C-Reactive Protein and Incident Cardiovascular Events Among Men with Diabetes

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C-Reactive Protein and Incident Cardiovascular Events Among Men With Diabetes

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Objective — Several large prospective studies have shown that baseline levels of C-reactive protein (CRP) are an independent predictor of cardiovascular events among apparently healthy individuals. However, prospective data on whether CRP predicts cardiovascular events in diabetic patients are limited so far.

Research Design and Methods — To investigate the association between plasma CRP levels and incidence of cardiovascular events among men with type 2 diabetes, we followed prospectively a cohort of 746 American men aged 46–81 years who were free of cardiovascular diseases at the time of blood collection in 1993–1994.

Results — During an average of 5 years of follow-up (3,986 person-years), we identified 103 incident cardiovascular events (18 myocardial infarction, 70 coronary artery bypass grafting or angioplasty, and 15 stroke), confirmed by medical records. After adjustment for age, BMI, smoking, alcohol consumption, physical activity, family history of coronary heart disease, history of high blood pressure, history of high serum cholesterol, aspirin use, and fasting status as well as for fibrinogen, creatinine, HbA1c, and non-HDL cholesterol levels, CRP remained significantly associated with an increased risk of cardiovascular events. The relative risks for quartiles were 1.00, 1.51, 2.52, and 2.62 (95% CI: 1.29–5.32; P for trend: 0.011). We observed no effect modifications by plasma levels of LDL cholesterol, HDL cholesterol, non-HDL cholesterol, apolipoprotein B, HbA1c, and fibrinogen or by BMI.

Conclusions — High plasma levels of CRP were associated with an increased risk of incident cardiovascular events among diabetic men, independent of currently established lifestyle risk factors, blood lipids, and glycemic control.

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Patients with type 2 diabetes have a markedly increased atherosclerotic risk (1). The risk of fatal coronary heart disease (CHD) among subjects with diabetes is comparable to that observed in subjects who have had a previous myocardial infarction (2,3). Although this increased risk has previously been attributed mainly to hyperglycemia, dyslipidemia, and a prothrombotic state (4), recent observations in apparently healthy individuals have focused attention on inflammatory mechanisms that may be relevant in patients with diabetes as well (5). Several large prospective studies have shown that baseline levels of C-reactive protein (CRP) are an independent predictor of cardiovascular events among apparently healthy individuals (6,7). However, data on CRP and cardiovascular disease (CVD) among diabetic subjects are limited, and in many cases, study size was too small to be informative (8,9). In the prospective Honolulu Heart Program (10,11), the associations between CRP and risk of thromboembolic stroke or myocardial infarction were generally weaker and not significant in diabetic compared with nondiabetic subjects. Given the relatively scarce data at hand, we conducted this study to determine whether CRP predicts cardiovascular events among subjects with diabetes, independent of currently established risk factors, e.g., blood lipid levels and glycemic control.

Research Design and Methods — The Health Professionals Follow-up Study is a prospective cohort study of 51,529 American male health professionals (dentists, veterinarians, pharmacists, optometrists, osteopathic physicians, and podiatrists) aged 40–75 years at study initiation in 1986. This cohort is followed through biennial mailed questionnaires focusing on various lifestyle factors and health outcomes. In addition, between 1993 and 1994, 18,159 study participants provided blood samples by overnight courier. Among participants who returned blood samples, 1,000 had a confirmed diagnosis of type 2 diabetes (as reported on a supplementary questionnaire sent to all men who reported a diagnosis of diabetes) at baseline or during follow-up through 1998. The present study included 746 men who did not report a diagnosis of angina pectoris, myocardial infarction, coronary artery bypass grafting (CABG) or percutaneous transluminal coronary angioplasty (PTCA), or stroke on any of the biennially questionnaires before blood collection.

