The Relation of Markers of Inflammation to the Development of Glucose Disorders in the Elderly

The Cardiovascular Health Study

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Several studies suggest that inflammation plays a role in the pathogenesis of some glucose disorders in adults. We tested this hypothesis in a longitudinal cohort study of older individuals who had normal fasting glucose (FG) values at baseline. We compared the baseline levels of six inflammatory markers in participants who had developed glucose disorders at follow-up with those of participants whose FG remained normal at follow-up. Participants were members of the Cardiovascular Health Study, a prospective study of risk factors for cardiovascular disease in adults ≥65 years. All 5,888 participants had baseline testing, including FG and markers of inflammation: white blood cell and platelet counts and albumin, fibrinogen, C-reactive protein (CRP), and factor VIIIc levels. At 3–4 years of follow-up, 4,481 (84.5%) of those who were alive had FG levels retested. Participants who developed diabetes (n = 45) had higher median levels of CRP at baseline than those who remained normoglycemic. On multivariate analysis, those with elevated CRP levels (75th percentile [2.86 mg/l] vs. 25th percentile [0.82 mg/l]) were 2.03 times (95% confidence intervals, 1.44–2.86) more likely to have diabetes on follow-up. Adjustment for confounders and other inflammatory markers did not appreciably change this finding. There was no relationship between the development of diabetes and other markers of inflammation. Inflammation, as measured by CRP levels, is associated with the development of diabetes in the elderly. Understanding the role of inflammation in the pathogenesis of glucose disorders in this age-group may lead to better classification and treatment of glucose disorders among them. *Diabetes* 50:2384–2389, 2001

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ADA, American Diabetes Association; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; FG, fasting glucose; IFG, impaired fasting glucose; OR, odds ratio.

It has been proposed that inflammation plays a role in the pathogenesis of some glucose disorders in adults. This hypothesis is based on four lines of evidence. First, a subset of nonobese adults without antecedent glucose abnormalities rapidly develop incident diabetes. Islet cell antibodies and antibodies to glutamic acid decarboxylase—both markers of autoimmune inflammation against the β-cell— occasionally are present (1–3). Second, in cross-sectional studies, markers of inflammation are elevated in those with diabetes compared with those without diabetes (4,5). In two longitudinal studies, baseline levels of certain inflammatory markers predicted incident diabetes in nondiabetic individuals (6,7). Third, mediators of inflammation, such as tumor necrosis factor α (which often are present before diabetes), decrease insulin sensitivity, possibly helping to precipitate diabetes (8–10). Last, inflammation has been implicated as part of the insulin resistance syndrome (11,12).

In this report, we examine the prospective relationship between baseline markers of inflammation and worsening glucose status during a period of 3–4 years. The study population is a cohort of adults who are ≥65 years and members of the Cardiovascular Health Study (CHS), an ongoing observational study of risk factors for cardiovascular disease (CVD). We tested the hypothesis that individuals with normal fasting glucose (FG) at baseline, who had impaired fasting glucose (IFG) or diabetes at follow-up, had elevated levels of six inflammatory markers at baseline.

RESEARCH DESIGN AND METHODS

Recruitment methods for the CHS have been previously published (13). A random sample of individuals from Medicare eligibility lists were invited to participate in the study. Potential participants were excluded if they were institutionalized or confined to a wheelchair in the home or had a severe illness that was expected to lead to early death. Participants were recruited in two phases. In the first, 5,201 eligible men and women (4,926 [94.7%] white, 244 [4.7%] black, 31 [0.6%] other), ≥65 years, agreed to participate (original cohort). In the second phase—initiated to enhance representation of black individuals—a total of 687 similarly aged participants (672 [97.8%] black, 15 [2.2%] other) were recruited (black cohort). The analyses done for this study are based on the updated CHS database, which incorporates minor corrections through June 1999. All participants signed consent forms upon entry into the study.

Baseline examination. During the interview for the baseline examination, information on prescription medications used in the preceding 2 weeks was collected directly from prescription bottles. Questionnaires regarding physical activity (14,15) and medical conditions (16) were administered.

