

# Associations of C-Reactive Protein With Measures of Obesity, Insulin Resistance, and Subclinical Atherosclerosis in Healthy, Middle-Aged Women

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**Abstract**—Obesity, the insulin resistance syndrome, and atherosclerosis are closely linked and may all be determinants of an increased acute-phase response. In this study, we examined the relationship of C-reactive protein (CRP) with measures of obesity, variables of the insulin resistance syndrome, and intima-media thickness of the common carotid arteries in 186 healthy, middle-aged women selected from the general population. Associations were assessed by regression analysis. CRP was strongly associated with body mass index (BMI) and waist circumference. CRP was also associated with other variables of the insulin resistance syndrome, including blood pressure, insulin, high density lipoprotein cholesterol, triglycerides, apolipoprotein A1 (inversely), plasminogen activator inhibitor-1 antigen, and tissue-type plasminogen activator antigen. Associations between CRP and the variables of the insulin resistance syndrome disappeared after controlling for BMI but remained significant for plasminogen activator inhibitor-1 antigen only. The association of CRP with common carotid artery intima-media thickness was weak and limited to ever-smokers. BMI explained 29.7% of the variance of CRP, whereas common carotid artery intima-media thickness explained only 3.7%. The results of this population-based study indicate that adiposity is strongly associated with CRP in healthy, middle-aged women. In this population, BMI accounted for the relationship between CRP and other variables of the insulin resistance syndrome. Further studies should determine whether losing weight ameliorates the inflammatory state. (*Arterioscler Thromb Vasc Biol.* 1999;19:1986-1991.)

**Key Words:** C-reactive protein ■ obesity ■ insulin resistance ■ carotid artery intima-media thickness ■ women

Recent data suggest that inflammation is involved in atherogenesis.<sup>1,2</sup> C-reactive protein (CRP), a major acute-phase protein, has been associated with the presence and severity of atherosclerosis<sup>3</sup> and has been found to predict cardiac events in subjects with<sup>4-6</sup> and without<sup>7-9</sup> prevalent cardiovascular disease. Raised concentrations of inflammatory mediators may reflect inflammation in the arterial wall associated with atherosclerosis but may also be causally involved in the disease process.<sup>10,11</sup> Sources of inflammation include infections<sup>10-12</sup> and smoking.<sup>13</sup> Moreover, levels of obesity have been shown to be associated with low-grade inflammation.<sup>14,15</sup>

Recent data also indicate that the insulin resistance syndrome is accompanied by an increased acute-phase response.<sup>16,17</sup> A link between the insulin resistance syndrome and the inflammatory state is further suggested by increased levels of the acute-phase proteins plasminogen activator inhibitor-1 (PAI-1) and fibrinogen in the insulin resistance syndrome<sup>18-20</sup> and by the finding that dyslipidemia in the insulin resistance syndrome and during the acute-phase response shows strong similarities.<sup>21-23</sup>

Obesity, the insulin resistance syndrome, and atherosclerotic disease are closely linked and may all be determinants of an increased acute-phase response. However, it is not clear whether these factors are independently associated with the inflammatory state. Previous studies on associations between CRP level as a measure of inflammation and cardiovascular risk factors were conducted in middle-aged men and elderly men and women, all of whom are at relatively high risk for atherosclerosis.<sup>14,15</sup> Atherosclerosis and smoking are potential sources of inflammation and possibly obscure the relation of CRP with other risk factors.

In the present study, we investigated the relationship between CRP and measures of obesity, the insulin resistance syndrome, and subclinical atherosclerosis in a population of healthy, middle-aged women with a low exposure to tobacco smoke.

## Methods

### Study Population

We studied a population of 186 women, aged 43 to 55 years, selected from the general population and participating in a study on the

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cardiovascular effects of natural menopause. Women were selected from respondents to a mailed questionnaire about menopause, which was sent to all women aged 40 to 60 years living in the town of Zoetermeer, the Netherlands (N=12 675). Women were considered premenopausal if they had experienced 1 or more regular bleeding episodes in the past 12 months and were free from climacteric symptoms, defined as perspiration and/or hot flushes. Women were considered postmenopausal if their menses had ceased naturally for at least 12 months. Exclusion criteria were diabetes mellitus, prevalent clinical cardiovascular disease, and use of antihypertensive medication or cholesterol-lowering drugs. Women reporting use of female hormones (hormone replacement therapy or oral contraceptives) within 6 months before the clinical examination were excluded, as were subjects currently smoking 5 or more cigarettes per day. Of the eligible subjects, 93 premenopausal and 93 postmenopausal age-matched women were selected (response rate 76% of eligible and invited women). All women gave written informed consent, and the study was approved by the medical ethics committee of the Erasmus University Medical School.