Diabetes and cardiovascular endpoints definitions
In accordance with the criteria of the National Diabetes Data Group (12), confir-
CRP and CVD among men with diabetes

mation of diabetes required at least one of the following self-reports on a supplementary questionnaire: 1) elevated plasma glucose concentration (fasting plasma glucose \( \geq 7.8 \) mmol/l, random plasma glucose \( \geq 11.1 \) mmol/l, and/or plasma glucose \( \geq 11.1 \) mmol/l after \( \geq 2 \) h during an oral glucose tolerance test) plus at least one classic symptom (excessive thirst, polyuria, weight loss, or hunger); 2) no symptoms but at least two elevated plasma glucose concentrations (by the above criteria) on different occasions; or 3) treatment with hypoglycemic medication (insulin or oral hypoglycemic agent).

We used the National Diabetes Data Group criteria to define diabetes because most of our subjects were diagnosed before the release of the American Diabetes Association criteria in 1997 (13). The validity of self-reported diabetes using the supplementary questionnaire has been documented in a subsample of 71 men from the Health Professionals Follow-up Study cohort. Of these, 12 had incomplete records, whereas the diagnosis of type 2 diabetes was confirmed in 57 (97%) of the remaining 59 subjects (14).

Cardiovascular end points consisted of fatal stroke, nonfatal stroke, fatal CHD, nonfatal myocardial infarction, and CABG/PTCA. The end points did not include angina pectoris. Nonfatal myocardial infarction was confirmed by reviewing medical records using the criteria of the World Health Organization of symptoms plus either typical electrocardiographic changes or elevated cardiac enzyme levels (15). Stroke was confirmed by reviewing medical records using the criteria of the National Survey of Stroke (16), requiring evidence of a neurological deficit with sudden or rapid onset that persisted for \( >24 \) h or until death. Physicians who reviewed the records had no knowledge of the self-reported risk factor status. Deaths were reported by next of kin, the postal system, and through records of the National Death Index. Using all sources combined, it is estimated that follow-up for deaths was \( >98\% \) complete (17). Cardiovascular deaths (fatal myocardial infarction, fatal stroke, and coronary disease) were confirmed by review of medical records or autopsy reports with the permission of the next of kin. The cause listed on the death certificate was not sufficient alone to confirm coronary death or stroke. Sudden deaths (i.e., death within 1 h of symptom onset in a man without known disease that could explain death) were included in the fatal CHD category.

**Blood collection and processing**

Each interested participant was sent a blood collection kit containing instructions and needed supplies (blood tubes, tourniquet, gauge, adhesive bandages, and needles). The participants made arrangements for the blood to be drawn. Blood samples were collected in three 10-ml liquid EDTA blood tubes, placed on ice packs stored in Styrofoam containers, and returned to our laboratory via overnight courier; >95% of the samples arrived within 24 h. After receipt, the chilled blood was centrifuged; aliquoted into plasma, erythrocytes, and buffy coat; and stored in continuously monitored nitrogen freezers at a temperature not higher than \(-130^\circ\)C. We requested information on the date and time of the blood sample collection and the time elapsed since the preceding meal to identify non-fasting (\( >8 \) h) subjects. All biomarker assays were performed using a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, IN). CRP was measured via an immunoturbidimetric assay using reagents and calibrators from Denka Seiken (Niigata, Japan). This assay has a sensitivity of 0.1 mg/l. The day-to-day variability of the assay at concentrations of 0.91, 3.07, and 13.38 mg/l were 2.8, 1.6, and 1.1%, respectively. Determination of HbA1c was based on turbidimetric immunoinhibition using hemolysed whole blood or packed red cells. The day-to-day variability at HbA1c concentrations of 5.5 and 9.1% was 1.9 and 3.0%, respectively.

