Prospective Study of C-Reactive Protein in Relation to the Development of Diabetes and Metabolic Syndrome in the Mexico City Diabetes Study

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OBJECTIVE — Recent evidence suggests that C-reactive protein (CRP) may predict development of diabetes in Caucasian populations. We evaluated CRP as a possible risk factor of the development of diabetes and metabolic syndrome in a 6-year study of 515 men and 729 women from the Mexico City Diabetes Study.

RESEARCH DESIGN AND METHODS — Baseline CRP, indexes of adiposity, and insulin resistance (homeostasis model assessment [HOMA-IR]) were used to predict development of the metabolic syndrome, defined as including two or more of the following: *1*) dyslipidemia (triglyceride ≥2.26 mmol/l or HDL cholesterol ≤0.91 mmol/l in men and ≤1.17 mmol/l in women; <35 and 40 mg/dl for men and women); *2*) hypertension (blood pressure >140/90 mmHg or on hypertensive medication); or *3*) diabetes (1999 World Health Organization criteria).

RESULTS — At baseline, CRP correlated significantly ($P < 0.001$) with all metabolic indexes in women, but less so in men. After 6 years, 14.2% of men and 16.0% of women developed the metabolic syndrome. Compared with tertile 1, women with CRP in the highest tertile had an increased relative risk of developing the metabolic syndrome by 4.0 (95% CI 2.0–7.9) and diabetes by 5.5 (2.2–13.5); these risks changed minimally after adjusting for BMI or HOMA-IR. The area under receiver-operating characteristic (ROC) curve for the prediction of the development of the syndrome was 0.684 for CRP, increasing to 0.706 when combined with BMI and to 0.710 for a complex model of CRP, BMI, and HOMA-IR.

CONCLUSIONS — CRP was not a significant predictor of the development of the metabolic syndrome in men. Our data strongly support the notion that inflammation is important in the pathogenesis of diabetes and metabolic disorders in women.

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C-reactive protein (CRP) is an acute-phase reactant that is a marker of inflammation in the body. Mild chronic elevations of CRP concentrations, even when within the clinically "normal" range, are independently predictive of future cardiovascular events (1,2). Recent cross-sectional studies show that elevated CRP levels correlate significantly with features of the metabolic syndrome (insulin resistance syndrome), including adiposity, hyperinsulinemia, insulin resistance, hypertriglyceridemia, and low HDL cholesterol (3,4). A recent study has also demonstrated that high levels of CRP are related to increased accumulation of visceral and subcutaneous fat depots measured by computerized tomography scan (5). Two studies have reported that CRP predicts development of diabetes in postmenopausal women (6) and the elderly (7). These studies require confirmation in other populations and extension to other features of the metabolic syndrome.

Therefore, we assessed the 6-year development of diabetes and the metabolic syndrome in relation to baseline CRP levels in 515 men and 729 women from the Mexico City Diabetes Study. The metabolic syndrome was defined as subjects developing two or more of the metabolic disorders (dyslipidemia, hypertension, or diabetes) (8). We also compared the clinical value of CRP with indexes of adiposity and insulin resistance as a simple risk factor for the development of metabolic disorders.

RESEARCH DESIGN AND METHODS

— The Mexico City Diabetes Study is a population-based study of diabetes and cardiovascular risk factors. Between 1990 and 1992, 3,326 men and nonpregnant women aged 35–64 years from several low income "colonias" in Mexico City were eligible for the survey. A home interview was completed by 93% of the eligible subjects, and 68% of these completed a medical examination in a clinic (8). The protocol was approved by the Institutional Review Boards of the University of Texas Health Science Center at San Antonio and the American British Cowdray Hospital in Mexico City, and all subjects gave written informed consent. As described in the present article, we studied 515 men and 729 women who, at

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Abbreviations: CRP, C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; ROC, receiver-operating characteristic.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

baseline, had serum CRP concentrations measured and were free of diabetes.