### Measurements

During a visit at the research center, a medical history was taken by a physician. Height, weight, and waist and hip circumferences were measured while the subjects wore indoor clothes without shoes. Body mass index (BMI, weight divided by height squared) and waist-to-hip ratio (WHR) were computed. Cigarette smoking history was obtained by a standardized questionnaire. Blood pressure was assessed with a DINAMAP automatic blood pressure recorder (Critikon, Inc). After a 5-minute rest in the supine position, blood pressure was measured 4 times at the right upper arm with an appropriately sized cuff, and the mean was used in the analyses. Venous blood samples were drawn from each subject after a 12-hour fast. The samples were stored at  $-80^{\circ}\text{C}$ . Total cholesterol was measured with an automated enzymatic method using the CHOD-PAP high-performance reagent kit from Boehringer Mannheim. HDL cholesterol was measured by the phosphotungstate method. LDL cholesterol was computed by the Friedewald formula.<sup>24</sup> Triglycerides were determined by using a reagent kit from Boehringer Mannheim after enzymatic hydrolysis of the triglycerides and subsequent determination of liberated glycerol by colorimetry. No correction was made for serum free glycerol. Apolipoproteins A1 and B were measured by an automated turbidimetric immunoassay with reagent kits from Orion Diagnostics. Glucose was enzymatically determined by the hexokinase method (Instruchemie). Serum insulin was determined by metric assay (Biosource Diagnostics). This assay has no cross-reactivity with either proinsulin or C-peptide. PAI-1 antigen and tissue-type plasminogen activator (tPA) antigen levels were determined by ELISA (Innotest PAI-1, Innogenetics NV, and Imulyse, Biopool, respectively). CRP was measured by an in-house ELISA with rabbit anti-CRP (Dako) as the catching and tagging antibody.<sup>25</sup> Intra-assay and interassay CVs for CRP were 3.8% and 4.7%, respectively. Fasting insulin levels were used as a measure of insulin resistance.<sup>26</sup> In addition, insulin sensitivity was calculated according to the formula of the homeostasis model assessment method (HOMA):  $\text{insulin resistance} = \text{fasting insulin} \times \text{fasting glucose} / 22.5$ .

### Carotid Artery Intima-Media Thickness (IMT)

Common carotid artery IMT was used as an indicator of generalized atherosclerosis.<sup>28</sup> Ultrasonography of the right common carotid artery was performed with a 7.5-MHz linear-array transducer (ATL UltraMark IV) as described in detail previously.<sup>29</sup> For each individual, the common carotid artery IMT was determined as the average of near- and far-wall measurements. Carotid artery IMT measurements have been shown to be reproducible.<sup>30</sup> In short, mean differences (and SDs) in far-wall IMT of the common carotid arteries between paired measurements of sonographers, readers, and visits were 0.040 mm (0.07), 0.069 mm (0.04), and 0.071 mm (0.09), respectively. The intraclass correlation coefficients were 0.63, 0.88, and 0.74, respectively. These results are in agreement with the reproducibility of IMT measurements found in other studies.<sup>31</sup> In the present study, all measurements were conducted by 1 sonographer and 1 reader.

**TABLE 1. Clinical and Biochemical Characteristics of 186 Middle-Aged Women**

Variable	All Subjects
Age, y	50.9±2.3
BMI, kg/m <sup>2</sup>	24.9±4.0
Waist circumference, cm	81.5±9.5
Hip circumference, cm	105.7±8.6
WHR, cm/cm	0.77±0.05
Smoking status, %	
Never	53.2
Past	40.3
Current*	6.5
Systolic blood pressure, mm Hg	121±14
Diastolic blood pressure, mm Hg	68±10
Hypertension, %†	2.2
Glucose, mmol/L	5.5±0.6
Insulin, picomol/L‡	44.0 (32.0–59.0)
HOMA, picomol×mmol/L <sup>2</sup> §	10.8 (7.7–15.5)
Total cholesterol, mmol/L	6.2±1.0
HDL cholesterol, mmol/L	1.6±0.4
LDL cholesterol, mmol/L	4.1±0.9
Triglycerides, mmol/L	1.0 (0.8–1.3)
Apolipoprotein A1, mg/dL	154.5±31.6
Apolipoprotein B, mg/dL	102.0±26.3
PAI-1 antigen, ng/mL‡	53.0 (34.0–85.3)
tPA antigen, ng/mL	6.3±2.4
CRP, mg/L‡	0.68 (0.33–1.44)
Common carotid artery IMT, mm	0.61±0.09

Data are mean±SD, median (interquartile range) for variables with skewed distributions, or percentages.

