, when indicated by a significant main effect, Newman-Keuls post hoc test for multiple comparisons was used to assess differences between specific group means. Importantly, per joint American College of Cardiology and Centers for Disease Control recommendations (23), the absolute values for CRP are presented to facilitate clinical interpretation. Simple and forward stepwise multiple regression analyses were used to determine relations between variables of interest. All data are expressed as means ± SD. Statistical significance was set at P < 0.05.

RESULTS

Subjects. There were no significant differences in age and resting blood pressure between the physically active and sedentary women. However, percent body fat, BMI, and waist circumference were lower (all P < 0.05), and VO$_2$max was higher (P < 0.05), in the physically active women (Table 1). There were no significant differences in any of these subject characteristics between women taking and not taking HRT. The average years of use were similar between the sedentary and physically active users of HRT (9 ± 9 vs. 9 ± 10 yr).

Table 1. Selected physical characteristics of the subjects grouped by physical activity status and subgrouped by hormone replacement status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonusers (n=32)</th>
<th>Users (n=43)</th>
<th>Mean (n=75)</th>
<th>Nonusers (n=17)</th>
<th>Users (n=22)</th>
<th>Mean (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>62 ± 9</td>
<td>59 ± 8</td>
<td>60 ± 8</td>
<td>58 ± 9</td>
<td>59 ± 6</td>
<td>58 ± 7</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>64.5 ± 9.3</td>
<td>66.7 ± 13.7</td>
<td>65.8 ± 12.1</td>
<td>59 ± 6.2</td>
<td>58.4 ± 5.5</td>
<td>58.7 ± 5.6</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>36.9 ± 7.2</td>
<td>37.2 ± 8.7</td>
<td>37.1 ± 8.0</td>
<td>26.2 ± 6.7</td>
<td>27.0 ± 6.2</td>
<td>26.7 ± 6.4</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>82.5 ± 9.4</td>
<td>81.4 ± 12.6</td>
<td>82.4 ± 11.3</td>
<td>75.3 ± 5.6</td>
<td>73.7 ± 4.9</td>
<td>74.6 ± 5.2</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>24.4 ± 3.4</td>
<td>25.9 ± 4.6</td>
<td>25.3 ± 4.2</td>
<td>22.4 ± 2.0</td>
<td>21.8 ± 1.8</td>
<td>22.0 ± 1.9</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>120 ± 11</td>
<td>125 ± 12</td>
<td>123 ± 12</td>
<td>120 ± 13</td>
<td>119 ± 13</td>
<td>119 ± 12</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>72 ± 9</td>
<td>71 ± 9</td>
<td>71 ± 9</td>
<td>72 ± 9</td>
<td>73 ± 7</td>
<td>73 ± 8</td>
</tr>
<tr>
<td>VO$_2$max, ml·kg$^{-1}$·min$^{-1}$</td>
<td>25.5 ± 6.6</td>
<td>24.4 ± 4.9</td>
<td>24.8 ± 5.7</td>
<td>35.1 ± 8.1</td>
<td>35.0 ± 6.0</td>
<td>35.0 ± 6.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; VO$_2$max, maximal oxygen consumption. *P < 0.05 vs. sedentary group.
there was no significant difference in CRP levels between the lower (both parts (Fig. 1).

0.4/H11006 nonusers of HRT (1.9/H11006). No significant differences were observed in LDL cholesterol, high-density lipoprotein cholesterol, or fasting glucose (Table 2). There were no differences in any of these characteristics in the users vs. nonusers of HRT.

Metabolic characteristics. Whereas the physically active women demonstrated statistically significantly lower plasma triglycerides, total cholesterol, and insulin concentrations, all values were within the clinically normal range (20). No significant differences were observed in LDL cholesterol, high-density lipoprotein cholesterol, or fasting glucose (Table 2). There were no differences in any of these characteristics in the users vs. nonusers of HRT.

Plasma CRP and IL-6 concentrations. Plasma CRP levels were ~75% higher (P < 0.01) in the sedentary users vs. nonusers of HRT (1.9 ± 1.8 vs. 1.1 ± 1.0 mg/l). However, there was no significant difference in CRP levels between the physically active users and nonusers of HRT (0.6 ± 0.2 vs. 0.4 ± 0.2 mg/l; P = 0.61). Plasma CRP concentrations in the physically active users and nonusers of HRT were 68 and 64% lower (both P < 0.01) than their respective sedentary counterparts (Fig. 1).