Creatinine was measured by a rate-blanketed method that is based on the Jaffe reaction using Roche Diagnostics reagents (Indianapolis, IN) with a day-to-day variability of 5.0 and 2.2% at concentrations of 106.1 and 565.8 \( \mu \)mol/l, respectively. Fibrinogen was measured with an immunoturbidimetric assay using reagents and calibrators from Kamiya Biomedical (Seattle, WA). The day-to-day variability of the assay at concentrations of 4.92, 9.51, and 16.29 \( \mu \)mol/l were 0.9, 1.1, and 1.5%, respectively. The determination of total cholesterol and HDL cholesterol concentrations was simultaneously performed using reagents and calibrators from Roche Diagnostics (Indianapolis, IN); coefficients of variation for these measurements were <1.8%. LDL cholesterol was measured by a homogenous direct method from Genzyme Corporation (Cambridge, MA). The day-to-day variability at LDL cholesterol concentrations of 2.33, 2.74, and 3.34 mmol/l was <3.1%. Measurement of apolipoprotein B-100 (apoB) was based on the immunonephelometric assay using reagents and calibrators from Wako Chemicals (Richmond, VA) with a day-to-day variability of <5%. We calculated non-HDL cholesterol as the difference between total and HDL cholesterol.

**Assessment of lifestyle exposures**

Participants provided information biennially on their age, weight, smoking status, aspirin use, and physical activity. If the weight was missing, we used the weight reported on the preceding questionnaire instead. We calculated BMI as the ratio of weight (in kg) to squared height (in m\(^2\)). Self-reports of body weight have been shown to be highly correlated with technician-measured weights \( r = 0.97 \) in this cohort (18). Physical activity was computed as metabolic equivalents per week using the duration per week of various forms of exercise, weighting each activity by its intensity level (19). History of high blood pressure, high serum cholesterol, and cancer was determined from self-reports preceding the blood collection. Family history of CHD was reported in 1986. Alcohol intake was estimated with a dietary questionnaire in 1994.

**Statistical analysis**

We used Cox proportional hazards analysis stratified on 5-year age categories and over each 2-year follow-up interval to estimate the relative risk (RR) for each category of CRP compared with the lowest category. Person-months of follow-up accumulated starting with the reported date of blood collection. Participants in whom CVD was diagnosed or who died during follow-up were censored at the date of diagnosis or death. All other participants were followed through January 2000 or the return date of the last questionnaire if no questionnaire was returned in 2000. Participants were divided according to the American Heart Association risk categories for CRP (<1.0, 1.0–3.0, and >3.0 mg/l) (20). Because these risk categories are largely based on studies among apparently healthy individuals and their relevance among patients with diabetes is...
unknown, we also used a population-based approach and divided participants into quartiles of CRP levels. Tests for trend were calculated by assigning the median values to increasing categories of CRP. Multivariate models included the following covariates: physical activity (quartiles), alcohol intake (0, 0.1–4.9, 5.0–9.9, 10.0–14.9, and ≥15.0 g/day), family history of CHD, history of high blood pressure at blood collection, history of high blood cholesterol at blood collection, aspirin use, smoking (never, past, and current), and BMI (missing, <23, 23–24, 25–27, 28–30, and ≥31 kg/m²).

Table 1—Baseline characteristics of case subjects and non-case subjects

<table>
<thead>
<tr>
<th></th>
<th>Case subjects</th>
<th>Non-case subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>103</td>
<td>643</td>
<td>0.005</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.4 ± 7.1</td>
<td>62.7 ± 8.6</td>
<td>0.020</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6 ± 3.9</td>
<td>27.9 ± 4.3</td>
<td>0.820</td>
</tr>
<tr>
<td>Physical activity</td>
<td>32.7 ± 36.2</td>
<td>29.2 ± 33.2</td>
<td>0.235</td>
</tr>
<tr>
<td>Currently smoking</td>
<td>7.8</td>
<td>5.9</td>
<td>0.467</td>
</tr>
<tr>
<td>Aspirin use</td>
<td>46.6</td>
<td>37.2</td>
<td>0.068</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>6.8 ± 8.0</td>
<td>7.4 ± 9.7</td>
<td>0.574</td>
</tr>
<tr>
<td>Family history of CHD</td>
<td>13.6</td>
<td>12.4</td>
<td>0.744</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>61.2</td>
<td>44.8</td>
<td>0.002</td>
</tr>
<tr>
<td>History of high serum cholesterol</td>
<td>47.6</td>
<td>42.6</td>
<td>0.346</td>
</tr>
</tbody>
</table>

Data are means ± SD or percentages. Tests were two-sided Wilcoxon's signed-rank test or χ² test. *98 case subjects and 619 non-case subjects due to missing values; 97 case subjects and 600 non-case subjects due to missing values; 99 case subjects and 600 non-case subjects due to missing values.

