Inflammatory Markers and Risk of Developing Type 2 Diabetes in Women

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We conducted a prospective, nested, case-control study of inflammatory markers as predictors of type 2 diabetes among 32,826 women who provided blood samples in 1989 through 1990 in the Nurses' Health Study. Among women free of diabetes, cardiovascular disease, or cancer at baseline, 737 had developed diabetes by 2000. Control women (n = 785) were selected matched on age, fasting status, race, and BMI for cases in the top BMI decile. Baseline levels of tumor necrosis factor (TNF)-α receptor 2, interleukin (IL)-6, and C-reactive protein (CRP) were significantly higher among case than control subjects (all $P \leq 0.001$). After adjusting for BMI and other lifestyle factors, all three biomarkers significantly predicted diabetes risk; the odds ratios (ORs) comparing extreme quintiles were 1.64 (95% CI 1.10–2.45) for TNF-αR2, 1.91 (1.27–2.86) for IL-6, and 4.36 (2.80–6.80) for CRP (P for trend <0.001 for all biomarkers). In a multivariate model simultaneously including the three biomarkers, only CRP levels were significantly associated with risk of diabetes (OR comparing extreme quintiles of CRP = 3.99, P for trend <0.001). These data support the role of inflammation in the pathogenesis of type 2 diabetes. Elevated CRP levels are a strong independent predictor of type 2 diabetes and may mediate associations of TNF-αR2 and IL-6 with type 2 diabetes. *Diabetes* 53:693–700, 2004

Excess adiposity is the most important risk factor for the development of insulin resistance and type 2 diabetes (1). However, mechanisms whereby body fat induces insulin resistance in distant tissues are not well understood. Recent evidence indicates that obesity may be an inflammatory condition (2). It has been proposed that inflammatory cytokines secreted by adipose tissue exert an endocrine effect conferring insulin resistance in liver, skeletal muscle, and vascular endothelial tissue, ultimately leading to the clinical expression of both type 2 diabetes and cardiovascular disease (CVD) (3,4). In particular, elevated production of adipocyte cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, leads to an acute-phase response with increased hepatic production of C-reactive protein (CRP), a sensitive marker of low-grade systemic inflammation (5–7). TNF-α, IL-6, and CRP not only directly promote insulin resistance, but also stimulate endothelial production of adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1), critical mediators of endothelial dysfunction in capillary and arteriolar endothelium (8).

Most previous epidemiological research has focused on the role of CRP in predicting risk of type 2 diabetes. While several studies observed a significant positive association between CRP levels and type 2 diabetes after adjusting for BMI or waist circumference (9–12), others suggested that the association was largely explained by obesity (13–16). In this large prospective, nested, case-control study, we simultaneously examined the role of TNF-α receptor 2 (TNF-αR2, as a measure of TNF-α in plasma), IL-6, and CRP in predicting the development of type 2 diabetes independent of obesity, diet, and other lifestyle factors in women.

RESEARCH DESIGN AND METHODS

Study population. The Nurses' Health Study cohort was established in 1976 when 121,700 female registered nurses aged 30–55 years and residing in 11 U.S. states responded to mailed questionnaires regarding their medical history and health practices. Since 1976, questionnaires have been administered biennially to update health information and to identify new cases of disease. During 1989 through 1990, 32,826 women free of diagnosed diabetes, coronary heart disease, stroke, or cancer provided blood samples. By 2000, 737 of these women had developed definite diabetes. Control women providing baseline blood samples were matched to diabetes cases by year of birth, date of blood draw, race, and fasting status (at least 8 h overnight) at blood draw. In addition, to improve statistical control for obesity at the upper extreme of the distribution, control subjects were also matched on BMI with case subjects in the top 10% of the BMI distribution, giving a sample of 785 control women (26 control subjects were selected twice in the nested case-control risk-set sampling). The study was approved by the Institutional Review Board of the Brigham and Women's Hospital, Boston.

Ascertainment of diabetes. A validated supplementary questionnaire regarding symptoms, diagnostic tests, and hypoglycemic therapy was mailed to women who indicated on any biennial questionnaire that they had been diagnosed with diabetes. A case of diabetes was considered confirmed if at least one of the following was reported on the supplementary questionnaire: *1*) classic symptoms plus elevated glucose levels (a fasting plasma glucose concentration ≥140 mg/dl or randomly measured concentration of at least 200 mg/dl); *2*) at least two elevated plasma glucose concentrations on different occasions in the absence of symptoms (levels ≥200 mg/dl after 2 or more hours on oral glucose tolerance testing); *3*) treatment with oral hypoglycemic

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CRP, C-reactive protein; CVD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; TNF, tumor necrosis factor; TNF-αR2, TNF-α receptor 2; VCAM-1, vascular adhesion molecule-1.

agents or insulin. These criteria are consistent with those proposed by the National Diabetes Data Group (NDDG) (17). The diagnostic criteria for type 2 diabetes were changed in 1997 (18) such that lower fasting glucose levels (>126 mg/dl) would now be considered diagnostic. Thus, we have used the American Diabetes Association criteria for diagnosis of diabetes cases after 1998.