\*Subjects who smoked 5 or more cigarettes a day were excluded from study participation.

†Hypertension was defined as systolic blood pressure  $\geq 160$  mm Hg and/or diastolic blood pressure  $\geq 95$  mm Hg.

‡Skewed data.

§HOMA=fasting insulin×fasting glucose/22.5.

||Highest level of triglycerides was 3.80 mmol/L.

### Statistical Analysis

The clinical and biochemical features of the population are presented as mean±SD, median (and interquartile range) for variables with a skewed distribution, or percentages. Because the distribution of CRP was highly skewed, it was natural-log-transformed for all analyses. The strength of the associations between CRP and clinical and biochemical variables was assessed by linear regression of ln CRP on each variable separately, adjusted for age. Because strong associations were found between CRP and measures of obesity, we adjusted for them in additional models. Regression analysis was further used to estimate the explained proportion of variance in CRP ( $R^2$ ). The difference in CRP between premenopausal and postmenopausal women adjusted for age and measures of obesity was studied with regression analysis. A probability value  $<0.05$  (2-tailed test) was considered significant. SPSS 7.5 for Windows was used for all analyses.

### Results

Characteristics of the population are described in Table 1. BMI ranged from 16.8 to 41.1 kg/m<sup>2</sup>; 42 subjects had a BMI  $>27$  kg/m<sup>2</sup>. CRP varied from 0.05 to 14.38 mg/L; 2 subjects had values  $>10$  mg/L (10.70 and 14.38 mg/L), the cutpoint

**TABLE 2. Regression Coefficients\* for ln CRP as the Dependent Variable and Measures of Obesity as Independent Variables in 186 Women**

	Adjusted for Age		Adjusted for Age+BMI	
	$\beta^*$	95% CI	$\beta^*$	95% CI
BMI, 1 kg/m <sup>2</sup>	0.14†	0.11 to 0.18	...	...
Waist circumference, 10 cm	0.62†	0.48 to 0.75	0.39‡	0.11 to 0.67
Hip circumference, 10 cm	0.65†	0.50 to 0.80	0.29	-0.051 to 0.67
WHR, 0.05	0.34†	0.20 to 0.49	0.12	-0.021 to 0.26

$\beta$  indicates regression coefficient.

\*An increase of the independent variable by 1 unit is associated with an increase of CRP by a factor of  $e^{\beta}$ .

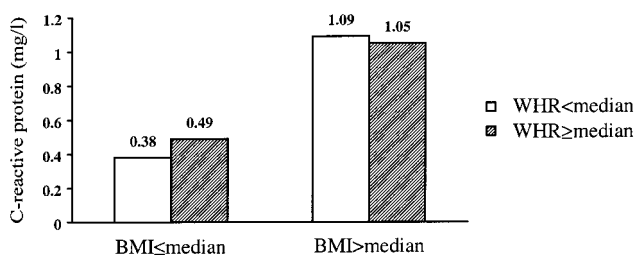
‡Regression significant at the  $\pm 0.01$  and  $\dagger 0.001$  levels, respectively (all 2-tailed).

generally used to identify clinically relevant inflammation.<sup>32</sup> Fasting insulin levels ranged from 18 to 232 pmol/L. Common carotid artery IMT ranged from 0.43 to 0.97 mm.

CRP was significantly associated with measures of obesity: BMI, waist and hip circumferences, and WHR (Table 2). Associations with CRP were stronger for BMI and waist and hip circumferences than for WHR ( $r=0.54$  for BMI,  $r=0.55$  for waist circumference,  $r=0.53$  for hip circumference, and  $r=0.33$  for WHR, all adjusted for age). After adjustment for BMI, hip circumference and WHR were no longer associated with CRP, whereas waist circumference still was. We next visualized this relationship between BMI, WHR, and CRP by subdividing the study population by the median BMI (23.9 kg/m<sup>2</sup>) and WHR (0.77) (the Figure, geometric means). BMI explained 29.7% of the variance of CRP; waist circumference, 31.3%; hip circumference, 28.7%; and WHR, 11.4%, after adjustment for age.

