High-Sensitivity C-Reactive Protein and Coronary Heart Disease Mortality in Patients With Type 2 Diabetes

A 7-year follow-up study

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OBJECTIVE — To investigate in a follow-up study whether high-sensitivity C-reactive protein (hs-CRP) predicts coronary heart disease (CHD) events in subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS — The original study population consisted of 1,059 patients with type 2 diabetes (age 45–64 years). Mean duration of diabetes was 8 years. CRP values were available from 1,045 subjects, of whom 878 were free of myocardial infarction (MI) at baseline. CHD mortality and the incidence of nonfatal MI were assessed in a 7-year follow-up.

RESULTS — Altogether, 157 patients died from CHD and 254 had a nonfatal or fatal CHD event. Patients with hs-CRP >3 mg/l had a higher risk for CHD death than patients with hs-CRP ≤3 mg/l (19.8 and 12.9%, respectively, $P = 0.004$). In Cox regression analysis, patients with high hs-CRP had a relative risk of 1.72 for CHD death even after the adjustment for confounding factors ($P = 0.002$). Among subjects who were free from MI at baseline, those with a high hs-CRP level had relative risks of 1.83 ($P = 0.003$) and 1.84 ($P = 0.004$) for CHD death in univariate and multivariate analyses, respectively.

CONCLUSIONS — In this large cohort of type 2 diabetic patients, hs-CRP was an independent risk factor for CHD deaths.

Inflammation has been established to play an important role in the pathogenesis of atherosclerosis (1,2). There are many systemic markers of inflammation, but most promising of these is high-sensitivity C-reactive protein (hs-CRP), which has been found to independently predict future coronary heart disease (CHD) events in several prospective studies that included nondiabetic subjects (3–5). Patients with type 2 diabetes have two- to fourfold increased risk for CHD, and >50% of all diabetic pa-

tients die of CHD (6). Levels of CRP are increased in type 2 diabetes (7). Prospective studies on the predictive value of hs-CRP for CHD events in patients with type 2 diabetes are scanty; there are only few studies reporting an association between elevated CRP and cardiovascular events in patients with type 2 diabetes (8–10).

In a large long-term follow-up study, we examined whether hs-CRP predicts CHD mortality and other CHD events in patients with type 2 diabetes.

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RESEARCH DESIGN AND METHODS

Baseline study

A detailed description of study participants has been given elsewhere (11). Patients with type 2 diabetes (age 45–64 years) who were born and living in the Turku University Central Hospital district in West Finland or in the Kuopio University Hospital district in East Finland were identified through a national drug reimbursement register. The final population consisted of 1,059 subjects with type 2 diabetes. Of these, 328 men and 221 women were from West Finland (participation rate 79%) and 253 men and 257 women were from East Finland (participation rate 83%). Type 1 diabetes was excluded in all insulin-treated patients by C-peptide measurements (11). Of the patients, 147 were treated with diet only, 762 with oral medication, and 150 with insulin.

In the present study, the final study population consisted of 1,045 patients with type 2 diabetes (572 men and 473 women) from whom serum was available for hs-CRP determination. Of these patients, 878 did not have a history of myocardial infarction (MI) at baseline. Only four women had hormone replacement therapy at baseline.

The baseline examination between 1982 and 1984 included an interview on the history of smoking, alcohol intake, physical activity, use of medication, and history of chest pain suggestive of CHD. The methods have been previously described in detail (11).

Chest pain symptoms suggestive for angina pectoris were recorded by specially trained nurses using the Rose cardiovascular questionnaire (12). All medical records of subjects who reported that they had been admitted to the hospital for chest pain were reviewed by two investigators (M.L. and T.R.). Methods were carefully standardized between the reviewers. The World Health Organization criteria for verified definite or possi-

Table 1—*hs-CRP, cardiovascular risk factors, and other clinical characteristics of men and women with type 2 diabetes at baseline in relation to CHD mortality*