The validity of this diagnostic procedure has been verified in a subsample of this study population (19). The diagnosis of type 2 diabetes was confirmed by medical records in 98.4% (60 of 61) of the women. In addition, another substudy assessing the prevalence of undiagnosed diabetes suggested a very low rate of false negatives (20).

Assessment of lifestyle factors. Every 2 years, participant exposure status has been updated by questionnaire, including smoking (never smoked, former smoker, or current smoker), menopausal status, use or nonuse of postmenopausal hormone therapy, and body weight. Reported weights have been shown to correlate with measured weights ($r = 0.96$) (21). We calculated BMI as weight in kilograms divided by the square of height (assessed in 1976) in meters. In 1986 we also assessed self-reported waist girth; this measure also strongly correlates with measured waist circumference ($r = 0.89$) in this cohort (22). The presence or absence of a family history of diabetes in first-degree relatives was assessed in 1982 and 1988. Information about physical activity was assessed in 1988 using a validated questionnaire (23).

Diet was assessed using a validated semiquantitative food frequency questionnaire (24). A composite score was constructed on the basis of a diet low in *trans* fat and glycemic load, high in cereal fiber, and with a high ratio of polyunsaturated to saturated fat (25). For each dietary factor, we assigned each woman a score of one to five corresponding to her quintile of intake, with five representing the most favorable quintile, and summed her quintile values for the four nutrients.

Laboratory procedures. Women willing to provide blood specimens were sent instructions and a phlebotomy kit (including sodium heparin blood tubes, needles, and a tourniquet). Blood specimens were returned by overnight mail in a frozen water bottle, and upon arrival they were centrifuged to separate plasma from buffy coat and red cells and frozen in liquid nitrogen until analysis. Ninety-seven percent of samples arrived within 26 h of phlebotomy. Quality control samples were routinely frozen along with study samples to monitor for plasma changes due to long-term storage and to monitor assay variability. Previous work has documented the long-term stability of plasma samples collected and stored under this protocol (26). Study samples were analyzed in randomly ordered case-control pairs to further reduce systematic bias and interassay variation.

Frozen plasma aliquots from case and control subjects were selected for simultaneous analysis. CRP levels were measured via a high-sensitivity latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring, Newark, DE). IL-6 was measured by a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay kit) and TNF-R2 levels by an enzyme-linked immunosorbent assay (ELISA) kit utilizing immobilized monoclonal antibody to human TNF-R2 (Genzyme, Cambridge, MA). Levels of E-selectin and ICAM-1 were measured by commercial ELISA (R & D Systems, Minneapolis, MN). Insulin levels were measured with a double antibody system with <0.2% cross-reactivity between insulin and its precursors (Linco Research, St. Louis, MO). HbA_{1c} was measured by immunoassay (Hitachi 911 Analyzer; Roche Diagnostics, Indianapolis, IN). The coefficients of variation for each analyte were: 3.8% for C-reactive protein, 9.5% for fasting insulin, 3.8% for HbA_{1c}, 5.9% for IL-6, 6.2% for TNF-αR2, and 6.6% for E-selectin.

Statistical analysis. Baseline characteristics of case and control subjects were compared by Student's *t* tests, Wilcoxon's rank-sum tests (for differences in median values), or χ^2 tests. Pearson correlation coefficients were used to evaluate associations among various biomarkers. We used conditional logistic regression to estimate the OR and 95% CIs for levels of biomarkers predicting type 2 diabetes, conditioning on matching factors such as age, fasting status, and race. We divided the distributions of the inflammatory markers in the control subjects into quintiles and used regression models to estimate the significance of trend in ORs across increasing biomarker quintiles and to estimate the OR of diabetes in each quintile using the lowest quintile as the referent category. In multivariate analyses, we adjusted for BMI (modeled as a continuously distributed covariate), further adjusted for family history of diabetes, smoking, alcohol intake, physical activity, postmenopausal hormone use (all modeled as categorical covariates), and the diet score. We then included CRP, IL-6, and TNF-αR2 simultaneously in the multivariate model to mutually adjust for each other. In a secondary analysis among women providing fasting blood samples, we also adjusted models for levels of fasting insulin and HbA_{1c}. In addition, we conducted analyses of inflammatory markers and diabetes risk stratified on levels of diabetes risk factors including obesity, physical activity, and the diet score. Likelihood ratio tests were conducted to test statistical interactions between inflammatory biomarkers and obesity, physical activity, regular aspirin use (approximately once per week or more), family history of diabetes, and diet score by comparing the −2 log(likelihood) between two nested models, one with only the main effects and the other with both the main effects and interaction terms. SAS Statistical (27) was used in all of the analyses.