Subjects' weight, height, and waist and hip circumferences were measured using standard methods for the calculations of BMI (kg/m^2) and waist-to-hip ratio (8). Systolic (first Korotkov phase) and diastolic (fifth Korotkov phase) blood pressures were measured in a seated position after 5 min at rest three times to the nearest even digit with a random-zero sphygmomanometer (Hawksley-Gelman, Lancing, Sussex, U.K.). The average of the second and third readings was defined as the subject's blood pressure.

Each subject's blood was sampled after a 12- to 14-h fast for the measurements of serum lipids, lipoproteins, CRP, insulin, and plasma glucose concentrations. Glucose and insulin concentrations were also measured 2 h after a 75-g oral glucose load. Plasma insulin was measured by a solid-phase radioimmunoassay (8). CRP concentrations were measured by an ultra-sensitive competitive immunoassay (antigens and antibodies from Calbiochem, Darmstadt, Germany) with an interassay coefficient of variation of 8.9% (9). Insulin resistance was calculated from the homeostasis model assessment (HOMA-IR): Insulin resistance = [fasting insulin (units/ml) × fasting glucose (mmol/l)/22.5] (8).

Information of subjects' lifestyle was obtained from their responses to questions about the number of cigarettes smoked, the number of alcoholic drinks consumed per week, and total (leisure and work) metabolic equivalents of activity performed.

Hypertension was defined as systolic blood pressure ≥140 or diastolic blood pressure ≥90 mmHg or hypertension diagnosed by a physician, and was confirmed by the use of antihypertensive medication that subjects were asked to bring to the examination (10). Hypertriglyceridemia was defined as ≥2.26 mmol/l (≥200 mg/dl). HDL cholesterol concentration was considered low if it was ≤0.91 mmol/l (≤35 mg/dl) in men and ≤1.17 mmol/l (≤40 mg/dl) in women (10). Dyslipidemia was defined as subjects with either hypertriglyceridemia or low HDL cholesterol. Diabetes was diagnosed as fasting plasma glucose ≥7 mmol/l (126 mg/dl) or a 2-h glucose level ≥11.1 mmol/l (200 mg/dl). Subjects who did not meet these criteria but who were receiving oral antidiabetic agents or insulin were also considered to have diabetes regardless of their plasma glucose value. The metabolic syndrome was defined as two or more metabolic disorders (dyslipidemia, hypertension, or diabetes) (10). All independent variables were stratified into sex-specific tertiles for analyses of the risks of the development of metabolic disorders and the metabolic syndrome. CRP was stratified into tertiles at 0.91 and 1.66 mg/l in men and at 1.24 and 2.17 mg/l in women. After 6 years, subjects were recalled to ascertain the incidence of dyslipidemia, diabetes, and hypertension. All anthropometric, biochemical, and physiological measurements were performed in an identical manner to those used at the baseline examination (10).

All statistical analyses were performed using SAS statistical package version 8.01 (SAS Institute, Cary, NC) for men and women separately and were adjusted for age and lifestyle factors. Spearman's correlations were used to assess the relationship between CRP and biochemical factors. Logistic regression analyses were carried out to calculate the odds ratios for the development of the metabolic syndrome and its components (dependent variables) in subjects with elevated baseline CRP (second and third tertiles) compared with those with low baseline CRP (lowest tertile, i.e., the reference group), with additional adjustments for baseline indexes of adiposity and insulin resistance (independent variables).

