Increased Levels of C-Reactive Protein After Oral Hormone Replacement Therapy May Not Be Related to an Increased Inflammatory Response

Antonello Silvestri, MD; Otavio Gebara, MD; Cristiana Vitale, MD; Mauricio Wajngarten, MD; Filippo Leonardo, MD; Josè A.F. Ramires, MD; Massimo Fini, MD; Giuseppe Mercuro, MD; Giuseppe M.C. Rosano, MD, PhD

Background—It has been suggested that hormone replacement therapy (HRT) in postmenopausal women is associated with an increased inflammatory response that may trigger acute cardiovascular events. This suggestion is mainly based on the finding of elevated C-reactive protein (CRP) levels after HRT. The aim of the present study was to evaluate a broad spectrum of vascular inflammation markers in 389 postmenopausal women with increased cardiovascular risk at baseline and after either 6 months of HRT (126 women) or no HRT (263 women).

Methods and Results—Compared with baseline, CRP levels significantly increased after HRT (0.9±0.2 versus 1.6±0.4 mg/L, $P<0.01$); on the contrary, soluble intracellular adhesion molecule-1 decreased from 208±57 to 168±37 ng/mL ($P<0.01$) after HRT. Similarly, vascular cell adhesion molecule-1 decreased from 298±73 to 258±47 ng/mL ($P<0.01$), plasma E-selectin levels were reduced from 17.8±5.6 to 14.8±3.9 ng/mL ($P<0.01$), interleukin-6 levels decreased from 1.51±0.22 to 1.29±0.28 pg/mL, and s-thrombomodulin plasma levels decreased from 4.8±0.7 to 4.3±0.9 ng/mL ($P<0.01$). No significant changes in either CRP or vascular inflammatory marker were detected in women not taking HRT.

Conclusions—The discrepancy between increased plasma levels of CRP and reduced plasma levels of all other markers of inflammation suggests that the increased CRP levels after oral HRT may be related to metabolic hepatic activation and not to an acute-phase response. HRT seems to be associated with an overall decrease in vascular inflammation. **(*Circulation*. 2003;107:3165-3169.)**

Key Words: cardiovascular diseases ■ inflammation ■ interleukins

Atherosclerosis is associated with chronic inflammation, and the adhesion between vascular endothelial cells and circulating leukocytes plays a key role in the inflammatory process.[1,2] A feature of most forms of inflammation or tissue damage is the increased serum concentration of acute-phase reactants such as C-reactive protein (CRP). This protein is secreted by hepatocytes in response to interleukin 6 (IL-6) and tumor necrosis factor α (TNFα). Besides, CRP may act as a procoagulant, because it induces the expression of monocyte tissue factor that plays an important role in atherosclerosis.[3] Although the underlying mechanisms triggering the inflammatory response in atherosclerosis are unknown, elevation of CRP levels in the context of cardiovascular disease is involved in the pathophysiology of progression of atherosclerosis and in its complications. Elevated serum CRP levels have been reported in patients with myocardial infarction, unstable angina, and chronic stable angina.[4] In these patients, high plasma levels of CRP predict a poor prognosis and a rapid progression of cardiovascular disease.[5,6] CRP activates the complement cascade, the components of which have been implicated in early stages of atherogenesis, and has been colocalized with complement components in atherosclerotic lesions of human coronary arteries.[7,8] CRP is an independent marker for the risk of cardiovascular disease in men with and without coronary artery disease and in postmenopausal women without clinically evident coronary artery disease.[9]

It is important to note, however, that the cutoff value above which individuals without known disease should be considered at elevated risk and below which level patients with coronary artery disease should be considered at low risk requires additional evaluation in population studies and a consensus. Also, it must be acknowledged that in some vascular disorders, CRP levels may remain elevated for years without ever leading to an acute cardiovascular event.[10,11]

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From the Cardiovascular Research Unit (A.S., C.V., F.L., M.F., G.M.C.R.), San Raffaele–Roma, Tosinvest Sanità, Italy, and INCOR (O.G., M.W., J.A.F.R., G.M.), University of Sao Paulo, Brazil.

Correspondence to Giuseppe M.C. Rosano, MD, PhD, Cardiovascular Research Unit, San Raffaele–Roma, via della Pisana 235, 00163 Roma, Italy. E-mail giuseppe.rosano@sanraffaele.it

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The aim of the present study was to evaluate the effect of hormone replacement therapy (HRT) on the overall inflammatory milieu and, in particular, to assess the relative importance in the change in CRP levels compared with that of other indices of inflammation in postmenopausal women at increased cardiovascular risk (>20%/10 years) receiving HRT.