RESULTS

As expected, women who developed diabetes during follow-up had significantly higher levels of BMI, waist circumference, and waist-to-hip ratio; had lower levels of physical activity and intake of cereal fiber; and were more likely to have a family history of diabetes than control subjects (Table 1). Case subjects were slightly more likely to use aspirin than control subjects. Among case subjects, levels of TNF-αR2, CRP, IL-6, ICAM-1, E-selectin, and fasting insulin were all significantly higher at baseline in women who developed diabetes than in those who remained nondiabetic during follow-up.

Among the healthy control subjects, BMI and waist circumference were significantly correlated with CRP levels, and the correlations between obesity and TNF-αR2, IL-6, and endothelial markers were somewhat weaker (Table 2). CRP was moderately but significantly correlated with TNF-αR2, IL-6, ICAM-1, and E-selectin.

Elevated levels of TNF-αR2, IL-6, and CRP measured at baseline were significantly associated with risk of incident type 2 diabetes. In crude analyses conditioned on matching covariates, the OR of diabetes comparing extreme quintiles were 2.59 for TNF-αR2, 3.25 for IL-6, and 7.08 for CRP (*P* for trend <0.001 for all of the biomarkers, Table 3). These ORs were substantially attenuated but remained statistically significant after adjustment for BMI. Further adjustment for other diabetes risk factors, including family history of diabetes, smoking, alcohol intake, physical activity, diet, and hormone use, did not appreciably alter the ORs (Table 3). A recent study suggested a significant association between inflammation and diabetes among nonsmokers but not smokers (28). However, we did not observe such an interaction between smoking and CRP levels ($P = 0.43$).

When TNF-αR2, CRP, and IL-6 were included simultaneously in the model, only CRP remained significant (ORs across quintiles were 1.0, 1.29, 1.59, 3.48, and 3.99, *P* for trend <0.001). The ORs for TNF-αR2 and IL-6 were substantially attenuated and did not reach statistical significance (ORs comparing extreme quintiles were 1.25, 95% CI 0.82–1.91 for TNF-αR2 and 1.16, 0.75–1.79 for IL-6). Because these biomarkers are in the same causal pathway of systemic inflammation and diabetes, these results suggest that the effects of TNF-αR2 and IL-6 may be mediated through elevated CRP. When the effects of the inflammatory markers were evaluated jointly, the increased diabetes risk tended to be additive (all *P* values for multiplicative interactions >0.05 between the biomarkers), with the highest risk of diabetes among women with the highest levels of CRP in combination with the highest levels of IL-6 (Fig. 1*A*) and TNF-αR2 (Fig. 1*B*). However, the stratified analyses indicate that the effects of CRP were more evident than those of IL-6 or TNF-αR2. In joint analyses, the effects of TNF-αR2 and IL-6 tended to be independent and additive (Fig. 1*C*).

In a previous analysis (29), we found that markers of endothelial dysfunction, especially E-selectin, significantly

TABLE 1
Comparison of diabetes risk factors between case and control subjects at baseline*

	Case	Control	*P*
n	737	785	
Age (years)	56.3 ± 6.9	56.2 ± 6.9	0.69
BMI (kg/m^2)	30.3 ± 5.6	26.2 ± 6.1	<0.001
Physical activity (METs/week)†	12.3 ± 15.2	15.6 ± 27.2	0.004
Alcohol consumption (g/day)	3.7 ± 7.1	6.5 ± 9.0	<0.001
Waist circumference (in)	35.3 ± 4.9	31.3 ± 4.9	<0.001
Diet score‡	7.8 ± 2.7	8.2 ± 2.7	<0.001
Current smoking (%)	14.1	13.3	0.62
Postmenopausal status (%)	36.0	42.3	0.01
Current postmenopausal hormone use (%)	39.8	36.0	0.35
Family history of diabetes in first-degree relative (%)	46.8	21.0	<0.001
Race (Caucasian) (%)	94.8	94.3	0.68
Aspirin use ≥1/week	25.1	20.9	0.05
Biomarkers (median values)			
TNF-αR2 (pg/ml)	2,646.5	2,383.8	<0.001
CRP (mg/dl)	0.36	0.16	<0.001
IL-6 (pg/ml)	2.38	1.84	<0.001
E-selectin (pg/ml)	61.25	45.37	<0.001
Fasting insulin (uU/ml)§	11.96	8.27	<0.001