	CHD death in men			CHD death in women		
	No (n = 476)	Yes (n = 96)	P	No (n = 412)	Yes (n = 61)	P
hs-CRP (mg/l)	3.12 ± 6.06	4.10 ± 4.86	0.002	3.89 ± 5.49	5.31 ± 8.92	0.139
Age (years)	57.1 ± 5.1	58.6 ± 4.6	0.008	58.8 ± 4.9	61.5 ± 2.8	<0.001
Duration of diabetes (years)	7.9 ± 4.1	8.9 ± 4.8	0.042	7.9 ± 3.8	9.3 ± 4.4	0.009
Total cholesterol (mmol/l)	6.3 ± 1.3	7.1 ± 1.5	<0.001	7.0 ± 1.9	7.3 ± 2.1	0.200
HDL cholesterol (mmol/l)	1.18 ± 0.34	1.10 ± 0.31	0.025	1.27 ± 0.37	1.19 ± 0.37	0.137
Triglyceride (mmol/l)	2.22 ± 1.84	3.02 ± 2.12	<0.001	2.72 ± 3.01	4.02 ± 5.25	0.032
HbA$_1$ (%)	9.6 ± 2.3	10.0 ± 1.9	0.150	10.2 ± 2.2	10.6 ± 2.0	0.179
BMI (kg/m^2)	28.2 ± 4.3	28.6 ± 4.8	>0.25	30.4 ± 5.7	30.3 ± 5.5	>0.25
Current smokers (%)	24.6	25.0	>0.25	7.0	1.6	0.106
Hypertension (%)	56.1	60.4	>0.25	70.1	73.8	>0.25
Hypolipidemic therapy (%)	1.3	1.0	>0.25	0.5	0	>0.25
Aspirin therapy (%)	2.3	4.2	>0.25	2.7	4.9	>0.25

Data are means ± SD unless otherwise indicated.

ble MI based on chest pain symptoms, electrocardiogram changes, and enzyme determinations were used to define previous MI (13). Electrocardiogram abnormalities were classified according to the Minnesota code (12).

BMI was calculated (weight in kilograms divided by the square of height in meters) and blood pressure measured with the person in the sitting position after a 5-min rest. Hypertension was defined as systolic blood pressure ≥160 mmHg, diastolic pressure ≥95 mmHg, or appropriate drug treatment.

All blood specimens were drawn at 8:00 A.M. after a 12-h fast. Samples were centrifuged within 1 h and the sera immediately frozen at −20°C. Fasting plasma glucose was determined by the glucose oxidase method (Boehringer Mannheim, Mannheim, Germany). HbA$_1$ (reference range in nondiabetic subjects 5.5–8.5%) level was determined by affinity chromatography (Isolab, Akron, OH). Serum lipid and lipoprotein cholesterol levels were measured in fresh serum samples. Serum total cholesterol and triglyceride levels were determined enzymatically (Boehringer Mannheim). Serum HDL cholesterol level was determined enzymatically after precipitation of LDLs and VLDLs with dextran sulfate MgCl$_2$ (14).

hs-CRP was analyzed from baseline samples with a latex turbidimetric immunoassay (Wako Chemicals, Neuss, Germany). The analytical detection limit for this method is 0.06 mg/l. In our analyses, the interassay coefficient of variation was 3.3% and 2.7% at mean hs-CRP levels of 1.52 (n = 116) and 2.51 (n = 168) mg/l, respectively.

Follow-up study

In 1990, a questionnaire about hospitalization for acute chest pain was sent to every surviving participant of the original study cohort. All medical records of those subjects who died between the baseline examination and 31 December 1989 or who reported in the questionnaire that they had been admitted to the hospital because of chest pain between the baseline examination and 31 December 1989 were reviewed by one of the investigators (S.L.). The modified World Health Organization criteria for definite or possible MI were used in a manner similar to that in the baseline study. In the final classification of the causes of death, hospital records and autopsy records were used, if available. Copies of death certificates of those patients who had died were obtained from the Central Statistical Office of Finland. To ensure that the data collection was complete, a computerized hospital discharge register was used to check for hospital admissions of all participants in the baseline study. In cases of diagnoses suggesting MI, medical records were also checked.