The other variables included in or associated with the insulin resistance syndrome were also significantly associated with CRP: blood pressure, insulin, HDL cholesterol, triglycerides, apolipoprotein A1 (inversely), PAI-1 antigen, and tPA antigen (Table 3). No associations were found with glucose or with total and LDL cholesterol, whereas an association with apolipoprotein B was present. Separate analyses after exclusion of subjects with levels of CRP >10 mg/L did not affect the results (data not shown).

After controlling for BMI, the associations between CRP and variables of the insulin resistance syndrome disappeared except for the association with PAI-1 antigen, although there was a substantial decline in the magnitude of this association (Table 3). Controlling for waist circumference gave the same results, whereas controlling for hip circumference decreased the described associations to a somewhat smaller extent. Controlling for WHR, on the other hand, had only a small influence on the described associations (data not shown). To



Levels of CRP (mg/L) according to BMI and WHR in 186 women. Values are geometric means.

evaluate whether the clustering of variables belonging to the insulin resistance syndrome might be a reflection of a general acute-phase response, associations between measures of insulin resistance (insulin and HOMA) and the other variables of the insulin resistance syndrome were adjusted for CRP. This adjustment did not modify the relation between insulin, HOMA, and the other variables (data not shown).

Measures of obesity and CRP in premenopausal and postmenopausal women separately are shown in Table 4. CRP did not differ significantly between premenopausal and postmenopausal women. Age-adjusted geometric means were 0.61 and 0.71 mg/L respectively (15% increase with menopause, 95% CI, -15% to 45%). Because menopause may be associated with changes in measures of obesity, we adjusted for these variables, which slightly influenced the results. Postmenopausal women had an age-adjusted level of cholesterol of 6.48 mmol/L versus 5.89 mmol/L in premenopausal women (10% difference; 95% CI, 5% to 14%). PAI-1 antigen increased with menopause, but the difference lacked statistical significance. In premenopausal women, the age-adjusted geometric mean of PAI-1 antigen was 52.9 ng/mL versus 61.1 ng/mL in postmenopausal women (13% increase with menopause; 95% CI, -8% to 33%). Because cholesterol and PAI-1 antigen are known to increase with menopause, these results indicate a correct selection of menopausal groups. The associations between CRP on the one hand and both measures of obesity and other variables of the insulin resistance syndrome on the other were found to be identical when examined in premenopausal and postmenopausal women separately (data not shown).

CRP was significantly associated with common carotid artery IMT. After stratification by smoking status, associations between CRP and common carotid artery IMT appeared to be present in ever-smokers only (Table 5). Common carotid artery IMT explained 3.7% of the variance of CRP after adjustment for age.

## Discussion

Our results indicate that in healthy, middle-aged women, CRP is strongly associated with measures of obesity. CRP was associated with BMI and waist and hip circumferences but not with WHR after adjustment for BMI. CRP was also associated with other variables included in the insulin resistance syndrome. After controlling for BMI, however, the associations disappeared. Although in this population CRP was associated with common carotid artery IMT in ever-

**TABLE 3. Regression Coefficients\* for ln CRP as the Dependent Variable and Clinical and Biochemical Characteristics as Independent Variables in 186 Women**