TABLE 1
Diabetes status at baseline and follow-up in the original and black cohorts based on the fasting ADA criteria

Baseline	Follow-up				
	Normoglycemia	IFG	Untreated diabetes	Known diabetes	Total
Original					
Normoglycemia	2,752 (93.4)	156 (5.3)	31 (1.1)	6 (0.2)	2,945
IFG	246 (46.9)	193 (36.8)	77 (14.7)	9 (1.7)	525
Newly diagnosed diabetes	39 (12.9)	46 (15.2)	115 (38.1)	102 (33.8)	302
Known diabetes	6 (2.2)	6 (2.2)	9 (3.3)	254 (92.4)	275
Total	3,043	401	232	371	4,047
Black					
Normoglycemia	262 (94.2)	8 (2.9)	5 (1.8)	3 (1.1)	278
IFG	20 (37.0)	19 (35.2)	4 (7.4)	1 (20.4)	54
Newly diagnosed diabetes	5 (16.1)	2 (6.4)	6 (19.3)	18 (58.1)	31
Known diabetes	1 (1.4)	1 (1.4)	1 (1.4)	67 (95.7)	70
Total	288	30	16	99	433

Numbers in parentheses are row percentages.

Venipuncture was done early during the clinic visit after an overnight fast. Plasma and serum were frozen at −70°C and shipped to the CHS Central Laboratory (University of Vermont, Burlington, VT). Fasting serum chemistry analyses were performed as described previously (17). C-reactive protein (CRP) was measured using an ultrasensitive enzyme-linked immunosorbent assay developed at the CHS Central Laboratory (15); it is a colorimetric competitive immunoassay that uses purified protein and polyclonal anti-CRP antibodies. The interassay coefficient of variation is 5.5% (18). Plasma fibrinogen was measured with a clot-based end point, using a BBL fibrometer (Becton-Dickinson, Cockeysville, MD) with a modification of the von Clauss method (19). Assays for factor VIII coagulant activity (VIIIc) were performed using factor VIII–deficient plasma on a General Diagnostics Coag-A-Mate X2 (Organon Teknika, Durham, NC) and standardized against World Health Organization materials (17).

The following cardiovascular tests were done early in the clinic visit: seated blood pressure, ankle arm index (20), duplex ultrasonography of the carotid arteries (21), and resting electrocardiogram (22).

Participants' baseline glycemic category was based on FG or the use of hypoglycemic agents. Self-report of a history of diabetes was not used as a defining criterion. Only participants who had not drunk or eaten within 9 h before blood drawing or whose diabetic status could be determined from medication history were analyzed. This led to the exclusion of 64 participants. Of the remaining participants, 139 were using insulin, 373 were using oral hypoglycemic agents, and 11 were using both.

Diabetes classification at baseline was based on the fasting criteria of the American Diabetes Association (ADA) (23). Participants who met the criteria for diabetes but who were not taking diabetic medications at baseline were classified as having newly diagnosed diabetes. Those who were taking diabetic medications at baseline were defined as having known diabetes.

Follow-up. FG was repeated after 3 years of follow-up (in 1992–1993) for the original cohort and after 4 years (in 1996–1997) for the black cohort. Of those who had baseline glucose data and were alive at follow-up, 4,481 (84.5%) had a second test. The glucose status of these individuals at follow-up again was classified by ADA criteria. Participants who were newly prescribed either oral hypoglycemic agents or insulin during follow-up were considered to have known diabetes at follow-up. Those who had newly diagnosed diabetes (or known diabetes at baseline but were not treated at the time of follow-up [24]) were classified as having untreated diabetes.

Potentially confounding factors. Conditions associated with inflammation that could confound the relation between glucose change and markers of inflammation were sought. These included chronic respiratory conditions (e.g., asthma, chronic bronchitis, emphysema) (25), current smoking (26), subclinical CVD (e.g., low ankle arm index, abnormal carotid wall thickness and/or stenosis, major electrocardiogram abnormalities) (27), and clinical cardiac disease (e.g., angina pectoris) (28).

Statistical methods. Three groups were of interest. The first was participants who had no glucose abnormality at baseline or at follow-up. The second was those who had no glucose abnormality at baseline and had IFG at follow-up. The third group was participants who were normoglycemic at baseline and had diabetes at follow-up. Tukey's t test and the χ^2 test with 2 degrees of freedom were used to compare continuous and categorical variables, respectively, from the two groups with glucose change to variables in the group that had no glucose abnormality at baseline and follow-up. Values with skewed distribution were log-transformed for analyses.

Previous studies (26,29,30) showed that certain markers of inflammation are related to BMI. As such, BMI might confound associations of interest in studies of obesity-related conditions such as diabetes. Analyses therefore were stratified by tertiles of BMI (<24.4, 24.4–26.8, ≥27.9 kg/m^2). Our cut point for overweight is similar to the standard used to define overweight (>24.9) (31). For those inflammation markers that were significantly different between participants with and without progression of glucose status, unconditional logistic regression was used to examine the associations after adjustment for potential confounding factors. Logistic regression also was used to test for differences in the association of CRP with glucose status change according to tertiles of BMI. The likelihood ratio test was used to compare the fit of the model augmented with interaction terms to the reduced model.