Data are means ± SE unless otherwise indicated. *Case and control subjects were matched on age, fasting status, and race. †MET, metabolic equivalent; 1 MET hour is equivalent to energy expended by sting quietly for 1 h. ‡Intakes for *trans* fat, cereal fiber, glycemic load, and P-to-S (polyunsaturated fat–to–saturated fat) ratio were categorized into quintiles and for each participant, the quintile value for each nutrient (a higher quintile score represents a lower risk) was summed and the sum was recategorized into quintiles. §A total of 432 case and 398 control subjects had fasting insulin measurement.

predicted risk of type 2 diabetes. To examine whether the association between CRP and risk of diabetes was mediated through markers of endothelial dysfunction, we simultaneously included CRP and E-selectin in the multivariate model. The association for CRP was independent of levels of E-selectin (OR for the highest versus lowest quintile = 3.52, *P* for trend <0.001), and the elevated risk with increasing CRP and E-selectin levels appeared to be additive and independent of each other (Fig. 1*D*) (*P* for interaction = 0.55).

In stratified analyses, levels of TNF-αR2 (without adjustment for CRP and IL-6) significantly predicted diabetes risk among nonobese women, women with lower physical activity levels, women with a lower diet score, and women with no family history of diabetes (Table 4). However, only the test for interaction between obesity and TNF-αR2 was statistically significant (*P* = 0.01). The association between IL-6 and diabetes risk appeared to be stronger for women with BMI <30 kg/m^2 and those with lower physical activity levels, although the tests for interactions were not statistically significant. The association between CRP levels and risk of diabetes was significant in stratified analyses according to levels of obesity, physical activity, the diet score, and family history of diabetes (Table 4).

In additional analyses, we adjusted for regular use (once a week or more) of aspirin and other nonsteroidal anti-inflammatory drugs and obtained similar results for CRP (OR comparing extreme quintiles = 4.33, 95% CI 2.78–6.74, *P* for trend <0.001). However, in stratified analyses (Fig. 2), the association between CRP levels was substantially stronger among participants who did not use aspirin (multivariate ORs across quintiles of CRP were 1.0, 1.13, 2.06, 4.86, and 8.85, *P* for trend <0.001) compared with women who reported regular aspirin use (corresponding ORs were 1.0, 1.49, 1.12, 3.33, and 2.94, *P* for trend <0.001, *P* for interaction between aspirin use and CRP = 0.0002).

We conducted several sensitivity analyses to address the issues of undiagnosed diabetes in the control subjects. First, we removed women with HbA_{1c} levels >6.0% and repeated the analyses; the results remained virtually unchanged. Second, adjustment for HbA_{1c} levels for both case and control subjects in the multivariate analyses had virtually no impact on the results (multivariate OR comparing extreme quintiles of CRP was 4.33, 95% CI 1.98–

TABLE 2
Spearman correlation coefficients* between BMI, waist circumference, fasting insulin, and inflammatory markers in control subjects

	BMI	Waist circumference	Fasting insulin	CRP	IL-6	TNF-αR2	E-selectin
BMI	1.0	—	—	—	—	—	—
Waist circumference	0.78	1.0	—	—	—	—	—
Fasting insulin	0.29	0.27	1.0	—	—	—	—
CRP	0.44	0.37	0.26	1.0	—	—	—
IL-6	0.30	0.29	0.18	0.37	1.0	—	—
TNF-αR2	0.24	0.25	0.15	0.27	0.28	1.0	—
E-selectin	0.28	0.27	0.26	0.24	0.25	0.23	1.0

*All correlation coefficients were statistically significant at $P < 0.05$.

TABLE 3
RR (95% CI) of type 2 diabetes according to quintiles of inflammatory markers estimated from conditional logistic regression models