Methods

Population

The study population included 389 consecutive postmenopausal women with increased cardiovascular risk (>20%/10 years) referred for a full routine cardiovascular assessment.

All women underwent full cardiac evaluation, including echocardiogram and exercise testing if needed, and were asked to complete a questionnaire regarding lifestyle practices and risk factors for both cardiovascular disease and cancer. Women with ischemic heart disease, primary valvular disease, or myocardial disease were excluded from the study as those with contraindications to HRT. Only women with no prior history of cancer or chronic inflammatory condition were included in the study. Cardiovascular risk was evaluated according to the European Society of Cardiology–European Atherosclerosis Society (ESC-EAS) joint guidelines.[12]

Study Protocol

The 389 patients who met the inclusion criteria for the study were proposed to start HRT. Women who decided to start HRT were given conjugated equine estrogens (0.625 mg) and continuous combined medroxyprogesterone acetate (MPA 2.5 mg). Women who decided not to take HRT were selected as the control group. Study subjects and controls underwent blood sampling for evaluation of vascular inflammation markers at baseline and at 3 and 6 months. Venous blood samples were taken after at least 10 hours of fasting in a supine position after 20 minutes of rest with a Vacutainer system (Becton Dickinson). Blood samples were collected in tubes containing EDTA or trisodium citrate (1:9 vol/vol) and were immediately placed on ice and centrifuged within 1 hour from collection. Plasma was divided into aliquots and stored at −80°C until laboratory analysis. All serum was assessed in duplicate. Plasma samples obtained were thawed and assayed for CRP by use of a high-sensitivity assay with a coefficient of variation below 5% (Dade Behring). An ELISA method was also used to measure IL-6, intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), E-selectin plasma concentrations (R&D Systems Inc), and s-thrombomodulin (Imubind, Thrombomodulin, American Diagnostica Inc). The lower limit for the detection of IL-6 was 0.2 ng/L; the blood sedimentation rate was measured according to the method of Wintrobe. Complement fractions C3 and C4 were measured by radial immunodiffusion method on agar gel plate (Immunotil, Chematil s.r.l). Normal values for C3 and C4 with this method are 138 mg/dL (95% CI, 84 to 193) and 28 mg/dL (95% CI, 20 to 40), respectively.

Statistical Analysis

Values are given as mean±SD or as percentages where appropriate. Because of the skewed distribution of CRP and IL-6 plasma levels, a nonparametric test (Wilcoxon signed-rank test) was used to compare median values at baseline and at follow-up assessment. A nonparametric repeated-measures ANOVA (Friedman test) was used to test statistical difference on the effect of treatment among groups, whereas the Wilcoxon signed-rank test was used to test statistical difference between groups. Correlation between variables was calculated with the Spearman's correlation coefficient. $P<0.005$ was considered for statistical significance.

Results

After baseline evaluation, 126 women decided to initiate HRT, whereas 263 refused to take HRT. The clinical features of the 2 groups are shown in Table 1. Women not taking HRT were slightly older and had a longer time since menopause. At baseline, body mass index (BMI) was significantly higher in patients not receiving HRT. At follow-up, no significant changes in BMI were noted within groups. The prevalence of risk factors for coronary artery disease was evenly distributed among groups; indeed, there were no significant differences between groups in terms of smoking status, hyperlipidemia, hypertension, exercise frequency, alcohol use, or family history of coronary artery disease. At 6 months, a complete set of blood samples was obtained in 124 subjects undergoing HRT and in 212 controls.

TABLE 1. Clinical Characteristics of Study Subjects

	Patients Undergoing HRT (n=126)	Patients Not Undergoing HRT (n=263)
Mean age, y	55.2±3.1	57.8±6.5
Time since menopause, y	2.3±1.1	5.2±4.1
Body mass index, kg/m^2	23.3±2.4	28.2±4.4
Patients with risk factors for coronary artery disease		
Cholesterol >220 mg/dL	45	84
Diabetes	19	32
Hypertension	35	63
Cigarette smoking	57	100
Family history	63	145