RESULTS — During an average of 5 years of follow-up (3,986 person-years), we identified 103 incident cases of CVD (18 myocardial infarction, 70 CABG/PTCA, and 15 stroke) confirmed by medical records. Among the study population of 746 men, case subjects had a significantly higher baseline age and higher levels of CRP, apoB, LDL cholesterol, and non-HDL cholesterol but lower levels of HDL cholesterol (Table 1). In addition, case subjects were more likely to have a history of hypertension at the time of blood collection. Although case subjects tended to use aspirin more frequently and to have higher HbA1c levels, these differences were not statistically significant.

Increasing CRP levels were strongly associated with progressively higher risk of CVD (Table 2). The age-adjusted RRs across quartiles were 1.00, 1.77, 2.52, and 2.23 (P for trend: 0.037). This association remained strong after further adjustment for BMI, alcohol consumption, smoking, family history of CHD, physical activity, history of high blood pressure, history of high serum cholesterol, aspirin use, and fasting status. Further adjustment for fibrinogen, creatinine, HbA1c, and non-HDL cholesterol levels did not materially change this observation. The RRs for quartiles were 1.00, 1.51, 2.52, and 2.62 (95% CI: 1.29–5.32; P for trend: 0.011). Adjustment for HDL cholesterol in addition to non-HDL cholesterol only slightly attenuated this association (RR for extreme quartiles: 2.25, 95% CI: 1.09–4.63; P for trend: 0.037). Furthermore, results remained similar after adjustment for apoB instead of non-HDL cholesterol.

Table 2—RRs of cardiovascular events according to quartiles of CRP among 746 men with diabetes

<table>
<thead>
<tr>
<th>Quartiles of CRP</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Median (mg/l)</td>
<td>0.53</td>
</tr>
<tr>
<td>Age-adjusted RR</td>
<td>1.00</td>
</tr>
<tr>
<td>Multivariate-adjusted RR*</td>
<td>1.00</td>
</tr>
<tr>
<td>Further adjustment for fibrinogen, creatinine, and HbA1c</td>
<td>1.00</td>
</tr>
<tr>
<td>Further adjustment for fibrinogen, creatinine, HbA1c*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Data are RR (95% CI). *Adjusted for age, physical activity (quartiles), alcohol intake (0, 0.1–4.9, 5.0–9.9, 10.0–14.9, and ≥15.0 g/day), family history of CHD, history of high blood pressure at blood collection, history of high serum cholesterol at blood collection, aspirin use, smoking (never, past, current), fasting status, and BMI (missing, <23, 23–24, 25–27, 28–30, and ≥31 kg/m²).
of non-HDL cholesterol (RR for extreme quartiles: 2.51; 95% CI: 1.23–5.09; P for trend: 0.020). We repeated our analysis considering CHD end points only, thus not including stroke. Results were very similar (RR for extreme quartiles adjusted for lifestyle covariates and fibrinogen, creatinine, HbA1c, and non-HDL cholesterol: 2.54; 95% CI: 1.16–5.58; P for trend: 0.045). Because inflammation might be related to cancer risk and survival (21), we excluded in a secondary analysis 16 study participants who had a diagnosis of cancer at the time of blood collection (n = 99 remaining CVD cases). CRP remained strongly associated with CVD risk (RR for extreme quartiles: 2.48; 95% CI: 1.21–5.05; P for trend: 0.015). Because our initial analysis combined men with both prevalent and incident diabetes but associations between CRP and CVD risk might differ between both groups, we excluded 198 participants who were diagnosed with diabetes after blood collection (n = 84 remaining CVD cases) in a further analysis. The RRs for quartiles were 1.00, 1.86, 3.21, and 2.90 (95% CI: 1.25–6.77; P for trend: 0.039).