The Joint Commission on Ethics of the Turku University and Turku University Central Hospital and the Ethics Committee of the University of Kuopio approved the study. Informed written consent was obtained from all participants.

Statistical analyses

All statistical analyses were performed using SPSS for Windows (version 10.0; SPSS, Chicago, IL). Data of continuous
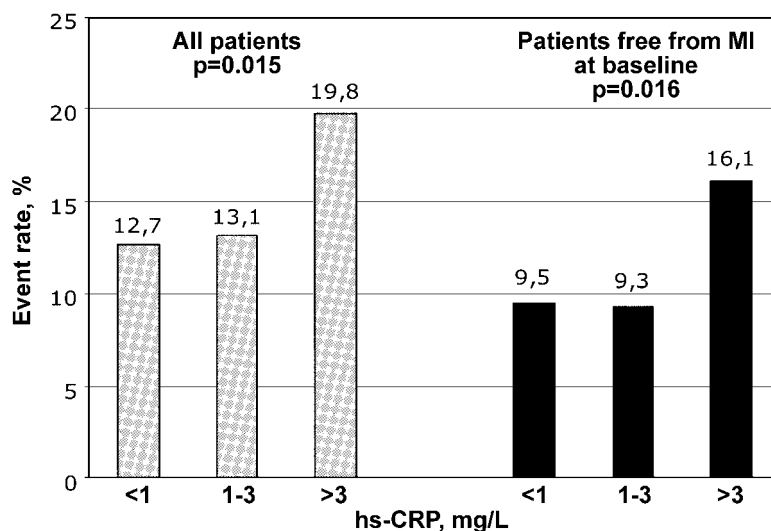

Figure 1—*CHD mortality by baseline hs-CRP level in men and women with type 2 diabetes.*

Table 2—*Relative risks for death from CHD in type 2 diabetic patients with hs-CRP level >3.0 vs. ≤3.0 mg/l*

	Relative risk (95% CI)	*P*
All patients		
Unadjusted	1.67 (1.22–2.30)	0.002
Adjusted for age and sex	1.72 (1.25–2.37)	0.001
Adjusted for multiple factors*	1.72 (1.23–2.41)	0.002
Patients without MI at baseline		
Unadjusted	1.83 (1.24–2.72)	0.003
Adjusted for age and sex	1.81 (1.22–2.69)	0.003
Adjusted for multiple factors*	1.84 (1.21–2.80)	0.004

*Adjusted for age, sex, duration of diabetes, total cholesterol level, HDL cholesterol level, triglyceride level, HbA_1, presence of hypertension, smoking, BMI, and area of residence.

variables are expressed as means (±SD). Differences between groups were assessed by the Student's *t* test for independent samples. hs-CRP and triglyceride levels were analyzed after logarithmic transformation because of their skewed distribution. American Heart Association recommendations (15) for hs-CRP cut points of low risk (<1.0 mg/l), average risk (1.0–3.0 mg/l), and high risk (>3.0 mg/l), which approximately corresponded to tertiles in our study, were used in statistical analyses. The χ^2 test was used to compare categorical variables. Univariate and multivariate Cox regression analyses were performed to investigate the association between CHD risk factors and the time to CHD events.

RESULTS — During the 7-year follow-up 157 patients died from CHD and 254 patients had a nonfatal or fatal CHD event. Table 1 presents hs-CRP levels and background data of the study sample in relation to CHD mortality during the 7-year follow-up. In men, the mean hs-CRP was statistically significantly higher in patients with type 2 diabetes who died of CHD than in those who did not. The difference in women was not statistically significant. In men, the mean hs-CRP level was statistically significantly higher in those who had the combined end point, a fatal or nonfatal MI, compared with those who did not (*P* = 0.024). In women, this difference was not statistically significant.

When we divided hs-CRP into low-, average-, and high-risk groups (<1.0, 1.0–3.0, and >3.0 mg/l, respectively, men and women combined), CHD mortality rates were 12.7, 13.1, and 19.8%, respectively (overall *P* = 0.015) (Fig. 1). CHD mortality or nonfatal MI rates in corresponding groups were 22.9, 22.8, and 27.6% (*P* = 0.262).