As shown in Table 2 and Figure 1, plasma levels of CRP increased significantly after 3 months of treatment with HRT and remained unchanged at 6 months (0.9±0.2 mg/L baseline versus 1.6±0.4 mg/L after 6 months of treatment; $P<0.01$). By contrast, no difference in plasma levels of CRP was detected in women not using HRT between baseline and follow-up evaluations (1.35±0.5 mg/L baseline versus 1.37±0.6 mg/L after 6 months of treatment; P=NS). The plasma levels of soluble ICAM-1 (sICAM-1), sVCAM-1, IL-6, E-selectin, and soluble thrombomodulin were all significantly reduced by HRT after both 3 and 6 months while remained unchanged in nonusers (Table 2). In detail, IL-6 plasma levels decreased by 12% and 14% after 3 and 6 months of therapy; similarly, sVCAM-1 plasma levels decreased by 9.2% and 13.4%, sICAM-1 plasma levels decreased by 26% and 24%, E-selectin plasma levels decreased by 16% and 18%, and soluble thrombomodulin plasma levels decreased by 8% and 10%. Figure 1 summarizes changes in inflammation markers at 3 and 6 months in women receiving HRT. Figure 2 shows changes in inflammatory markers in patients with diabetes mellitus (n=19) and in those taking aspirin (n=21) and statins (n=32). After 6 months, plasma levels of CRP increased significantly in all subgroups of women receiving HRT, but no significant differences between subgroups were detected. In detail, CRP plasma levels increased by 82% in patients with diabetes, by 68% in patients taking statins, and by 78% in patients taking aspirin. On the other hand, plasma levels of sICAM-1, sVCAM-1, E-selectin, s-thrombomodulin, and IL-6 were significantly reduced by HRT in women in these subgroups. In detail,

TABLE 2. Plasma Levels of Inflammatory Markers and Complement Subfractions at 6 Months in Women Receiving or Not Receiving HRT

	HRT		No HRT	
	Baseline	6 Months	Baseline	6 Months
CRP, mg/L	0.9±0.2	1.6±0.4*	1.35±0.5	1.37±0.6
sICAM-1, ng/mL	208±57	168±37*	262±102	280±64
sVCAM-1, ng/mL	298±73	258±47*	277±65	278±91
E-selectin, ng/mL	17.8±5.6	14.8±3.9*	18.2±6.1	18.3±5.9
s-Thrombomodulin, ng/mL	4.8±0.7	4.3±0.9*	5.1±0.8	5.05±0.6
IL-6, pg/mL	1.51±0.22	1.29±0.28*	1.48±0.20	1.49±0.25
C3, mg/dL	34±7.4	36±11.1	39±6.2	40±7.4
C4, mg/dL	127±21	121±37	134±34	129±21
SED, mm at 1 hour	7.7±3.5	7.2±4.6	8.3±6.7	8.4±7.28

SED indicates sedimentation velocity.
*$P<0.01$.

sICAM-1 plasma levels decreased by 18% in patients with diabetes, by 14% in patients taking aspirin, and by 16% in patients taking statins. Similarly, sVCAM-1 decreased by 12% in all of the subgroups; E-selectin plasma levels decreased by 15% in patients with diabetes and those taking statins and by 16% in patients taking aspirin; s-thrombomodulin plasma levels decreased by 12% in patients taking aspirin and those taking statins and by 9% in patients with diabetes; and IL-6 plasma levels decreased by 14% in patients taking aspirin and those with diabetes and by 16% in patients taking statins (Figure 2). In this latter group, a smaller increase in CRP but not in all other vascular inflammation markers was noted. Plasma levels of complement fractions remained unchanged at 3 and 6 months. No correlations between increase in plasma CRP and changes in plasma levels of complement fractions C3 and C4 were found.

Discussion

The present study shows that in postmenopausal women with increased cardiovascular risk (>20%/10 years), short-term HRT significantly increases plasma concentration of CRP. However, despite a significant increase in CRP, a reduction of all other markers of inflammation was noted with HRT, suggesting that the increase in CRP may be metabolic and possibly related to the hepatic first-pass effect. The present study has not evaluated the effect of transdermal HRT on vascular inflammation markers that are thought not to be affected by the transdermal route of administration.[13] The regulation of CRP synthesis in the liver is mainly mediated by IL-6, TNFα, and IL-1β.[14] Both IL-6 and TNFα have been shown to be modulated by estrogens. A negative correlation has been found between IL-6 levels and plasma estradiol, and significantly higher IL-6 levels were observed in postmenopausal women compared with premenopausal women.[15] These findings have been confirmed in the experimental setting, because ovariectomy raised IL-6 plasma levels in the mice model and HRT reversed this rise.[16] Therefore, it seems likely that HRT may increase CRP through an IL-6–independent and TNFα-independent mechanism.[17,18] In accordance with our findings, Pradhan et al[19] in a prospective analysis from the Women's' Health Initiative (WHI) have reported that long-term HRT use was associated with increased CRP levels and with a decrease in IL-6 and that baseline levels of CRP and IL-6 are independently associated with cardiovascular risk in postmenopausal women.