We examined the joint effect of CRP and non-HDL cholesterol by cross-classifying participants by both variables. The effect of CRP was independent but additive to that of non-HDL cholesterol. The RR for the combination of a CRP level above the population median (≥1.63 mg/l) and a non-HDL cholesterol level above the population median (≥4.34 mmol/l) compared with the opposite extreme was 3.57 (95% CI: 1.84–6.92).

The probability of event-free survival according to American Heart Association risk categories (CRP <1.0, 1.0–3.0, and >3.0 mg/l) is shown in Fig. 1. Multivariate adjusted RRs for these categories were 1.00, 1.50, and 2.09 (95% CI: 1.12–3.89, P for trend: 0.028). We used stratified analysis to assess whether BMI, hypertension, LDL cholesterol, HDL cholesterol, apoB, HbA1c, and fibrinogen modified this association (Table 3). The association between CRP and CVD appeared to be stronger among subjects with higher BMI, HDL cholesterol, and HbA1c, with lower apoB, and with a history of hypertension. However, none of the tests for interaction were statistically significant, although admittedly, we had limited statistical power.

CONCLUSIONS — We found that among men with type 2 diabetes, high plasma levels of CRP were associated with an increased risk of incident cardiovascular events. Although subjects who developed CVD during follow-up were older and had a higher prevalence of hypertension at baseline, this association was independent of these and other lifestyle and clinical risk factors. HbA1c and non-HDL cholesterol did not account for the observed association between CRP and risk of cardiovascular events. This suggests that inflammation might be associated with cardiovascular complications in diabetic subjects independent of hyperglycemia and dyslipidemia.

Although the increased cardiovascular risk in patients with type 2 diabetes (2,3) has previously been mainly attributed to hyperglycemia, dyslipidemia, and a prothrombotic state, our results confirm the hypothesis that inflammatory mechanisms may be relevant in patients with diabetes (4). In addition to advanced glycation end product–mediated cytokine release, other pathogenic phenomena in subjects with diabetes, such as an increase in formulation of immune complexes with modified lipoproteins, might be relevant contributors to CRP release (4). It is likely that the associated risk is mediated through multiple pathways, including insulin resistance and hyperglycemia as well as direct effects on the vessel wall (22). CRP levels were found to be associated with insulin resistance (23,24) and risk of type 2 diabetes (25,26). In addition, recent findings suggest that inflammation might not only play a possible role in diabetogenesis but also in hyperglycemia after diabetes has been established (27). Furthermore, CRP is associated with several aspects of the metabolic syndrome (28) and is not only a marker of low-grade chronic systemic inflammation but also causes expression of endothelial cell adhesion molecules (29) and activation of complement (30). It furthermore mediates LDL uptake to macrophages (31) and promotes atherothrombosis by increasing plasminogen activator inhibitor 1 (32).

Several large prospective studies show that CRP is an independent predictor of cardiovascular events among healthy individuals (6,7). However, evidence that CRP is a robust predictor among patients with type 2 diabetes is less consistent and very limited. In contrast to case-control studies, which found CRP to be associated with CHD risk (8,9), in the prospective Honolulu Heart Program (10,11), the associations between CRP and risk of thromboembolic stroke or myocardial infarction were generally weaker and not significant in diabetic
In conclusion, our findings support the hypothesis that high CRP levels increase the risk of cardiovascular events among men with type 2 diabetes, independent of currently established lifestyle and metabolic risk factors in diabetic subjects, e.g., obesity, cholesterol levels, and HbA1c.

Acknowledgments — This study was supported by research grants (HL-63582, HL-39464, and CA-59075) from the National Institutes of Health as well as a European Association for the Study of Diabetes/American Diabetes Association Trans-Atlantic fellowship. and a fellowship within the Postdoc-Program of the German Academic Exchange Service (DAAD) (to M.B.S.). F.B.H. is the recipient of an American Heart Association Established Investigator Award.