Table 1—*Baseline characteristics of 1,244 men and women in the Mexico City Study*

	Men (n = 515)	Women (n = 729)
Age (years)	46.0 ± 0.4	46.0 ± 0.3
Weight (kg)	72.6 ± 0.5	65.6 ± 0.4
Height (cm)	164.5 ± 0.2	151.4 ± 0.2
BMI (kg/m^2)	26.8 ± 0.2	28.6 ± 0.2
Waist circumference (cm)	93.1 ± 0.4	97.4 ± 0.5
Waist-to-hip ratio	0.964 ± 0.002	0.964 ± 0.003
CRP (mg/l)	2.29 ± 0.32	2.74 ± 0.20
Fasting insulin (IU/ml)	14.7 ± 0.6	16.7 ± 0.6
Fasting glucose (mmol/l)	4.72 ± 0.03	4.73 ± 0.02
Insulin resistance (HOMA)*	3.22 ± 0.13	3.68 ± 0.14
Triglycerides (mmol/l)	2.79 ± 0.09	1.98 ± 0.05
HDL cholesterol (mmol/l)	0.78 ± 0.01	0.90 ± 0.01
Systolic blood pressure (mmHg)	117.5 ± 0.6	114.4 ± 0.6
Diastolic blood pressure (mmHg)	74.9 ± 0.5	71.2 ± 0.4
Current smoking (%)	48.2	20.3

Data are means ± SE. *Insulin resistance calculated using the HOMA as fasting insulin (units/ml) × fasting glucose (mmol/l)/22.5.

RESULTS — Table 1 shows the baseline characteristics of 515 men and 729 women who had similar age. Compared with men, women were lighter and shorter, but they had higher BMI and larger waist circumference. Women had higher concentrations of CRP, HOMA-IR, and HDL cholesterol, and they had lower levels of triglyceride and systolic and diastolic blood pressures. Of the women, 4% received hormonal replacement therapy.

CRP correlated significantly ($P < 0.01$) with BMI ($r = 0.34$), waist circumference ($r = 0.32$), HOMA-IR (0.22), fasting glucose ($r = 0.11$), triglyceride ($r = 0.23$), HDL cholesterol ($r = -0.17$), and systolic blood pressure ($r = 0.16$) in women but only significantly ($P < 0.05$) in men with BMI ($r = 0.14$), waist circumference ($r = 0.12$), HOMA-IR ($r = 0.12$), and triglyceride ($r = 0.10$).

Six years after the baseline examination of subjects who were free of diabetes but had one or none of other metabolic disorders (hypertension or dyslipidemia), 14.2% of men and 16.0% of women developed the metabolic syndrome, 45.4% of whom (44.1% of men and 46.2% of women) developed diabetes. The incidence of the metabolic syndrome adjusted for age, cigarette smoking, alcohol consumption, and physical activity was significantly higher in women with higher levels of CRP: 7.5, 18.1, and 23.8% in the first, second, and third CRP tertiles, respectively ($P < 0.001$ for trend). This relationship was not significant in men (14.2, 11.9, and 14.1%, respectively, trend $P = 0.54$).

Table 2—*Multivariate logistic regression analysis of baseline CRP in the prediction of features of the development of the metabolic syndrome with adjustment for age, cigarette smoking, alcohol consumption, and physical activity*

	CRP tertile 2			CRP tertile 3		
Dependent variable*	OR	95% CI	*P*	OR	95% CI	*P*
Men						
Hypertriglyceridemia	2.2	0.92–5.4	0.078	1.2	0.5–3.3	0.69
Low HDL cholesterol	1.2	0.4–3.6	0.71	1.07	0.3–3.5	0.92
Hypertension	0.8	0.3–2.1	0.71	1.0	0.4–2.4	0.99
Diabetes	0.7	0.3–1.6	0.37	0.8	0.4–2.0	0.70
Metabolic syndrome	0.8	0.4–1.7	0.54	0.9	0.5–2.0	0.88
Women						
Hypertriglyceridemia	1.2	0.6–2.3	0.67	1.9	0.97–3.6	0.064
Low HDL cholesterol	2.3	1.01–5.2	0.047	2.2	0.92–5.3	0.077
Hypertension	2.1	0.98–4.7	0.058	2.1	0.96–4.7	0.064
Diabetes	2.8†	1.1–7.3	0.036	5.4‡	2.2–13.4	<0.001
Metabolic syndrome	2.8†	1.4–5.5	0.003	4.1‡	2.1–8.0	<0.001

*In each model, subjects were free of the corresponding disorder under analysis, but they might have had none or one of the other disorders at baseline (e.g., for the prediction of the development of hypertriglyceridemia, subjects were free of baseline hypertriglyceridemia, but they might have had none or just one of the other disorders). †$P < 0.05$, ‡$P < 0.01$ for heterogeneity by sex.