We then compared the CHD mortality rate in patients with type 2 diabetes whose hs-CRP levels were >3.0 mg/l with those whose hs-CRP levels were ≤3.0 mg/l. The CHD mortality rate was ~1.6-fold higher in patients with hs-CRP levels

>3.0 mg/l (*P* = 0.004). Results were similar if we excluded patients who already had a history of MI at baseline (*P* = 0.004).

In Cox regression analysis (men and women combined), patients with type 2 diabetes whose hs-CRP levels were >3.0 mg/l had higher CHD mortality rate when adjusted for age and sex (*P* = 0.001) (Table 2). Further adjustment for total cholesterol level, HDL cholesterol level, triglyceride level, duration of diabetes, HbA_1 level, hypertension, smoking, BMI, and area of residence did not alter the association (*P* = 0.002). Corresponding analyses were performed in patients who did not have a history of MI at baseline, and the results were similar to those obtained in the whole cohort (*P* = 0.004). Adding aspirin to the multivariate analysis did not alter the results in our study (*P* = 0.002 in the entire study population, *P* = 0.005 in patients with no MI at baseline).

Figure 2 shows the Kaplan-Meier estimates for the probability of CHD death in men and women with type 2 diabetes whose hs-CRP levels were >3.0 mg/l and ≤3.0 mg/l. Those patients with hs-CRP levels >3.0 mg/l at baseline had a poorer prognosis than those with hs-CRP levels ≤3.0 mg/l. A similar result was obtained also when we excluded study patients who already had MI at baseline.

When we excluded those patients with type 2 diabetes whose hs-CRP was >10 mg/l, the results were essentially similar to those in the whole cohort. For example, the relative risk for CHD mortality rate in those patients with type 2 diabetes whose hs-CRP was >3.0 mg/l but not >10 mg/l (*n* = 238) compared
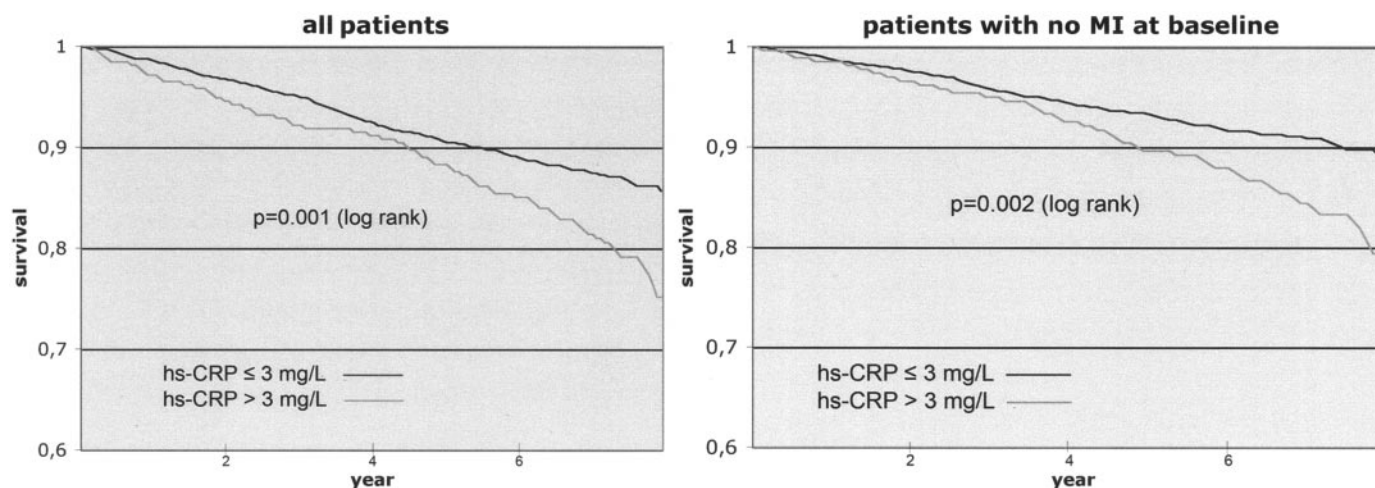

Figure 2—*Kaplan-Meier survival curves for CHD mortality in type 2 diabetic subjects with hs-CRP concentration >3 mg/l or ≤3 mg/l.*

with those whose hs-CRP was ≤3.0 mg/l ($n = 722$) was 1.60 without adjustment and 1.68 after multiple adjustment ($P = 0.007$).

