Hormone Replacement Therapy and Increased Plasma Concentration of C-Reactive Protein

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Background—It has been hypothesized that postmenopausal hormone replacement therapy (HRT) may increase levels of C-reactive protein (CRP), a marker of inflammation associated with increased risk of future cardiovascular events. However, data evaluating this hypothesis are sparse and limited to older women.

Methods and Results—CRP levels were evaluated in a cross-sectional survey of 493 healthy postmenopausal women; mean age was 51 years. Overall, median CRP levels were 2 times higher among women taking HRT than among women not taking HRT (0.27 versus 0.14 mg/dL; $P=0.001$). This difference was present in all subgroups evaluated, including those with no history of hypertension, hyperlipidemia, obesity, diabetes, or cigarette consumption or a family history of premature coronary artery disease (all $P< 0.01$). Compared with nonusers of HRT, median CRP levels were higher among women using estrogen alone ($P=0.003$) and women using estrogen plus progesterone ($P=0.03$); however, there was no significant difference in CRP levels between users of different HRT preparations. In multivariate analysis, the relationship between HRT use and CRP remained significant after control for body mass index, age, diabetes, hypertension, hyperlipidemia, alcohol use, and cigarette consumption ($P=0.001$).

Conclusions—In this cross-sectional survey, CRP levels were increased among apparently healthy postmenopausal women taking HRT. The potential impact of HRT on inflammatory parameters should be investigated in ongoing clinical trials. **(*Circulation*. 1999;100:713-716.)**

Key Words: inflammation ■ hormones ■ C-reactive protein ■ risk factors

In the recent Heart and Estrogen/progestin Replacement Study (HERS), an increase in early cardiovascular events was reported in association with randomized assignment to hormone replacement therapy (HRT) (relative risk during year 1=1.5, 95% CI 1.0 to 2.3).[1] Although this observation may represent a chance effect and must be interpreted with caution, data from the HERS trial have been of concern because they raise the possibility that initiation of HRT might be associated with transiently increased thrombotic risk.

A potential explanation of this effect has been proposed by Cushman and colleagues,[2] who reported that levels of C-reactive protein (CRP) were elevated among elderly women undergoing HRT. Because elevated levels of CRP are a risk factor for future cardiovascular events,[3–9] this observation raises the possibility that HRT may have proinflammatory effects that might increase plaque vulnerability.

To date, available data suggesting that HRT is associated with elevated CRP levels derive primarily from elderly women enrolled in the Cardiovascular Health Study (CHS) (mean age 73 years).[2] In that study, the association of HRT and CRP was most apparent in women with elevated body mass index. Thus, to provide further data regarding this hypothesis, we evaluated whether HRT was associated with increased levels of CRP in a group of otherwise healthy postmenopausal women representative of those deciding whether or not to use HRT.

Methods

We measured CRP levels in 493 postmenopausal women participating in the Women's Health Study (WHS), an ongoing trial of aspirin and vitamin E in the primary prevention of cancer and cardiovascular disease among apparently healthy women.[10] Baseline blood samples collected in EDTA were obtained before randomization and were stored at −170°C until laboratory analysis. At enrollment, participants provided questionnaire data concerning lifestyle practices and potential risk factors for both cardiovascular disease and cancer. Included in the baseline data collection were questions concerning use of HRT and whether any such use consisted of estrogen alone or of estrogen plus progesterone. All women evaluated had no prior history of cardiovascular disease, cancer, or chronic inflammatory condition. None of the women had been participants in a prior evaluation of inflammation in the WHS.[5]

Plasma samples obtained at baseline were thawed and assayed for CRP by use of a high-sensitivity assay with a coefficient of variation below 5% (hs-CRP, Dade Behring). Because distributions of CRP are skewed, differences in median levels according to HRT status were tested with the rank sum test. We used the Student *t* test and the

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TABLE 1. Baseline Clinical Characteristics of Study Participants, According to HRT Status

	No HRT (n=311)	Current HRT (n=182)	Estrogen Alone (n=99)	Estrogen Plus Progesterone (n=83)
Age, y (SD)	49.6 (4.9)	52.4 (5.3)*	53.2 (6.0)	51.5 (4.2)
Body mass index, kg/m^2 (SD)	25.2 (4.7)	24.1 (4.3)*	24.2 (4.2)	23.9 (4.4)
Smoking status, %				
Current	15.8	12.6	14.1	10.8
Never	53.7	51.6	52.5	50.6
Past	30.6	35.7	33.3	38.6
Hyperlipidemia, %†	16.1	21.4	26.3	15.7
Hypertension, %†	20.4	17.7	23.2	11.0
Family history of CAD, %	17.9	11.7	11.3	12.2
Diabetes, %†	13.2	4.9*	3.0	7.2
Exercise frequency, %				
Rarely/never	31.8	34.6	34.3	34.9
<1 time/wk	24.8	22.0	21.2	22.9
1–3 times/wk	31.8	33.5	35.4	31.3
4+ times/wk	11.6	9.9	9.1	10.8
Alcohol use, %				
Rarely/never	40.8	42.9	46.5	38.6
1–3 drinks/mo	16.7	17.0	19.2	14.5
1–6 drinks/wk	33.4	26.9	23.2	31.3
1+ drink/d	9.0	13.2	11.1	15.7