Table 2 shows that compared with women with low baseline CRP levels (lowest tertile, i.e., reference group), those with high baseline CRP levels (highest tertile) had a significant ($P < 0.01$) increase in the risk for the development of diabetes and for the metabolic syndrome. There were no significant relationships shown in men.

Table 3 shows that compared with women in the lowest tertile of baseline CRP, the risk for developing the metabolic syndrome was increased by 2.8 (1.4–5.5) in women with baseline CRP in the second tertile and by 4.0 (2.0–7.9) in the third tertile. These odds ratios did not change substantially after adjusting for indexes of adiposity and insulin resistance. There were no significant relationships between CRP and the development of the metabolic syndrome in men. There were little changes in the relationship after adjusting for BMI and insulin resistance. We repeated this analysis in "lean" and "overweight" subjects (stratified by median of baseline BMI) separately, and found that the incidence was higher in overweight subjects in each CRP tertile and highest in overweight subjects with the highest levels of CRP (Fig. 1). In both lean and over-

Table 3—*Odds ratio for the development of metabolic syndrome*

	CRP					
	Tertile 2*			Tertile 3*		
	OR	95% CI	*P*	OR	95% CI	*P*
Men						
CRP alone	0.8	0.4–1.7	0.590	1.0	0.5–2.1	0.696
CRP + BMI	0.7	0.3–1.6	0.408	0.9	0.4–2.0	0.869
CRP + waist circumference	0.8	0.4–1.7	0.610	1.0	0.5–2.1	0.992
CRP + waist-to-hip ratio	0.8	0.4–1.7	0.607	1.0	0.5–2.1	0.980
CRP + insulin resistance	0.8	0.4–1.7	0.563	1.0	0.5–2.1	0.972
CRP + BMI + insulin resistance	0.8	0.3–1.7	0.480	1.0	0.5–2.1	0.946
CRP + waist circumference + insulin resistance	0.8	0.4–1.8	0.634	1.0	0.5–2.2	0.996
Women						
CRP alone	2.8	1.4–5.5	0.004	4.0	2.0–7.9	<0.001
CRP + BMI	2.6	1.3–5.1	0.008	3.4	1.7–6.9	<0.001
CRP + waist circumference	2.6	1.3–5.1	0.007	3.7	1.9–7.3	<0.001
CRP + waist-to-hip ratio	2.6	1.3–5.2	0.007	3.7	1.9–7.3	<0.001
CRP + insulin resistance	3.1	1.5–6.4	0.002	3.8	1.9–7.7	<0.001
CRP + BMI + insulin resistance	3.0	1.5–6.1	0.003	3.5	1.7–7.2	<0.001
CRP + waist circumference + insulin resistance	3.1	1.5–6.3	0.002	3.7	1.8–7.4	<0.001

Data was pooled with sex interaction with baseline CRP levels in the second and third (highest) tertiles compared with those in the first (lowest) tertile, after adjustment for indexes of adiposity and insulin resistance as covariates in various models. Data were adjusted for baseline age, cigarette smoking, alcohol consumption, and physical activity. *Reference group = CRP tertile 1.