RESULTS

Table 1 shows the relation of baseline glucose status with glucose status on follow-up in the original and black cohorts. Of those with normal FG status at baseline, 5.1% ([156 + 8]/[2,945 + 278]) and 1.4% ([31 + 6 + 5 + 3]/[2,945 + 278]) had IFG and diabetes, respectively, on follow-up.

Table 2 shows baseline characteristics of participants whose FG status changed compared with those whose FG status remained unchanged. Those who progressed to IFG or diabetes more often were men, were heavier, and were more likely to have established hypertension and subclinical CVD at baseline than those who remained normoglycemic. They also had higher baseline triglyceride, glucose, and insulin levels. They did not differ with regard to age (mean: 72.3, 72,6, and 71.0 years, respectively), total cholesterol levels (mean: 6.1, 5.9, 5.8 mmol/l, respectively), baseline presence of lung disease (mean: 8.4, 9.8, and 8.9%, respectively) or angina pectoris (mean: 13.5, 14.6, and 17.8%, respectively), or energy expenditure (median: 1,870.1, 1,848.9, and 1,805.4 kcal/week, respectively).

With regard to baseline markers of inflammation, only CRP levels were higher in those whose status changed as compared with those whose status remained the same (Fig. 1). None of the other markers showed a significant difference between glycemic groups (fibrinogen [g/l], 3.2 [0.6], 3.1 [0.7], and 3.3 [0.8]; albumin [g/l], 40.0 [3.0], 40.0 [3.0], and 40.0 [3.0]; white blood cell count [10^3], 6.1 [1.8], 6.3 [1.4], and 6.3 [1.7]; factor VIIIc [g/l] [done only in the original cohort], 1.2 [0.4], 1.2 [0.3], and 1.2 [0.4]; and platelet count [10^3], 245 [69], 244 [63], and 245 [77]).

The cohort was stratified by tertiles of BMI to investigate markers of inflammation with regard to change in FG

TABLE 2
Baseline characteristics of the cohort categorized by glucose status on follow-up

	No change in status (n = 3,014)	Normal → IFG (n = 164)	Normal → diabetes (n = 45)
Men (%)	38.6	53.7†	44.4
BMI (kg/m^2)	25.8 (4.2)	27.8 (4.3)†	28.5 (6.0)†
HDL cholesterol (mmol/l)	1.5 (0.4)	1.4 (0.4)*	1.4 (0.3)
Triglycerides (mmol/l)§	1.3 (0.7)	1.4 (0.9)*	1.4 (0.7)
Fasting glucose (mmol/l)	5.3 (0.4)	5.7 (0.3)†	5.6 (0.5)†
2-h Glucose (mmol/l)‡	7.2 (2.1)	8.5 (2.3)†	9.6 (3.0)†
Fasting insulin (pmol/l)§	65.9 (35.9)	77.9 (56.9)*	77.9 (47.9)*
2-h Insulin (pmol/l)‡§	365.6 (350.7)	488.5 (419.6)*	407.6 (359.6)
Hypertension (%)	36.9	45.1*	48.9
Use of thiazide diuretics (%)	15.6	23.2*	13.3
Any subclinical CVD (%)	60.8	68.9*	80.0*
Current smoker (%)	11.5	9.8*	15.6

Values are means (SD) except for skewed variables, which are log-transformed and presented as medians with interquartile ranges. All participants had normal glucose at entry. *$P < 0.05$; †$P < 0.001$, versus the group that remained normoglycemic; ‡these variables were not measured in the black cohort; §median and interquartile range.

status within strata of BMI. Table 3 shows that among those with BMI of <24.4 (the thinnest group), there was a significant stepwise increase in CRP levels with progression of glucose disorders. There was an indication of a similar trend in those with BMI of >27.9 (the heaviest group), but it did not reach statistical significance. Fibrinogen showed a significant difference in the highest tertile of BMI among those who developed IFG as compared with those who did not change glycemic status or those who developed diabetes. No other marker of inflammation showed a significant difference between those who remained normoglycemic and those who developed IFG or diabetes. In general, levels of CRP and fibrinogen were higher in those who were heavy as compared with those who were not heavy.