*$P<0.05$.

†Presence of hyperlipidemia, hypertension, and diabetes based on self-report with confirmation of appropriate treatment from medical records.

χ^2 statistic to compare differences in baseline cardiovascular risk factors among study women according to HRT status. Multivariate analysis was used to evaluate the relationship between HRT use and log-normalized levels of CRP after adjustment for any baseline differences between study groups.

For comparison, we also evaluated CRP levels using the high-sensitivity Dade Behring assay in a group of 291 middle-aged men participating in the Physicians' Health Study (PHS).[11] Like participants in the WHS, men in the PHS were free of cardiovascular disease, cancer, or chronic inflammatory condition at the time of blood sampling.

Results

Table 1 shows baseline characteristics of the WHS participants according to HRT status. Women undergoing HRT were older (52.4 versus 49.6 years), less likely to have diabetes (4.9% versus 13.2%), and had a lower mean body mass index (24.1 versus 25.2 kg/m^2) than women not using HRT (all $P< 0.05$). There were no significant differences between groups in terms of smoking status, hyperlipidemia, hypertension, exercise frequency, alcohol use, or a family history of coronary artery disease (CAD).

Overall, median CRP levels were 2 times higher among women using HRT than among women not using HRT (0.27 versus 0.14 mg/dL; $P=0.001$). This difference was present in all low-risk subgroups evaluated, including those with no history of hypertension, hyperlipidemia, family history of CAD, cigarette consumption, diabetes, or obesity (all $P< 0.01$) (Table 2).

As shown in the Figure, the distribution of CRP was higher for women using HRT than for those not using HRT, both for the total cohort ($P=0.001$) and for those using estrogen alone ($P=0.003$) or estrogen plus progesterone ($P=0.03$). By contrast, there was no difference in the distribution of CRP in comparisons of women not using HRT and men participating in the PHS. Furthermore, there was no difference in CRP distributions between women taking estrogen alone compared with estrogen plus progesterone.

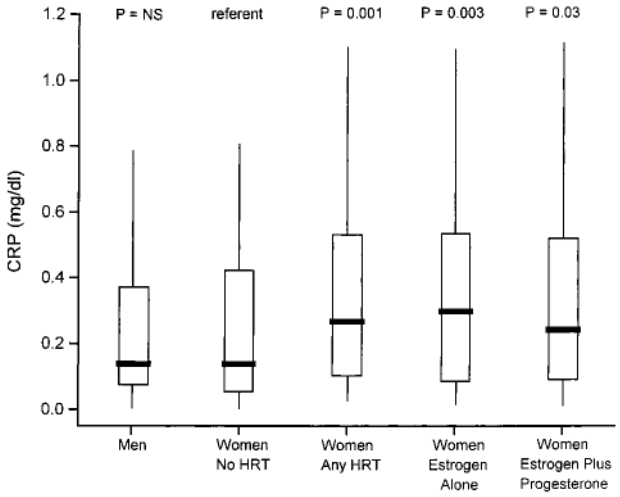
To evaluate further the potential impact of HRT on CRP distributions, we computed cutpoints for each increasing quartile of CRP for each of the major study groups. Quartile cutpoints for men and women not using HRT were similar;

TABLE 2. Median CRP Values (mg/dL) Among Women Using and Not Using HRT

Study Group	No HRT	Any HRT	*P*
All participants (n=493)	0.14	0.27	0.001
No history of hypertension (n=391)*	0.12	0.25	0.0001
No history of hyperlipidemia (n=404)*	0.14	0.27	0.002
No family history of premature CAD (n=406)	0.14	0.28	0.0003
Nonsmokers (n=423)	0.13	0.24	0.007
No history of diabetes (n=443)*	0.14	0.27	0.0009
Body mass index <27.3 kg/m^2 (n=372)	0.10	0.23	0.0001

Data are shown for the total study group and for several low-risk subgroups.