	Adjusted for Age		Adjusted for Age+BMI	
	$\beta^*$	95% CI	$\beta^*$	95% CI
Systolic blood pressure, 10 mm Hg	0.16†	0.057 to 0.27	0.025	-0.071 to 0.12
Diastolic blood pressure, 10 mm Hg	0.20†	0.043 to 0.35	0.029	-0.11 to 0.17
Glucose, 1 mmol/L	0.23	-0.055 to 0.51	-0.069	-0.32 to 0.18
Insulin, 10 picomol/L	0.11‡	0.057 to 0.16	0.024	-0.025 to 0.074
HOMA, 5 picomol×mmol/L <sup>2</sup> §	0.18‡	0.089 to 0.26	0.028	-0.058 to 0.12
Cholesterol, 1 mmol/L	0.11	-0.045 to 0.26	0.080	-0.050 to 0.21
HDL cholesterol, 0.5 mmol/L	-0.37‡	-0.56 to -0.17	-0.092	-0.28 to 0.093
LDL cholesterol, 1 mmol/L	0.15	-0.020 to 0.31	0.090	-0.051 to 0.23
Triglycerides, 1 mmol/L	0.64‡	0.38 to 0.90	0.23	-0.027 to 0.49
Apolipoprotein A1, 10 mg/dL	-0.063	-0.11 to -0.015	-0.027	-0.068 to 0.015
Apolipoprotein B, 10 mg/dL	0.078	0.021 to 0.14	0.029	-0.022 to 0.080
PAI-1 antigen, 1 ng/mL#	0.65‡	0.45 to 0.84	0.30	0.082 to 0.51
tPA antigen, 1 ng/mL	0.13‡	0.067 to 0.19	0.052	-0.005 to 0.11

$\beta$  indicates regression coefficient.

\*An increase of the independent variable by 1 unit is associated with an increase of CRP by a factor of  $e^\beta$ .

§HOMA=fasting insulin×fasting glucose/22.5.

#PAI-1 was ln-transformed to obtain a better-model fit as assessed by residual analysis; an increase of PAI-1 by 1% yields an increase of CRP by  $\beta\%$ .

Regression significant at the †0.05, ||0.01, and ‡0.001 levels, respectively (all 2-tailed).

smokers, measures of obesity explained a much larger part of the variance of CRP than did carotid artery IMT.

One hypothesis explaining these results is that adipose tissue might be the common antecedent of both CRP and insulin resistance. The associations between CRP and variables of the insulin resistance syndrome may thus be due to the association of BMI with both insulin resistance and the acute-phase response. This idea is consistent with experimental evidence indicating that adipocytes produce tumor necrosis factor (TNF)- $\alpha$ .<sup>33</sup> TNF- $\alpha$  induces interleukin-6 (IL-6) synthesis,<sup>34</sup> a prime regulator of CRP synthesis.<sup>35,36</sup> Additional support for this hypothesis comes from the observation that weight reduction leads to a decrease of TNF- $\alpha$  mRNA expression<sup>37</sup> and of serum levels of TNF- $\alpha$  in diabetic subjects.<sup>38</sup> We found that CRP was strongly related to BMI and to waist and hip circumferences separately, but less to WHR. These results are compatible with previous studies, in

which BMI but not WHR was related to TNF- $\alpha$  expression or TNF- $\alpha$  levels.<sup>33,39</sup> However, after adjustment for BMI, waist circumference was still related to CRP, whereas hip circumference was not. This suggests that abdominal fat deposition is most important in inducing inflammation.

Associations between CRP concentrations and fasting serum insulin concentrations, which persisted after adjustment for BMI, have been observed in a population of male patients with angina pectoris.<sup>18</sup> In addition, in healthy, middle-aged men, relationships between CRP and cardiovascular risk factors like HDL cholesterol and triglycerides persisted after adjustment for BMI.<sup>14</sup> One possible explanation for these discrepant results might be that the relationships between obesity, the insulin resistance syndrome, and the acute-phase response are different between men and women. Support for this hypothesis comes from the observation that sex steroids influence the metabolic activity of adipose tissue.<sup>40</sup> Addition-

**TABLE 4. Measures of Obesity and CRP in Premenopausal and Postmenopausal Women**

	Premenopausal (n=93)		Postmenopausal (n=93)	
	Mean	SE	Mean	SE
Age, y	50.6	0.24	51.1	0.24
BMI, kg/m <sup>2</sup> *	24.7	0.41	25.0	0.41
Waist circumference, cm*	81.3	1.00	81.6	0.99
Hip circumference, cm*	105.3	0.90	106.1	0.89
WHR, cm/cm*	0.77	0.005	0.77	0.005
CRP, mg/L*†	0.61	0.49 to 0.76	0.71	0.58 to 0.88
CRP, mg/L†‡	0.62	0.52 to 0.74	0.69	0.58 to 0.84

\*Adjusted for age.

†Geometric mean (95% CI) are shown for CRP because its distribution is highly skewed.

‡Adjusted for age and BMI.