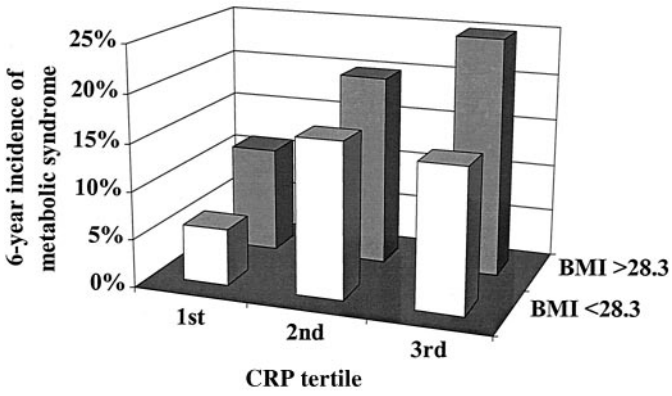

Figure 1—*Six-year incidence of the metabolic syndrome in "lean" and "overweight" women (stratified by median of baseline BMI) by tertiles of baseline CRP levels. Data were adjusted for age, cigarette smoking, alcohol consumption, and physical activity.*

weight groups, high CRP levels remained significantly predictive of the metabolic syndrome (results not shown).

Figure 2 shows that the area under the receiver-operating characteristic (ROC) curve was 0.684 for CRP in the prediction of the development of metabolic syndrome, and increased to 0.706 when CRP was combined with BMI, and a further 0.004 increase was obtained when insulin resistance was added to this model.

CONCLUSIONS — The findings in the present study showed that Mexican women with elevated CRP levels had a significantly increased risk of the development of both diabetes and the metabolic syndrome independent of their adiposity and insulin resistance. In addition, the results showed that CRP alone was as good as other single risk factors in the prediction of the syndrome. When CRP was combined with indexes of adiposity, the area under the ROC curve improved by ~0.02, but further inclusion of biochemical measures such as insulin resistance added only another 0.004.

Thus, our data in Mexican women complement the recent study by Pradhan et al. (6) who demonstrated a similar observation in predominantly Caucasian women where diabetes was initially identified by self-report and subsequently verified by a spectrum of criteria. Another study (7) demonstrates that CRP levels are associated with the development of diabetes in the elderly. This latter study did not examine men and women separately, was based on fewer individuals (n = 45) who developed diabetes, and only assessed fasting glucose on one occasion after baseline at 3–4 years of follow-up. Our analysis, which identified a larger number of middle-aged subjects of both sexes with a more complete diagnosis of diabetes on the basis of both fasting glucose and 2-h post–glucose tolerance test data, is a significant addition to the above observations.

The other novel and important aspect of our study is that, in addition to the development of diabetes, we examined for the development of other features of the metabolic syndrome. In line with our expectations, CRP also predicted the development of the syndrome in women independently of obesity and insulin resistance, thereby strengthening its potential role in predicting metabolic disorders. The clinical relevance of our results in women is that CRP is the most commonly used and best standardized inflammatory marker and thus has the potential to be used in the prediction of diabetes and the metabolic syndrome. This issue has become more important since recent reports have suggested prevention/delay of diabetes with lifestyle intervention (11,12). A recent report (13) suggested that a fasting prediction equation may identify individuals at high risk of developing diabetes without requiring an oral glucose tolerance test. The ability of CRP to improve such diabetes risk assessments should be studied.

CRP is an inflammatory marker produced and released by the liver under the stimulation of cytokines including interleukin-6, interleukin-1, and tumor necrosis factor-α. The relationships between CRP levels and adiposity could be explained by recent findings of adipose tissue as a source for the production and release of cytokines (14). These inflammatory factors have also been linked to dyslipidemia, hypertension, and insulin action in previous cross-sectional studies (3,4) and such observations have been extended in the present prospective study. There exist plausible biochemical mechanisms for linking some of these components. For example, cytokines promote de novo hepatic fatty acid synthesis and interfere with lipoprotein lipase activity (the endothelial enzyme responsible for catabolism of triglyceride-rich lipoproteins) (15). Cytokines may also directly impede insulin-stimulated glucose uptake (16).