*Presence of hyperlipidemia, hypertension, and diabetes based on self-report with confirmation of appropriate treatment from medical records.



Distributions of CRP among study participants according to sex and HRT status. Box plots show 10th, 25th, 50th, 75th, and 90th percentile cutpoints of CRP distribution for each study group.

however, for women using HRT, the cutpoints determining each successive quartile of CRP were higher, particularly at the upper end of the distribution (Table 3).

In multivariate analysis, the relationship between HRT and log-normalized levels of CRP among WHS participants remained significant after control for body mass index (kg/m^2), age (years), diabetes, hypertension, hyperlipidemia, alcohol use, and smoking. This was true for the total study group ($P=0.001$) and for those taking estrogen alone ($P=0.001$), as well as for those taking combined estrogen plus progesterone ($P=0.002$).

Discussion

These cross-sectional data indicate that plasma concentrations of CRP are significantly higher among healthy postmenopausal women using HRT than among similar women not using HRT. This association was present in all subgroups of women evaluated, including those with no history of cigarette consumption or obesity, 2 factors known to influence CRP levels. Furthermore, although women using HRT had significantly higher levels of CRP than women not using HRT, the distribution of CRP in this latter group was not different from that observed among apparently healthy middle-aged men. We found no difference in CRP levels between women using estrogen alone and women using estrogen plus progesterone.

The current data corroborate findings from the CHS that suggested that CRP levels were elevated among elderly women using HRT compared with nonusers.[2] That study evaluated older women who had been undergoing HRT for long periods of time. Thus, the current data extend these observations to a group of women representative of those who have more recently started HRT.

The current data and those from the CHS are cross-sectional and cannot address causality. Nonetheless, evidence supporting an association between HRT and CRP is accumulating. For example, in randomized data deriving from the Postmenopausal Estrogen/Progestin Intervention (PEPI) study, assignment to HRT compared with placebo was associated with increased levels of CRP, which were maintained on long-term follow-up.[12] Similarly, in a short-term study of postmenopausal women initiating HRT, CRP levels were found to increase among those assigned to micronized estradiol.[13] Neither of these interventional studies found a concomitant increase in plasma fibrinogen level associated with HRT use. Thus, although these latter studies suggest that any effect of HRT on CRP is unlikely to reflect a generalized acute-phase response or simple hepatic induction, this possibility cannot be excluded and requires direct experimental testing.

In the present study, estrogen alone and estrogen plus progesterone were associated with increased levels of CRP. These data thus suggest that the addition of progesterone does not have a major influence on CRP levels, at least when given in combination with estrogen.

The clinical impact of these accumulating data is uncertain but may have implications for women initiating HRT. Specifically, because elevated levels of CRP are associated with increased cardiovascular risk among otherwise healthy women,[5] it has been hypothesized that the initiation of HRT increases CRP levels and results in heightened plaque instability and a propensity to thrombosis.[2,12] In a previous report from the WHS in which a different high-sensitivity assay for CRP was used, those women with CRP levels in the highest quartile had a 7-fold increase in risk of subsequent myocardial infarction and stroke compared with women with CRP levels in the lowest quartile (95% CI 2.7 to 19.9; $P=0.0001$).[5] However, the number of clinical events accrued in the WHS to date limits the power to detect any evidence of interaction between HRT, CRP, and incident cardiovascular events. On the other hand, in the HERS trial, a potential increase in early cardiovascular events was reported among women initiating HRT.[1] Although this observation may simply reflect the play of chance and must be interpreted with caution, accumulating data concerning CRP raise the possibility that HRT may have proinflammatory effects.

TABLE 3. Range of CRP Values (mg/dL) According to Quartile of CRP Distribution for Men, Women Not Using HRT, and Women Using HRT

	Quartile of CRP			
Study Group	1	2	3	4
Men (n=291)	≤0.08	0.09–0.15	0.16–0.37	≥0.38
Women, no HRT (n=311)	≤0.06	0.07–0.14	0.15–0.42	≥0.43
Women using HRT (n=182)	≤0.10	0.11–0.27	0.28–0.53	≥0.54

In summary, in this cross-sectional survey, CRP levels were significantly increased among apparently healthy postmenopausal women taking HRT, regardless of whether the HRT formulation consisted of estrogen alone or estrogen plus progesterone. Whether these observations have clinical relevance requires further investigation. We therefore believe the potential impact of HRT on inflammatory parameters should be directly investigated in ongoing clinical trials, particularly those that can provide longitudinal data and differentiate between transdermal and oral estrogen preparations. In this regard, the successful completion of ongoing large-scale randomized trials of HRT, such as the Women's Health Initiative, is crucial.

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