Early observational studies have suggested that HRT may reduce cardiovascular risk.[20] In contrast, the Heart and

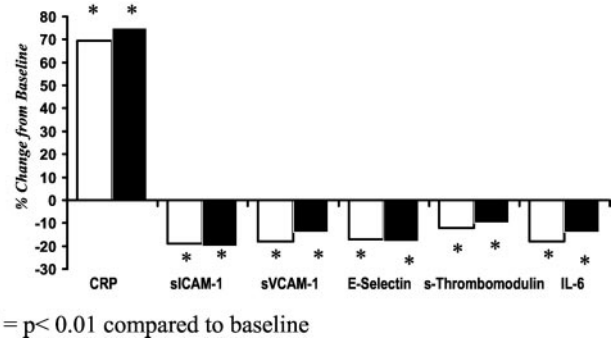

Figure 1. Percent change in CRP and inflammatory markers after 3 (white bars) and 6 (black bars) months of oral HRT. Despite an increase in CRP, all other vascular markers of inflammation were reduced by HRT.

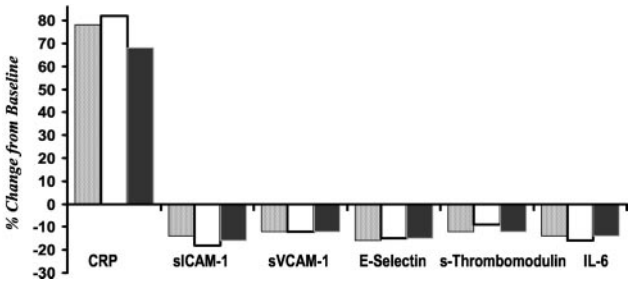

Figure 2. Percent change in CRP and inflammatory markers after 6 months of oral HRT in postmenopausal women with diabetes (n=19), taking aspirin (n=21), or taking statins (n=32). No significant changes were detected between groups. Dashed bars indicate patients taking; white bars, diabetic patients; black bars, patients taking statins.

Estrogen/Progestin Replacement Study (HERS) failed to show a reduction in the rate of coronary events in elderly women with coronary disease who were randomly assigned to estrogen plus MPA or placebo.[21] More recently, the estrogen-progestin arm of the WHI has been stopped because of an increased incidence of breast cancer.[22] In this study, an increased incidence of thrombotic events was detected in women assigned to conjugated estrogens and MPA. Although stopped early and with an incidence of cardiovascular events lower than expected, the WHI investigators suggested that the estrogen plus progestin association tested in the study did not confer benefit for preventing CHD among healthy postmenopausal women. Both HERS and WHI have suggested that initiation of HRT may be associated with an early increase of cardiovascular events. The reasons for the increased cardiovascular event rate observed in women with advanced cardiovascular disease (included in the HERS) and in women without apparent cardiovascular disease (in the WHI) are unclear and controversial, although it has been suggested that the prothrombotic and proinflammatory effects of ovarian hormones may be of importance.

The long-term follow-up of HERS concluded, after 6.8 years, that the hormone therapy did not reduce risk of cardiovascular events in women with coronary heart disease. However, because of the large drop out and drop in rates, the results of this study are difficult to interpret.[23]

The population reported in the present study differs from the populations of both HERS and WHI. Our patients, unlike those included in HERS, did not have coronary artery disease; in addition, our patients much younger and had a lower time since menopause compared with those included in WHI. Also, patients included in our study had a lower BMI than those included in WHI and HERS.[21,22]

The Postmenopausal Estrogen/Progestin Interventions (PEPI) study, a prospective, double-blind, randomized study, has shown that women receiving oral HRT had higher CRP levels compared with placebo over 12 and 36 months of follow-up. Although the correlation between elevated CRP and cardiovascular events was not investigated in the PEPI study, the authors hypothesized that the increased CRP with HRT may explain the increased cardiovascular event rate detected in the first year of HRT.[24,25]