**TABLE 5. Regression Coefficients\* for ln CRP as the Dependent Variable and Common Carotid Artery IMT as the Independent Variable in 186 Women According to Smoking Status**

	All Subjects		Ever-Smokers (n=87)		Never-Smokers (n=99)	
	$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI
Adjusted for age	0.021†	0.003 to 0.039	0.040‡	0.013 to 0.067	0.004	-0.020 to 0.028
Adjusted for age+BMI	0.014	-0.001 to 0.030	0.036‡	0.014 to 0.059	-0.006	-0.026 to 0.015

$\beta$  indicates regression coefficient.

\*A 1-mm increase of common carotid artery IMT is associated with an increase of CRP by a factor of  $e^\beta$ .

Regression significant at the †0.05 and ‡0.01 levels, respectively (2 tailed).

ally, the described studies differ from ours in that those subjects were likely to suffer from more pronounced atherosclerosis because they were male or suffering from angina pectoris. Atherosclerosis might have spuriously induced the relation between CRP and other cardiovascular risk factors. Because in our population women had a low burden of atherosclerosis, as estimated from carotid artery IMT, the potential for confounding by atherosclerosis in our study is less likely.

Associations between measures of insulin resistance and other variables included in the insulin resistance syndrome were not attenuated by adjusting for CRP levels. Therefore, our data do not suggest that the clustering of variables belonging to the insulin resistance syndrome might be a reflection of a general acute-phase response.<sup>16</sup> Also, because the association between insulin resistance and measures of obesity was not affected by adjustment for CRP, our data do not support the hypothesis that adipose-tissue-derived cytokines may mediate the relation between obesity and the insulin resistance syndrome.<sup>17,33,37,39</sup> However, this hypothesis encompasses a causal role for TNF- $\alpha$ ; therefore, this inference might have been more valid had we adjusted for TNF- $\alpha$  instead of CRP.

The selection of premenopausal and postmenopausal women is likely to be accurate, as reflected by an age-adjusted increase of cholesterol of 10%, which is in agreement with other studies.<sup>41,42</sup> We did not find a clear influence of menopause on CRP levels. Both age- and age-and-BMI-adjusted levels of CRP were slightly higher in postmenopausal (0.71 mg/L) than in premenopausal (0.61 mg/L) women, but this 15% difference was not statistically significant. This result can probably be attributed to the large variation of this measure. To the best of our knowledge, no published data on the association between menopause and CRP levels are available from other studies. Estrogen replacement therapy in postmenopausal women has been shown to lower TNF- $\alpha$ <sup>43</sup> and acute-phase reactants other than CRP.<sup>44</sup> Experimental data suggest an inhibitory effect of estrogens on IL-6 gene expression.<sup>45</sup> Recent data from the Cardiovascular Health Study, however, suggest an increase of CRP with hormone replacement therapy.<sup>46</sup> Further studies are needed to address the association between inflammation, estrogens, and menopause.

We are the first to describe an association between CRP and common carotid artery IMT in healthy, middle-aged women, which association was limited to ever-smokers (Table 4). In a study by Tracy et al<sup>15</sup> in a population of elderly men and women, CRP was not related to internal carotid wall thickness but was related to ankle-arm index in ever-smokers

only. Data from the MRFIT (Multiple Risk Factor Intervention Trial) study also show a stronger association of CRP with coronary heart disease deaths in middle-aged male smokers than in nonsmokers, as defined at baseline.<sup>8</sup> Taken together, these and the present data suggest that CRP may mark permanent, underlying vascular damage due in part to smoking. This may explain why the associations between inflammation and atherosclerosis are more pronounced not only in current but also in former smokers. In the Physicians' Health Study, however, smoking did not modify the relation between CRP and the risk of cardiovascular events.<sup>7</sup>

Some issues of our study need to be addressed. First, we did not measure exposure to infectious agents such as *Helicobacter pylori* and *Chlamydia pneumoniae*, which may be weak determinants of CRP levels.<sup>12,17</sup> However, it appears unlikely that exposure to these agents would confound the association between BMI and CRP level. Second, in this study we measured atherosclerosis at only 1 location in the vascular system. Although we assume that common carotid artery IMT is a measure of generalized atherosclerosis,<sup>28</sup> assessment of the degree of atherosclerosis might have been more accurate had we used measurements at multiple locations. Finally, this study was conducted in healthy, middle-aged women without clinical cardiovascular disease, no medication use, and a low, current exposure to tobacco. Smoking and atherosclerosis are potential determinants of CRP, and therefore, the choice of our population facilitates the investigation of other factors associated with CRP. However, in this population, ever-smoking was also found to modify the association between CRP and atherosclerosis.