In an unadjusted logistic regression model, higher baseline CRP was associated with an increased risk of developing IFG (75th percentile [2.86 mg/l] vs. 25th percentile [0.82 mg/l]; odds ratio [OR], 1.28 [95% confidence intervals, 1.05–1.55]). This association was not significant after adjustment for BMI and other confounders. Increasing baseline CRP levels also were associated with an increased risk of developing diabetes (OR, 2.03 [1.44–2.86]). This risk remained significant after adjustment for BMI, age, sex, fasting insulin, FG, subclinical CVD, and use of thiazide diuretics (OR 1.83 [1.24–2.86]). Further adjustments for other inflammatory factors did not significantly alter the association of CRP with worsening FG status (data not

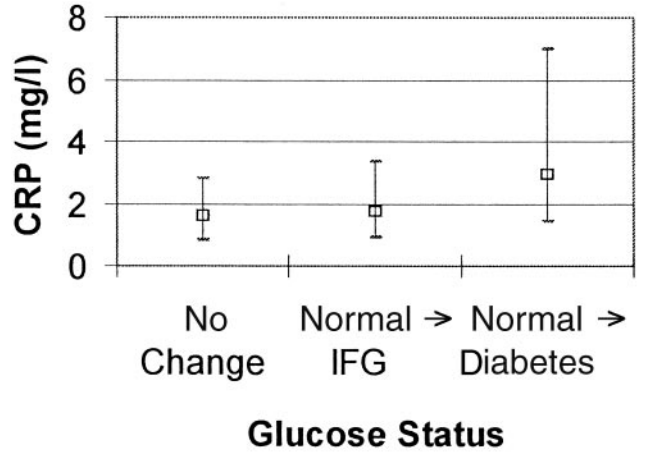

FIG. 1. Median values of CRP (mg/l) and interquartile ranges in those with no change in FG status (1.62 [0.81, 2.81]), those who progressed to IFG (1.73 [0.93, 3.34]; $P < 0.05$ compared with those with no change in status), and those who progressed to diabetes (2.94 [1.44, 7.0]; $P < 0.001$ compared with those with no change in status).

TABLE 3
Values of inflammation markers stratified by tertiles of BMI and FG status at follow-up

	No change in status	Normal → IFG	Normal → diabetes
BMI < 24.4			
n	1,168	35	11
CRP (mg/l)	1.2 (1.7)	1.4 (2.5)*	3.3 (10.4)*†
Fibrinogen (g/l)	3.1 (0.6)	3.2 (0.7)	3.0 (0.8)
Albumin (g/l)	40.0 (3.0)	40.0 (3.0)	40.0 (3.0)
WBC count (10^3/mm^3)	6.0 (2.0)	6.6 (1.4)	6.5 (1.2)
Factor VIIIc (g/l)	1.2 (0.3)	1.3 (0.4)	1.3 (0.4)
Platelet count (10^3/mm^3)	244 (73)	265 (74)	273 (73)
24.4 < BMI < 27.9			
n	1,076	54	13
CRP (mg/l)	1.6 (1.9)	1.7 (2.5)	1.6 (1.5)
Fibrinogen (g/l)	3.2 (0.6)	3.2 (0.7)	3.1 (0.4)
Albumin (g/l)	40.0 (3.0)	41.0 (3.0)	41.0 (3.0)
WBC count (10^3/mm^3)	6.1 (1.8)	6.2 (1.5)	5.5 (1.9)
Factor VIIIc (g/l)	1.2 (0.4)	1.1 (0.3)	1.2 (0.4)
Platelet count (10^3/mm^3)	242 (66)	235 (56)	220 (57)
BMI > 27.9			
n	760	75	21
CRP (mg/l)	2.2 (2.3)	2.3 (2.3)	3.1 (4.7)
Fibrinogen (g/l)	3.3 (0.6)	3.0 (0.6)*	3.5 (0.8)+
Albumin (g/l)	40.0 (3.0)	40.0 (3.0)	40.0 (3.0)
WBC count (10^3/mm^3)	6.1 (1.6)	6.3 (1.4)	6.7 (1.8)
Factor VIIIc (g/l)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)
Platelet count (10^3/mm^3)	248 (66)	240 (60)	249 (88)

Values are means (SD) except for CRP, which is presented as median with interquartile ranges. *Significantly different ($P < 0.05$) than the group without change in glycemic status; †significantly different from the Normal → IFG group. For participants with no diabetes at baseline, data were missing for the following: CRP, 16 (0.5%); fibrinogen, 20 (0.6%); WBC count, 11 (0.3%); factor VIII, 314 (9.7%); platelet count, 24 (0.7%). Ten participants were missing BMI values and are not included in this analysis. WBC, white blood cell.

shown). In the bivariate analysis (Table 3), the association between baseline CRP and development of diabetes was stronger in participants in the lowest tertile of BMI than in heavier participants. This difference in association across strata of BMI, however, did not reach statistical significance in a formal test for interaction ($P > 0.10$), suggesting that the difference in association according to BMI could be a chance finding.