Most studies of CRP have analyzed men and women together and have been adjusted for sex, although a recent article by Hak et al. (17) did show a stronger association of inflammatory markers with

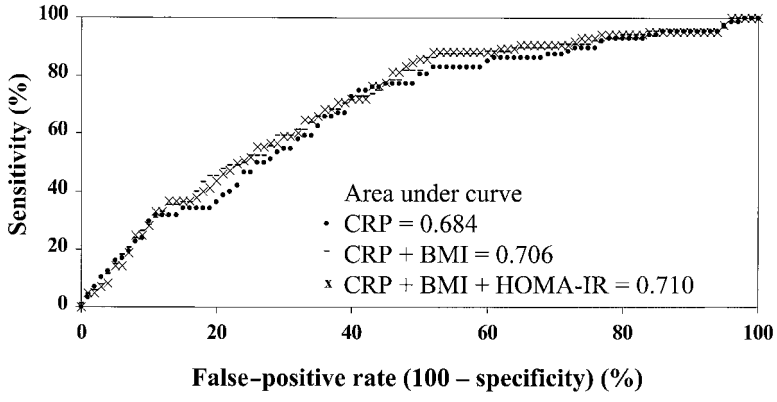

Figure 2—*ROC analyses of CRP, BMI, and insulin as covariates in the prediction of 6-year development of the metabolic syndrome in women. Data were adjusted for age, cigarette smoking, alcohol consumption, and physical activity. The higher the area under the curve (i.e., the greater the curvature away from the 50% line [zero prediction]), the greater the predictive power.*

insulin concentrations in elderly women than in men. The present study explicitly tested for sex-CRP interactions. Elevated CRP was a significant predictor of the development of diabetes and the metabolic syndrome in women only, independent of obesity and insulin resistance. This relationship was not significant in men. There are several possible explanations for these sex differences. Firstly, it should be noted that many more women were included in this study and that more women developed both diabetes and the metabolic syndrome. Thus, the study had greater statistical power to detect meaningful results in women. Secondly, since CRP correlated with indexes of adiposity and other metabolic parameters more strongly in women than in men, low-grade inflammation may have a greater role in perturbing insulin action in women. Inflammatory factors may interact with female sex hormones such that chronic inflammation may reduce the protective effects of estrogen on body fat distribution and insulin action. Data from the present study cannot clarify this suggestion. Nevertheless, there is some evidence for an interaction between these two pathways. Previous studies have suggested that cytokines may interfere with estradiol secretion (18), whereas oral, but not transdermal (which avoids hepatic first-pass metabolism) estrogens, can increase CRP levels (19,20). Only 4% of women in the present study received hormonal replacement therapy, and adjustment for this factor (or any previous hormone use) did not change the results (data not presented). Clearly, further prospective data are needed to examine the potential of CRP to predict diabetes and metabolic disorders in men. In the present study, <50% of subjects who developed the metabolic syndrome had developed diabetes as a manifestation of the syndrome; thus, the risks attributable to the metabolic syndrome cannot simply be accounted for by diabetes alone.

It is now known that both lipid-lowering therapies (statins) and ACE inhibitors have anti-inflammatory properties (21,22), and preliminary findings have suggested that they may lower the risk of diabetes (23,24). In addition, insulin-sensitizing agents, such as thiazolidinediones (25), and physical exercise (26) also appear to have anti-inflammatory properties. More recent animal data suggest that that salicylates prevent obesity and diet-induced insulin resistance (27). Thus, reducing inflammation may have beneficial effects on the development of diabetes and other metabolic disorders. Future investigations are clearly needed to examine this possibility.

In conclusion, we show that the predictive value of CRP for the development of diabetes extends to Mexican women 35–64 years of age. We also show for the first time that CRP predicts the development of the metabolic syndrome independently of the levels of adiposity and insulin resistance in Mexican women, but this risk was not evident in men. We suggest that measurement of CRP alone or combined with BMI or waist circumference can be used instead of complicated measures (e.g., fasting insulin, which requires a fasting state) as a risk factor for developing diabetes and the metabolic syndrome. Thus, CRP may be of clinical value for monitoring steps for the prevention of diabetes and metabolic disorders.

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