In summary, our results indicate that adipose tissue is strongly associated with CRP in healthy, middle-aged women. In this population with a low burden of atherosclerosis and current smoking, BMI accounts for the association between CRP and variables of the insulin resistance syndrome. Because inflammatory mediators may be directly involved in atherogenesis, these results suggest an important mechanism through which obesity might affect the risk of coronary heart disease. Further studies should determine whether losing weight ameliorates the inflammatory state.

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## References

- Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*. 1993;362:801–809.
- Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ. Atherosclerosis: basic mechanisms: oxidation, inflammation, and genetics. *Circulation*. 1995;91:2488–2496.
- Heinrich J, Schulte H, Schonfeld R, Kohler E, Assmann G. Association of variables of coagulation, fibrinolysis and acute-phase with atherosclerosis in coronary and peripheral arteries and those arteries supplying the brain. *Thromb Haemost*. 1995;73:374–379.
- Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuffi AG, Pepys MB, Maseri A. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *N Engl J Med*. 1994;331:417–424.
- Thompson SG, Kienast J, Pyke SD, Haverkate F, van de Loo JC. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris: European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *N Engl J Med*. 1995;332:635–641.
- Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina: European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *Lancet*. 1997;349:462–466.
- Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336:973–979.
- Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study: Multiple Risk Factor Intervention Trial. *Am J Epidemiol*. 1996;144:537–547.
- Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M, Meilahn EN, Kuller LH. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly: results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol*. 1997;17:1121–1127.
- Nieto FJ, Adam E, Sorlie P, Farzadegan H, Melnick JL, Comstock GW, Szklo M. Cohort study of cytomegalovirus infection as a risk factor for carotid intimal-medial thickening, a measure of subclinical atherosclerosis. *Circulation*. 1996;94:922–927.
- Gupta S, Leatham EW, Carrington D, Mendall MA, Kaski JC, Camm AJ. Elevated *Chlamydia pneumoniae* antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. *Circulation*. 1997;96:404–407.
- Patel P, Mendall MA, Carrington D, Strachan DP, Leatham E, Molineaux N, Levy J, Blakeston C, Seymour CA, Camm AJ, et al. Association of *Helicobacter pylori* and *Chlamydia pneumoniae* infections with coronary heart disease and cardiovascular risk. *BMJ*. 1995;311:711–714.
- Palosuo T, Husman T, Koistinen J, Aho K. C-reactive protein in population samples. *Acta Med Scand*. 1986;220:175–179.
- Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. *BMJ*. 1996;312:1061–1065.
- Tracy RP, Psaty BM, Macy E, Bovill EG, Cushman M, Cornell ES, Kuller LH. Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. *Arterioscler Thromb Vasc Biol*. 1997;17:2167–2176.
- Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia*. 1997;40:1286–1292.
- Yudkin JS, Stehouwer CDA, Emeis JJ, Coppel SW. Insulin resistance syndrome and endothelial damage: role of adipose tissue derived proinflammatory cytokines. *Arterioscler Thromb Vasc Biol*. In press.
- Juhan-Vague I, Thompson SG, Jespersen J. Involvement of the hemostatic system in the insulin resistance syndrome: a study of 1500 patients with angina pectoris: the ECAT Angina Pectoris Study Group. *Arterioscler Thromb*. 1993;13:1865–1873.
- Lindahl B, Asplund K, Eliasson M, Evrin PE. Insulin resistance syndrome and fibrinolytic activity: the northern Sweden MONICA study. *Int J Epidemiol*. 1996;25:291–299.
- Mohamed-Ali V, Gould MM, Gillies S, Goubet S, Yudkin JS, Haines AP. Association of proinsulin-like molecules with lipids and fibrinogen in non-diabetic subjects: evidence against a modulating role for insulin. *Diabetologia*. 1995;38:1110–1116.
- Cabana VG, Siegel JN, Sabesin SM. Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoproteins. *J Lipid Res*. 1989;30:39–49.