When the changes in FG levels from baseline to follow-up for those with IFG and diabetes were calculated (ΔFG), those whose ΔFG was above the median of ΔFG (0.72 mmol/l) had a median CRP level of 2.21 mg/l; those with ΔFG below the median had a median CRP level of 1.64 mg/l ($P = 0.06$, Mann-Whitney U test). In each of the BMI tertiles, the median CRP level was higher among those with ΔFG above the median for that tertile compared with those whose ΔFG was below the median for that tertile (lowest tertile of BMI, median CRP 1.93 for high ΔFG, 1.18 for low ΔFG, $P = 0.15$; middle tertile of BMI, 1.88 vs. 1.70, $P = 0.72$; highest tertile of BMI, 2.75 vs. 2.34, $P = 0.12$).

DISCUSSION

In this study, elevated baseline CRP levels were associated with the development of diabetes over a 3- to 4-year period. This association was present after adjustment for known baseline predictors of FG status change and was not confounded by other inflammatory conditions. Such an association is consistent with the possibility that the development of diabetes may in part be inflammatory. Baseline white blood cell and platelet counts and levels of albumin, fibrinogen, and factor VIIIc were not associated with development of diabetes.

CRP is an acute-phase reactant that is part of the immune response to injury and infection (32). It is regulated by interleukin-6, a cytokine associated with recruitment of macrophages and monocytes. We showed previously that CRP shows little short-term fluctuation (18) and that a single determination of CRP can predict future clinical disease (33,34). Consistent with previous studies (5), the "elevated" serum CRP concentrations in this study were within the conventional healthy reference range. Whether low-level inflammation was the cause of the change in FG status or was the result of the process that caused the change in FG status cannot be determined.

That none of the other inflammatory markers measured in this study was associated with worsening of glucose status is not inconsistent with previous findings. Only a few inflammatory markers were investigated, representing a limited part of the inflammatory cascade. The stronger association of inflammation with glucose progression in those with lower BMI suggests that our results were not due to the production of proinflammatory cytokines by adipose cells (a major source of such cytokines [35,36]).

Two additional points should be noted. First, participants with worsening FG status had mildly higher baseline triglyceride and lower HDL cholesterol levels than participants with no change in status. Previous studies (37–39) showed that these lipids increase and decrease, respectively, in association with inflammation. It is therefore possible that some of the lipid abnormalities observed in diabetes may be a consequence of low-level inflammation. Second, fibrinogen levels were lower in those who developed IFG as compared with those who had no change in glucose status in the highest BMI tertile. Previous studies showed that baseline fibrinogen levels are higher in those who develop diabetes, although not independently associated with the development of diabetes (6,7). The reason for our finding is unclear.

This study has several strengths. Medical conditions that could have confounded the relation of inflammatory markers and change in FG status were controlled for, and effect modification by BMI was considered. Participants in CHS were chosen to represent a cross-section of the U.S. population ≥65 years. Our results are therefore generalizable. Only those who had terminal illnesses and were in nursing homes were excluded. This has the effect of excluding very sick individuals whose high levels of inflammatory markers might have generated misleading results. This study also examined the association of inflammatory markers to change in FG status within 3–4 years of baseline. This short follow-up has the advantage of not attenuating the association of inflammatory markers with incident disease.

This study has limitations. Classification of glucose status was based on a single FG level (as done in many epidemiological studies) rather than two readings as recommended by the ADA. Many of the participants who were defined as having IFG and newly diagnosed diabetes at baseline had normal glucose status on follow-up. This suggests that some of the participants who were classified as having worsening FG status may not have been so categorized on repeat testing. Although this is true, it should be borne in mind that variation occurred in both directions. Bidirectional misclassification should weaken the association found in this study, biasing the results to the null hypothesis. Moreover, baseline CRP was associated with a major change in FG status, i.e., it was predictive for those who developed diabetes (requiring a large change in FG) but not for those who developed IFG (which required a smaller change in FG). Last, the higher CRP levels in those with the greater change in FG levels argues that the relation of CRP to glucose status deterioration was not due to chance. Another possible weakness of this study is the variability inherent in the markers of inflammation that may distort their association with FG status change (40). The stability of CRP over time may help explain its positive association with FG status change.

In conclusion, inflammation, as measured by CRP, was found to be associated with the development of diabetes during 3–4 years of follow-up in the elderly. Given the increasing representation of older adults in the population and the high prevalence of glucose disorders among them, understanding the role of inflammation in the development of diabetes may be relevant to future classification and treatment of diabetes.

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