	Q1	Q2	Q3	Q4	Q5	*P* for trend
TNFαR2 (pg/ml)						
Range	≤1,927.7	1,827.8–2,224.4	2,224.5–2,530.0	2,530.1–3,023.5	≥3,023.6	
Median	1,705.6	2,085.5	2,385.9	2,737.5	3,448.4	
n	96/167	98/155	124/153	192/155	227/155	
Crude RR*	1.00	1.14 (0.79, 1.65)	1.29 (0.90, 1.84)	2.10 (1.49, 2.95)	2.59 (1.84, 3.65)	<0.001
BMI-adjusted RR†	1.00	1.00 (0.68, 1.48)	1.11 (0.76, 1.62)	1.56 (1.08, 2.24)	1.67 (1.16, 2.42)	<0.001
Multivariate RR‡	1.00	0.89 (0.58, 1.37)	1.09 (0.73, 1.64)	1.47 (0.99, 2.18)	1.64 (1.10, 2.45)	0.001
IL-6 (pg/ml)						
Range	≤1.12	1.13–1.62	1.63–2.13	2.15–3.07	≥3.08	
Median	0.88	1.34	1.84	2.55	4.24	
n	88/185	110/150	134/150	176/151	229/149	
Crude RR	1.00	1.60 (1.11, 2.31)	1.77 (1.24, 2.53)	2.44 (1.71, 3.46)	3.25 (2.30, 4.58)	<0.001
BMI-adjusted RR	1.00	1.29 (0.87, 1.90)	1.21 (0.83, 1.78)	1.55 (1.06, 2.26)	1.95 (1.34, 2.84)	<0.001
Multivariate RR	1.00	1.24 (0.82, 1.88)	1.23 (0.81, 1.86)	1.43 (0.95, 2.15)	1.91 (1.27, 2.86)	0.001
CRP (mg/dl)						
Range	≤0.055	0.056–0.118	0.119–0.208	0.209–0.404	≥0.405	
Median	0.03	0.083	0.158	0.291	0.627	
n	55/176	58/152	89/153	216/152	319/152	
Crude RR	1.00	1.36 (0.87, 2.13)	2.11 (1.38, 3.22)	4.93 (3.33, 7.31)	7.08 (4.84, 10.37)	<0.001
BMI-adjusted RR	1.00	1.20 (0.75, 1.91)	1.68 (1.08, 2.62)	3.64 (2.42, 5.49)	4.18 (2.78, 6.29)	<0.001
Multivariate RR	1.00	1.30 (0.80, 2.13)	1.62 (1.01, 2.06)	3.61 (2.33, 5.60)	4.36 (2.80, 6.80)	<0.001

*Conditioned on matching variables: age, fasting status, and race; †BMI as a continuous variable; ‡adjusted for time at blood drawn, alcohol consumption (nondrinkers, 0–4.9, 5–10, and >10 g/day), physical activity (quintiles of METs/week), smoking status (never, past, and current smoking of 1–14, 15–24, and ≥25 cigarettes per day), BMI (continuous), family history of diabetes, postmenopausal hormone use and menopausal status (never/unknown user or premenopausal status, past, and current user), and diet score (in quintiles).

9.44, *P* for trend <0.001). To ensure that the association of inflammatory biomarkers with incident diabetes was not due to subclinical CVD at baseline, we repeated the analysis after removing all women with incident CVD that developed during follow-up; again, results were unchanged (multivariate OR comparing extreme quintiles of CRP 4.43, 2.83–6.93, *P* for trend <0.001).

To examine whether the associations between inflammatory markers and risk of diabetes were mediated through hyperinsulinemia, we conducted additional anal-