- Alvarez C, Ramos A. Lipids, lipoproteins, and apoproteins in serum during infection. *Clin Chem*. 1986;32:142–145.
- Fahie-Wilson M, Mills R, Wilson K. HDL cholesterol and the acute phase reaction following myocardial infarction and acute pancreatitis. *Clin Chim Acta*. 1987;167:197–209.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.
- Highton J, Hessian P. A solid-phase enzyme immunoassay for C-reactive protein: clinical value and the effect of rheumatoid factor. *J Immunol Methods*. 1984;68:185–192.
- Laakso M. How good a marker is insulin level for insulin resistance? *Am J Epidemiol*. 1993;137:959–965.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and  $\beta$ -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419.
- Bots ML, de Jong PT, Hofman A, Grobbee DE. Left, right, near or far wall common carotid intima-media thickness measurements: associations with cardiovascular disease and lower extremity arterial atherosclerosis. *J Clin Epidemiol*. 1997;50:801–807.
- Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation*. 1997;96:1432–1437.
- Bots ML, Mulder PG, Hofman A, van Es GA, Grobbee DE. Reproducibility of carotid vessel wall thickness measurements: the Rotterdam Study. *J Clin Epidemiol*. 1994;47:921–930.
- Kanters SD, Algra A, van Leeuwen MS, Banga JD. Reproducibility of in vivo carotid intima-media thickness measurements: a review. *Stroke*. 1997;28:665–671.
- Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. *Clin Chim Acta*. 1981;117:13–23.
- Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor- $\alpha$  in human obesity and insulin resistance. *J Clin Invest*. 1995;95:2409–2415.
- Gauldie J, Richards C, Northemann W, Fey G, Baumann H. IFN  $\beta$ 2/BSF2/IL-6 is the monocyte-derived HSF that regulates receptor-specific acute phase gene regulation in hepatocytes. *Ann NY Acad Sci*. 1989;557:46–58.
- Baumann H, Gauldie J. The acute phase response. *Immunol Today*. 1994;15:74–80.
- Steel DM, Whitehead AS. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today*. 1994;15:81–88.
- Hotamisligil GS, Spiegelman BM. Tumor necrosis factor- $\alpha$ : a key component of the obesity-diabetes link. *Diabetes*. 1994;43:1271–1278.
- Katsuki A, Sumida Y, Murashima S, Murata K, Takarada Y, Ito K, Fujii M, Tsuchihashi K, Goto H, Nakatani K, Yano Y. Serum levels of tumor necrosis factor- $\alpha$  are increased in obese patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*. 1998;83:859–862.
- Nilsson J, Jovinge S, Niemann A, Reneland R, Lithel H. Relation between plasma tumor necrosis factor- $\alpha$  and insulin sensitivity in elderly men with non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 1998;18:1199–1202.
- Bjorntorp P. Hormonal control of regional fat distribution. *Hum Reprod*. 1997;12:21–25.
- Hjortland MC, McNamara PM, Kannel WB. Some atherogenic concomitants of menopause: the Framingham Study. *Am J Epidemiol*. 1976;103:304–311.
- Davis CE, Pajak A, Rywik S, Williams DH, Broda G, Pazucha T, Ephross S. Natural menopause and cardiovascular disease risk factors: the Poland and US Collaborative Study on Cardiovascular Disease Epidemiology. *Ann Epidemiol*. 1994;4:445–448.
- Aune B, Oian P, Omsjo I, Osterud B. Hormone replacement therapy reduces the reactivity of monocytes and platelets in whole blood: a beneficial effect on atherogenesis and thrombus formation? *Am J Obstet Gynecol*. 1995;173:1816–1820.
- Tuck CH, Holleran S, Berglund L. Hormonal regulation of lipoprotein(a) levels: effects of estrogen replacement therapy on lipoprotein(a) and acute phase reactants in postmenopausal women. *Arterioscler Thromb Vasc Biol*. 1997;17:1822–1829.
- Ray P, Ghosh SK, Zhang DH, Ray A. Repression of interleukin-6 gene expression by 17  $\beta$ -estradiol: inhibition of the DNA-binding activity of the transcription factors NF-IL6 and NF- $\kappa$ B by the estrogen receptor. *FEBS Lett*. 1997;409:79–85.
- Cushman M, Meilahn EN, Kuller LH, Psaty BM, Tracy RP. Hormone replacement therapy (HRT) and markers of hemostasis and inflammation in elderly women. [Abstract.]. Santa Fe, NM: 38th Annual Conference on Cardiovascular Disease Epidemiology and Prevention, March 18–20, 1998. *Circulation*. 1998;97:814.