CONCLUSIONS — In our prospective study of a large cohort of patients with type 2 diabetes, we found that elevated hs-CRP level predicted CHD death independently of other cardiovascular risk factors. However, the risk was increased only at higher hs-CRP level (>3 mg/l).

Previous studies on the predictive value of hs-CRP in subjects with type 2 diabetic patients are scanty. In the Health Professionals Follow-up Study, high CRP level was associated with increased incidence of cardiovascular events in men, but the sample size was small and CHD events were few in number (8). The Hoorn study showed an association between CRP and cardiovascular mortality in patients with type 2 diabetes, but the association was not independent of other CHD risk factors (9). In patients with type 2 diabetes who had acute coronary syndrome, CRP seemed to be an independent predictor for cardiovascular death (10). In the Honolulu Heart Program, the association with elevated CRP and MI was weaker in diabetic than in nondiabetic men (16). It is possible that other CHD risk factors typical for patients with type 2 diabetes like high triglyceride level, low HDL cholesterol level, hypertension, and hyperglycemia per se partially mask the role of hs-CRP as a risk factor for CHD in this population. In nondiabetic subjects, increased level of hs-CRP seems to be a strong risk factor in apparently healthy individuals (3–5), but it also seems to predict future outcomes in patients with established CHD (17). In our study, we also found an independent association between CHD death and elevated hs-CRP in patients with type 2 diabetes without MI at baseline, suggesting that inflammation also plays an important role in this high-risk group before severe clinical outcomes of CHD have occurred. The association of elevated hs-CRP with CHD events was more clear in relation to fatal events than fatal and nonfatal events combined. This may imply that patients with high hs-CRP have had more severe atherosclerotic coronary disease with a poorer clinical outcome of MI than those with lower CRP-levels.

There are many possible mechanisms by which hs-CRP enhances atherosclerosis. hs-CRP activates the complement pathway (18) and induces adhesion molecule expression by human endothelial cells (19). hs-CRP also has been found to play a role in monocyte recruitment into the arterial wall (20). hs-CRP enhances the entry of LDL particles into macrophages (21), and it has been found to induce plasminogen activator inhibitor-1 expression (22). Elevated CRP is also associated with endothelial dysfunction (23). Type 2 diabetic patients have increased CRP values, and inflammation has been found to be related to insulin resistance syndrome, which may partly explain the high incidence of CHD in this group (24). However, we did not observe any attenuation in the relative risk for CHD events in patients with high CRP after adjustment for BMI and other variables related to metabolic syndrome. One explanation for this may be that existing atherosclerosis may induce an increase in circulating CRP levels (25).

In several studies, statins have been found to decrease CRP concentration and CHD events independently of serum lipid concentration (26,27). In the present study, only eight participants received hypolipidemic medication at baseline and none received statins, which were not available for clinical use before the end of the follow-up period. Thus, statin treatment did not cause any bias in our cohort. Aspirin, currently used in both primary and secondary prevention of MI, also has anti-inflammatory activities (4), but only a small proportion of our patients were on aspirin therapy.

A limitation of our study was that we had only one baseline CRP value and no follow-up values. However, there are studies showing that CRP concentrations are relatively constant in an individual over time and that there is no marked diurnal variation (28,29). Moreover, our findings were largely unaltered when we excluded patients with CRP values >10 mg/l, i.e., those with probable acute infection at baseline.

In summary, our study shows that CRP is an independent risk factor for CHD mortality in patients with type 2 diabetes. This suggests that inflammation plays an important role in fatal CHD events also among this high-risk population. This might partly explain why statins and aspirin, in addition to their LDL-lowering and antiplatelet effects, have had favorable effects in studies on the prevention of CHD events in patients with type 2 diabetes.

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