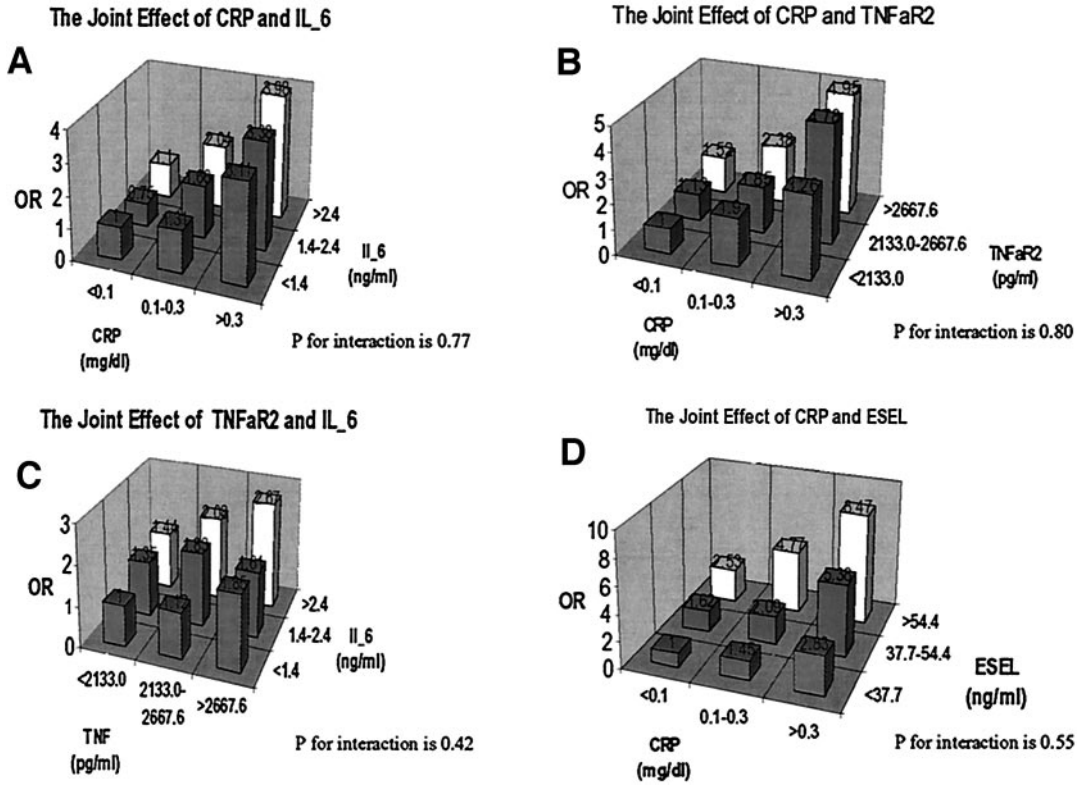

FIG. 1. *A–D*: Multivariate OR of type 2 diabetes according to joint classifications of plasma levels of inflammatory and endothelial markers. Adjusted for the same variables as in Table 3.

TABLE 4
Multivariate RR (95% CI) of type 2 diabetes according to quintiles of inflammatory markers stratified by BMI, physical activity, diet score, and family history of diabetes*

	Q1	Q2	Q3	Q4	Q5	*P* for trend	*P* for interaction
TNFαR2							
BMI <30 kg/m^2	1.00	0.95 (0.58–1.57)	1.16 (0.72–1.87)	1.62 (1.02–2.59)	2.25 (1.39–3.62)	<0.001	
BMI ≥30 kg/m^2	1.00	0.67 (0.26–1.68)	1.33 (0.53–3.35)	1.05 (0.45–2.44)	0.93 (0.41–2.09)	0.99	0.01
Physical activity levels							
Low	1.00	1.02 (0.58–1.77)	1.74 (1.02–2.98)	1.96 (1.18–3.28)	2.14 (1.29–3.54)	<0.001	
High	1.00	0.75 (0.41–1.38)	0.86 (0.48–1.54)	1.29 (0.74–2.26)	1.30 (0.72–2.34)	0.11	0.72
Diet score							
Low	1.00	0.74 (0.42–1.29)	1.04 (0.61–1.76)	1.45 (0.89–2.36)	2.05 (1.25–3.36)	<0.001	
High	1.00	1.06 (0.58–1.94)	1.32 (0.73–2.38)	1.78 (0.98–3.23)	1.18 (0.64–2.18)	0.48	0.21
Family history of diabetes							
Yes	1.00	0.73 (0.38–1.39)	0.75 (0.38–1.45)	1.15 (0.60–2.19)	1.51 (0.75–3.07)	0.07	
No	1.00	0.93 (0.54–1.62)	1.45 (0.87–2.41)	1.82 (1.12–2.96)	1.88 (1.16–3.04)	0.002	0.46
IL-6							
BMI <30 kg/m^2	1.00	1.20 (0.75–1.92)	1.14 (0.70–1.84)	1.44 (0.90–2.30)	1.84 (1.16–2.94)	0.006	
BMI ≥30 kg/m^2	1.00	0.90 (0.31–2.62)	1.11 (0.41–3.00)	1.07 (0.41–2.78)	1.46 (0.57–3.76)	0.17	0.29
Physical activity levels							
Low	1.00	1.31 (0.75–2.29)	1.27 (0.73–2.23)	1.70 (1.00–2.91)	2.47 (1.44–4.24)	<0.001	
High	1.00	1.38 (0.77–2.45)	1.63 (0.93–2.86)	1.44 (0.81–2.53)	1.77 (1.01–3.08)	0.09	0.73
Diet score							
Low	1.00	0.96 (0.56–1.65)	1.36 (0.80–2.29)	1.30 (0.78–2.16)	1.86 (1.12–3.09)	0.004	
High	1.00	1.74 (0.96–3.15)	1.28 (0.69–2.36)	1.85 (1.01–3.37)	2.16 (1.19–3.92)	0.024	0.38
Family history of diabetes							
Yes	1.00	1.99 (1.02–3.88)	1.79 (0.90–3.55)	2.19 (1.14–4.20)	1.97 (1.04–3.73)	0.16	
No	1.00	0.90 (0.54–1.50)	1.15 (0.70–1.87)	1.29 (0.80–2.10)	1.95 (1.21–3.13)	<0.001	0.13
CRP							
BMI <30 kg/m^2	1.00	0.80 (0.46–1.37)	0.98 (0.57–1.69)	2.42 (1.49–3.95)	3.57 (2.13–5.96)	<0.001	
BMI ≥30 kg/m^2	1.00	1.74 (0.48–6.27)	1.42 (0.49–4.18)	2.76 (1.01–7.58)	3.38 (1.31–8.77)	0.004	0.13
Physical activity levels							
Low	1.00	1.28 (0.69–2.37)	1.51 (0.83–2.74)	3.71 (2.14–6.43)	4.28 (2.47–7.43)	<0.001	
High	1.00	0.86 (0.42–1.78)	1.34 (0.68–2.62)	2.81 (1.49–5.30)	4.01 (2.09–7.68)	<0.001	0.87
Diet score							
Low	1.00	1.03 (0.55–1.92)	1.28 (0.72–2.27)	2.91 (1.70–5.01)	4.33 (2.50–7.49)	<0.001	
High	1.00	1.23 (0.61–2.49)	1.69 (0.83–3.46)	3.89 (2.05–7.36)	4.10 (2.13–7.88)	<0.001	0.94
Family history of diabetes							
Yes	1.00	1.49 (0.66–3.36)	1.84 (0.89–3.79)	3.27 (1.61–6.61)	3.73 (1.85–7.56)	<0.001	
No	1.00	0.94 (0.51–1.72)	1.15 (0.64–2.11)	3.30 (1.97–5.52)	4.66 (2.74–7.89)	<0.001	0.15