There was no significant influence of HRT use on plasma IL-6 concentrations in either the sedentary or physically active women. Although we observed a main effect of training status on plasma IL-6 levels, only the physically active women not taking HRT demonstrated significantly lower IL-6 concentrations compared with their sedentary controls (1.0 ± 0.7 vs. 1.6 ± 1.0 pg/ml) (Fig. 2).

Correlation analysis. Significant univariate correlations were observed between CRP and IL-6 (r = 0.348, P < 0.01), VO2max (r = 0.378, P < 0.01), fat mass (r = 0.293, P < 0.01), BMI (r = 0.363, P < 0.01), percent fat (r = 0.405, P < 0.01), and body mass (r = 0.344, P < 0.01). When multiple regression was applied for assessment of the independence of observed relationships, only VO2max was significantly associated with CRP (R2 = 0.12).

DISCUSSION

The salient findings of the present study are that 1) habitually physically active postmenopausal women demonstrate lower plasma CRP concentrations than their sedentary peers and 2) the HRT-related elevation in plasma CRP concentrations observed in the sedentary women was absent in the physically active postmenopausal women. To our knowledge, this is the first study to examine the influence of regular physical activity on the HRT-associated rise in CRP concentrations.

Regular aerobic exercise provides known cardiovascular benefits and reduces the incidence of adverse cardiovascular events in both healthy and diseased populations (4, 26, 42). Whereas acute bouts of intense exercise (i.e., marathon running) induce elevations in IL-6 and CRP (7, 13), cross-sectional (1, 9, 15) and prospective (19) studies have revealed a decrease in baseline CRP levels with habitual physical activity. Our finding of lower CRP concentrations in habitually physically active postmenopausal women is consistent with these.
Previous reports. Obtaining samples at >24 h after the last bout of exercise has been shown to be representative of steady-state serum values (17), thus portraying the focus of the present study on the chronic effects of exercise. However, the mechanism by which regular physical activity reduces CRP concentrations is unknown. In the present study, we observed lower plasma IL-6 levels associated with physical activity, which suggest a lower level of inflammation in this population. These results are consistent with previous studies that show an inverse relation between exercise and IL-6 concentrations (3).

IL-6, a proinflammatory cytokine, has been shown to stimulate CRP synthesis in the liver, and it is secreted from adipocytes in direct proportion to fat mass. The lower levels of body fatness observed in the physically active women in the present study are consistent with the differences in body composition observed in prior studies evaluating the influence of physical activity on CRP levels (1, 9). Thus it is plausible that lower CRP levels observed in the physically active women in the present study may be due, at least in part, to lower IL-6 concentrations as a result of less body fat. However, several studies have observed an increase in CRP concentration after adjustment for indexes of general obesity (1, 9). The BMI observed in the sedentary women of the present study is comparable or lower than that in the highest activity groups in these studies, suggesting the potential for a lower contribution of body fatness to CRP concentrations. Consistent with the lack of correlation between IL-6 and CRP in the sedentary women, there is evidence that CRP and IL-6 may not always track one another, especially with regard to HRT (3). This suggests that estrogen causes CRP elevation through a noninflammatory mechanism, such as a direct effect of CRP production by the liver (39). Regardless of the mechanism, lower plasma CRP concentrations in habitually physical active women may predict the reduced cardiovascular events previously noted in this population (4, 26, 42).

The HRT-associated elevation in CRP levels observed in the present investigation is consistent with previous studies (2, 10, 11, 30, 44). Although retrospective evaluations have reported cardiovascular benefit associated with HRT, prospective randomized trials for the primary and secondary prevention of cardiovascular disease demonstrated increased cardiovascular event rates in women taking HRT (24 37, 18). Recently, the combined therapy (estrogen + progesterone) arm of the Women’s Health Initiative (WHI) study was terminated because of an increased risk of adverse cardiovascular events with no apparent cardiovascular benefit (37). It has been suggested that vascular inflammation induced by HRT may contribute to this increased rate of cardiovascular events (10, 30, 43). Although our results and those of others (39) would not support a generalized inflammatory response to estrogen, there is evidence that CRP may directly destabilize vascular plaque, predisposing to plaque rupture and thrombosis (8, 21, 22, 40, 41).