References


<table>
<thead>
<tr>
<th>CRP (mg/l)</th>
<th>Case subjects</th>
<th>&lt;1.0</th>
<th>1.0–3.0</th>
<th>&gt;3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &lt;27.2 kg/m²*</td>
<td>48</td>
<td>1.00</td>
<td>1.56 (0.75–3.25)</td>
<td>1.66 (0.68–4.03)</td>
</tr>
<tr>
<td>BMI 27.2 kg/m²</td>
<td>50</td>
<td>1.00</td>
<td>1.94 (0.75–5.02)</td>
<td>2.70 (0.90–8.05)</td>
</tr>
<tr>
<td>LDL &lt;3.36 mmol/l</td>
<td>42</td>
<td>1.00</td>
<td>2.31 (0.92–5.80)</td>
<td>2.11 (0.72–6.18)</td>
</tr>
<tr>
<td>LDL 3.36 mmol/l</td>
<td>61</td>
<td>1.00</td>
<td>1.40 (0.68–2.91)</td>
<td>2.48 (1.05–5.83)</td>
</tr>
<tr>
<td>HDL 1.03 mmol/l</td>
<td>39</td>
<td>1.00</td>
<td>1.22 (0.52–2.89)</td>
<td>3.38 (1.20–9.50)</td>
</tr>
<tr>
<td>HDL &lt;1.03 mmol/l</td>
<td>64</td>
<td>1.00</td>
<td>1.78 (0.82–3.85)</td>
<td>1.90 (0.81–4.48)</td>
</tr>
<tr>
<td>apoB &lt;1.10 g/l</td>
<td>52</td>
<td>1.00</td>
<td>2.53 (1.14–5.64)</td>
<td>2.99 (1.15–7.80)</td>
</tr>
<tr>
<td>apoB 1.10 g/l</td>
<td>51</td>
<td>1.00</td>
<td>0.82 (0.34–1.97)</td>
<td>1.62 (0.58–4.46)</td>
</tr>
<tr>
<td>HbA1c &lt;7.0%</td>
<td>47</td>
<td>1.00</td>
<td>0.89 (0.42–1.87)</td>
<td>1.20 (0.51–2.84)</td>
</tr>
<tr>
<td>HbA1c 7.0%</td>
<td>56</td>
<td>1.00</td>
<td>2.78 (1.17–6.57)</td>
<td>3.77 (1.43–9.96)</td>
</tr>
<tr>
<td>Fibrinogen &lt;13.07 μmol/l</td>
<td>50</td>
<td>1.00</td>
<td>1.62 (0.79–3.34)</td>
<td>2.10 (0.77–5.74)</td>
</tr>
<tr>
<td>Fibrinogen 13.07 μmol/l</td>
<td>53</td>
<td>1.00</td>
<td>1.36 (0.54–3.38)</td>
<td>1.86 (0.74–4.65)</td>
</tr>
<tr>
<td>Without history of hypertension</td>
<td>40</td>
<td>1.00</td>
<td>0.66 (0.28–1.55)</td>
<td>2.03 (0.77–5.36)</td>
</tr>
<tr>
<td>With history of hypertension</td>
<td>63</td>
<td>1.00</td>
<td>3.06 (1.29–7.28)</td>
<td>3.50 (1.35–9.11)</td>
</tr>
</tbody>
</table>

Data are RR (95% CI). RRs were adjusted for age, physical activity (quartiles), alcohol intake (0, 0.1–4.9, 5.0–9.9, 10.0–14.9, and ≥15.0 g/day), family history of CHD, history of high blood pressure at blood collection, history of high serum cholesterol at blood collection, aspirin use, smoking (never, past, current), lasting status, BMI (continuous for BMI strata; five categories otherwise), and quartiles of fibrinogen, creatinine, and HbA1c. *Analysis of BMI strata was limited to 715 subjects due to missing values; categories were based on baseline median value; † stratification based on population median. 

Table 3 — RRs of cardiovascular events according to levels of CRP by BMI, LDL, HDL, apoB, HbA1c, fibrinogen, and hypertension among 746 men with diabetes.
CRP and CVD among men with diabetes