*Physical activity and diet score were classified into low and high groups according to median values. RRs were adjusted for the same variables as in table 3.

yses adjusting for fasting insulin levels in the subset of women who provided fasting samples (432 case and 398 control subjects), and the association was only slightly attenuated (OR comparing extreme quintiles of CRP 3.85, 95% CI 2.08–7.13, *P* for trend <0.001). Finally, in the subset of women who provided waist circumference measures, we adjusted for both BMI and waist circumference (continuous variables); again, the association for CRP did not substantially change (OR comparing extreme quintiles of CRP 3.61, 2.29–5.68, *P* for trend <0.001).

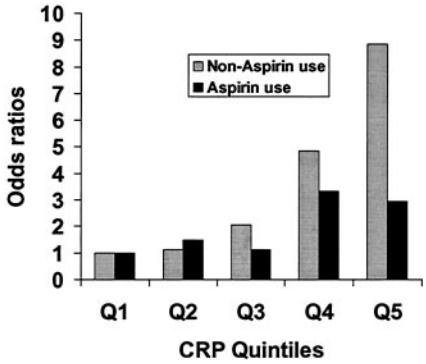

FIG. 2. Multivariate OR of type 2 diabetes according to quintiles of CRP levels stratified by regular aspirin use (≥1/week). *P* for interaction = 0.0002. Adjusted for the same variables as in Table 3.

DISCUSSION

In this large prospective, nested, case-control study, elevated baseline levels of TNF-αR2, IL-6, and CRP were significantly associated with the risk of developing type 2 diabetes over 10 years of follow-up. These associations were independent of BMI, physical activity, and other conventional diabetes risk factors and were not explained by hyperinsulinemia. Among the three inflammatory markers, CRP had the strongest and most robust relationship with diabetes.

The concept of atherosclerosis as an inflammatory disease is now well established (30). Recent cross-sectional data have demonstrated that elevated levels of CRP

are associated with obesity, insulin resistance, and glucose intolerance (31–33), suggesting that inflammation is also involved in the etiology of type 2 diabetes. However, prospective (or nested case-control) studies evaluating the association between CRP levels and risk of type 2 diabetes have been inconsistent. Several studies have reported a significant positive association, even after adjustment for BMI (9–12). However, in the Insulin Resistance Atherosclerosis Study (14) and the MONICA Augsburg Cohort Study (15), the association of CRP with diabetes disappeared after adjustment for BMI. In the Mexico City Diabetes Study, CRP was an independent predictor of metabolic syndrome and type 2 diabetes in women but not in men (13,34). In a nested case-control study of 71 pairs of diabetes case and control subjects (matched on BMI) in the Pima Indians (16), CRP was not related to risk of diabetes. Our study, with a much larger sample size, found a strong and independent association between CRP levels and type 2 diabetes. The magnitude of association between CRP and diabetes appears to be comparable or even stronger than the association of CRP with coronary heart disease observed in previous studies (30). These data support the hypothesis that low-grade systemic inflammation is a common antecedent for both type 2 diabetes and CVD (35). Consistent with previous results with coronary disease (36), we found that the association between CRP and diabetes was significantly stronger among nonaspirin users than aspirin users, suggesting that the inflammatory role of CRP may be mitigated by aspirin use.

The biological mechanisms through which CRP increases risk of type 2 diabetes are not well understood. CRP is a marker of low-grade inflammation and may have indirect influence on insulin resistance and insulin secretion through altered innate immune response due to heightened systemic inflammation (37,38). Elevated CRP also stimulates endothelial production of E-selectin, ICAM-1, and VCAM-1, important mediators of impaired vascular reactivity, reduced insulin delivery, and increased peripheral insulin resistance (8). Thus, the positive association between CRP and type 2 diabetes may simply reflect underlying endothelial dysfunction and subclinical atherosclerosis. However, our results show that the effects of CRP are independent of levels of E-selectin, and that the combination of higher CRP and E-selectin levels confers the greatest risk of diabetes. Our results did not change after excluding subjects with incident CVD during the follow-up, suggesting that the positive association of diabetes with CRP was unlikely due to subclinical CVD.