CRP has been shown to be a strong independent predictor of cardiovascular events in women (29). For example, it has been estimated that elevated plasma CRP concentrations in women taking HRT predicts as much as a sevenfold increase in the risk of myocardial infarction and stroke (28). A recent nested case-control study from the WHI population revealed that CRP levels independently predict subsequent coronary heart disease events irrespective of HRT use (27). Although the clinical significance of the HRT-related elevation in CRP is still unclear, it is possible that HRT use contributed to an elevation in CRP levels observed in these women, thus predicting an increased incidence of cardiovascular events.

The salient and novel finding of the present study is that the HRT-related elevation in CRP levels observed in the sedentary women was absent in their physically active peers. In terms of relative risk, the higher CRP levels in the sedentary users of HRT are predictive of a 1.5- to 3.7-fold increase in the risk of future cardiovascular events (27, 28, 34, 35). In contrast, the CRP values of the physically active users of HRT predicted no increase in relative risk (27, 28, 34). The mechanism(s) responsible for the lack of an HRT-related increase in plasma CRP concentrations in physically active women is unclear. Although the exercise-related reduction in IL-6 levels observed in this group may have contributed to their lower plasma concentrations of CRP, it is unlikely that this mechanism contributed to the lack of an HRT-associated increase in CRP in these women. Indeed, the elevation in plasma CRP concentration observed in the sedentary women taking HRT occurred without a concomitant rise in IL-6 levels, suggesting an IL-6 independent mechanism, such as a direct effect on production by the liver (28, 39). However, the potential mechanism(s) behind the lack of elevation with exercise is unclear. Although several studies report a decrease in CRP with physical activity after accounting for changes in indexes of body fatness (1, 9), it is possible that the lower levels of body fatness observed in the habitually physically active women may underlie the lower CRP levels. Although this does not negate the salient finding of the present study, future studies are needed to elucidate the interaction between regular exercise and HRT use on plasma CRP concentrations.

The clinical importance of reducing circulating CRP concentrations with regular physical activity remains to be verified. However, a recent statin intervention trial suggests that CRP may be a modifiable marker of risk (33). Statin therapy has been shown to lower CRP levels independent of cholesterol lowering (32, 33) and has reduced cardiovascular risk in patients with normal LDL and elevated CRP (31). Multiple statin intervention studies, for as short as 14 days to as long as 5 yr, report a 15–30% reduction in CRP concentrations (25, 31–33). In the present study, CRP levels in the habitually physically active women were more than 60% lower than their sedentary peers. Given the expense and potential adverse drug reactions of the prolonged statin therapy needed to produce only modest decreases in CRP levels, increased attention to prevention through regular physical activity is clinically attractive.

There are a number of limitations of the present study. First, given the cross-sectional design, we cannot overlook the inherent possibility that constitutional and genetic factors may have influenced our findings. However, it is important to note that the HRT- and physical activity-related differences in CRP levels observed in the present study are similar to those noted in interventional trials (19, 43). Second, we relied on a single baseline blood sample and therefore cannot account for changes in plasma CRP levels that may occur over time. For example, variability in CRP concentrations can be seen within 2 wk of acute inflammatory or infectious insults (33). However, all subjects in the present study denied any recent events in response to a validated infection questionnaire. Third, be-
cause of the relatively small sample size in the physically active groups, we are unable to address an exercise dose-response influence. Finally, our study was neither designed nor powered to evaluate hard cardiovascular end points. Larger prospective trials are necessary to determine whether preventing or reducing HRT-associated elevations in CRP is associated with a lower risk of cardiovascular events. However, the recent findings of the WHI (37) may prohibit (because of ethical considerations) this type of evaluation in healthy postmenopausal women.

In conclusion, physically active postmenopausal women exhibit lower plasma CRP levels compared with sedentary controls. Importantly, the HRT-related elevation in plasma CRP concentration observed in sedentary women was not evident in women who perform regular aerobic exercise. These data provide further support for the cardioprotective effects of a physically active lifestyle. Given the recent unfavorable clinical findings of the WHI, regular physical activity may be an important cotherapy in women using HRT for menopausal symptoms.

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