Elevated plasma levels of CRP are associated with adverse outcome in patients with acute ischemic syndromes and in patients with cardiovascular disease.[26] Ridker et al[27] have shown that elevated CRP levels are associated with increased cardiovascular risk in postmenopausal women, but the clinical relevance of the increase of CRP within the limits of normal that occurs with postmenopausal hormones remains to be clarified. Whether increased CRP plays a causative role in early cardiovascular events after HRT is a possibility that deserves careful investigation. It has been suggested that the increased plasma levels of CRP during HRT could reflect an increased inflammatory status as a mechanism for a putative adverse cardiovascular effect of postmenopausal hormones. However, data evaluating this hypothesis are sparse and limited to older women. The PEPI study investigators have considered as elevated a threshold level of CRP equal to 2.1 mg/L, which is therefore within the physiological range (because it is under the cutoff value of 3.0 mg/L). In a recent study in patients with chronic stable angina, Garcia-Moll et al[4] found that even though women taking HRT had significantly higher plasma levels of CRP than men or women not taking HRT, they had a similar event rate during follow-up.

Although not tested in the present study, it has been suggested that postmenopausal use of selective estrogen receptor modulators may have cardioprotective effects. Several studies have reported that these agents (tamoxifen, raloxifene, and losofoxifene) have a positive impact on cardiovascular health, possibly as a result of their effect on cholesterol and fibrinogen levels.[28] The possible cardioprotective effect of these substances is presently being tested by a randomized placebo-controlled study (Raloxifene Use for the Heart [RUTH]).[29]

In the present study, we found a significant decrease of the cytokines levels in the women undergoing HRT. The important finding of our study is that, despite a significant increase in CRP, all other markers of vascular inflammation (s-ICAM-1, s-VCAM-1, E-selectin, and s-thrombomodulin) were significantly lowered by HRT. Of importance, IL-6, a key mediator of production of CRP, is also decreased by HRT, and IL-6 levels did not change over time in postmenopausal women not receiving HRT. Therefore, the changes induced by HRT in postmenopausal women are bidirectional. On the one hand, HRT induces an increase of CRP; on the other hand, it lowers most markers of vascular inflammation, including IL-6, which is the modulator of the liver production of CRP, thereby suggesting that the increase in CRP occurring with HRT is metabolic and not related to an inflammatory response. This is additionally supported by a recent study from Koh et al[30] that demonstrated no correlation between CRP and TNF-α in hypertensive or obese postmenopausal women receiving HRT. Furthermore, the fact that the increase in CRP is less pronounced in women taking statin therapy while statin therapy does not affect the HRT-induced changes in all other inflammatory markers, including IL-6, suggests a possible metabolic explanation for the increase of CRP after oral HRT. Our findings in women taking statins are supported by recent findings from Koh et al,[31] who found that HRT and simvastatin do not correlate with baseline or treatment-induced changes in levels of IL-6, lipoproteins, or flow-mediated dilatation of the brachial artery as a measure of nitric oxide bioactivity.

One might argue that regardless of the inflammatory or metabolic nature of CRP, an increase in peptide plasma levels may be detrimental because of its effect on complement pathway. We have also shown in this study that complement fractions are not affected by HRT and that there is no correlation between the increase in CRP and changes in complement fractions. The noninflammatory origin of the high CRP levels during HRT may explain the increase of CRP levels without cardiovascular events as found by Garcia-Moll et al.[4]

We did not find any difference in the increase in CRP in patients taking aspirin, additionally suggesting the noninflammatory nature of the CRP increase. We found a less-pronounced decrease in inflammatory markers with HRT in women with type II diabetes. These findings are in partial

agreement with a recent study by Koh et al[32] that found a decrease in VCAM-1 similar to that reported in our study but a null effect on ICAM.

Limitations of this study are the fact that it is not randomized and placebo-controlled and its medium-term duration. The lack of randomization and placebo has not in our view affected the results seen, ie, the clear changes in women taking HRT and the absence of any change in those refusing HRT. The medium-term duration of the study was selected because it is the time within which cardiovascular events occur in women receiving HRT and is also the time at which most women decide to continue HRT or discontinue treatment.

In conclusion, HRT increases CRP levels but reduces all other markers of vascular inflammation, including IL-6, suggesting that the HRT-induced increase in CRP levels may be metabolic and not related to a heightened vascular inflammatory status. Additional studies are required to elucidate the metabolic mechanism responsible for the higher CRP concentration in postmenopausal women taking HRT.

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