The production of CRP is regulated by inflammatory cytokines such as TNF-α and IL-6. Thus, the observed association between CRP levels and diabetes may partly reflect the detrimental effects of cytokines, such as IL-6 and TNF-α, on insulin resistance. Adipose tissue is a major source of endogenous TNF-α production; elevation in levels of TNF-α may be a critical mechanism by which fat cells induce peripheral insulin resistance (39). Elevations in TNF-α levels then reflect both upregulated adipocyte signaling and systemic inflammation (6,40). TNF-α may mediate insulin resistance through indirect effects, including increasing free fatty acid oxidation, stimulation of insulin counterregulatory hormones or cytokines (e.g., IL-6 and CRP), impairment of endothelial function, or direct inhibitory effects on glucose transporter protein GLUT4, insulin receptor substrates, or glucose-stimulated insulin release by pancreatic β-cells. The results from our study suggest that the effects of TNF-α were accounted for by elevated CRP levels and may be mediated through obesity and elevated production of CRP. Interestingly, the positive association between TNF-αR2 was more evident in nonobese than obese women. This result is consistent with previous findings that TNF-αR2 was more strongly correlated with leptin, insulin, and C-peptide among normal weight than overweight subjects (41).

Consistent with the Women's Health Study (9), we found that the predictive role of IL-6 was weaker than that of CRP, and the association was also accounted for by elevated levels of CRP. Weaker associations of diabetes with TNF-αR2 and IL-6 may result from larger variability and thus greater random misclassification in the measurement. Clinically, CRP is a more stable and integrated measure of low-grade inflammation. Biologically, however, IL-6 may serve an inflammatory role by modulating the production of TNF-α, downregulating the immune response, and upregulating the production of CRP (8).

The strengths of our study include the large sample size, a long follow-up, and detailed measures of diet and lifestyle. Several limitations should be considered, however. First, because all our participants were health professionals, our findings may not directly apply to the general population. Second, diabetes diagnoses were reported by the nurses but confirmed by a validated supplementary questionnaire regarding symptoms, diagnostic tests, and treatment. Our previous study found this confirmation highly accurate compared with medical record review (19). Nonetheless, the findings of our study may only apply to the clinically diagnosed case subjects because it was not feasible to screen the entire cohort. Because our control subjects were not uniformly screened for glucose intolerance, some cases of diabetes may have been undiagnosed. However, when the analyses were restricted to subjects with HbA_{1c} <6.0% at baseline or adjusted for baseline HbA_{1c} levels, the findings were not appreciably altered, suggesting that bias due to undiagnosed diabetes is unlikely. Third, while direct TNF-α measurement in plasma is not possible using frozen specimens, TNF-α signals through at least two known cell surface receptors, TNF-αR1 (receptor 1) and TNF-αR2 (receptor 2). These receptors are shed into the plasma, and TNF-αR2, a specific marker of TNF-α–related insulin resistance, is easily and reliably measured in frozen plasma (42).

The clinical implications of these data are twofold. First, elevated CRP levels in apparently healthy subjects may help to identify high-risk populations for both type 2 diabetes and CVD. So far, CRP appears to be the most consistent and strongest nonconventional predictor of both conditions. Second, elevated CRP can serve as a common target for lifestyle and therapeutic interventions for these two conditions. Lifestyle interventions such as exercise and weight loss are effective in lowering CRP and other inflammatory markers (43,44) and in preventing type 2 diabetes in subjects with impaired glucose tolerance (45,46). Therapy with statins and ACE inhibitors have been shown to reduce CRP and improve insulin sensitivity and

endothelial function (47,48) and, in secondary analyses of CVD prevention studies, have been associated with a reduced risk of incident type 2 diabetes (49–51). However, whether a reduction in CRP per se will lead to a reduction in risk of type 2 diabetes and CVD remains to be seen in randomized trials.

In conclusion, we found that elevated plasma levels of inflammatory markers, especially CRP, were independent predictors of type 2 diabetes in apparently healthy women. Our findings support the hypothesis that low-grade systemic inflammation is an underlying factor in the pathogenesis of type 2 diabetes. These findings may have important implications for the prevention and treatment of type 2 diabetes. Modification of unhealthy diet and lifestyle factors, fundamental causes of heightened inflammatory response, and type 2 diabetes should remain the cornerstone for the prevention and management of diabetes. Pharmacological agents with anti-inflammatory properties may also have a role in diabetes prevention and treatment.

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