Serum Zinc Level and Coronary Heart Disease Events in Patients With Type 2 Diabetes

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OBJECTIVE — Low serum zinc level may predispose nondiabetic subjects to cardiovascular diseases. Our aim was to investigate whether serum zinc level 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, aged 45–64 years. Mean duration of diabetes was 8 years. Serum zinc values were available from 1,050 subjects. CHD mortality and the incidence of nonfatal myocardial infarction (MI) were assessed in a 7-year follow-up.

RESULTS — During the follow-up, 156 patients died from CHD and 254 patients had a fatal or nonfatal MI. Patients with serum zinc concentration ≤14.1 μmol/l at baseline had a higher risk for death from CHD than patients with serum zinc level >14.1 μmol/l (20.8 and 12.8%, respectively; $P = 0.001$) The risks for fatal or nonfatal MI were 30.5 and 22.0%, respectively ($P = 0.005$). In Cox regression analyses, low serum zinc concentration was significantly associated with CHD mortality (relative risk [RR] 1.7, $P = 0.002$) and all CHD events (RR 1.37, $P = 0.030$), even after adjustment for confounding variables.

CONCLUSIONS — In this large cohort of type 2 diabetic patients, low serum zinc level was an independent risk factor for CHD events.

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Coronary heart disease (CHD) is a major cause of death in type 2 diabetic patients, and the risk of CHD is two- to fourfold higher among patients with type 2 diabetes than in nondiabetic subjects. This enhanced risk is partly explained by traditional risk factors, e.g., hypertension, plasma lipid and lipoprotein abnormalities, smoking, and obesity, but all of the excess risk cannot be explained by these conventional risk factors. Nontraditional risk factors may be important in the pathogenesis of CHD in patients with type 2 diabetes.

Because serum zinc is a micronutrient with known antioxidant activity (1), it might be relevant to assess its role in the atherogenesis in type 2 diabetes. Patients with diabetes have lower serum levels of zinc (2).

In nondiabetic subjects there are studies suggesting that low serum level of zinc is associated with increased incidence of cardiovascular disease (3–5). To our knowledge, there are no studies in type 2 diabetic patients on the association between low serum levels of zinc and cardiovascular events. In this large prospective study with type 2 diabetic patients, we examined the association between serum zinc level and CHD events.

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Abbreviations: CHD, coronary heart disease; GFR, glomerular filtration rate; MI, myocardial infarction.

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

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RESEARCH DESIGN AND METHODS

Baseline study

A detailed description of study participants has been given elsewhere (6). Patients with type 2 diabetes, aged 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 type 2 diabetic subjects. 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 (6). 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 1,050 patients with type 2 diabetes whose serum zinc value was available. A total of 544 patients were from West Finland (326 men and 218 women), and 506 were from East Finland (250 men and 256 women).

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 (6).

Chest pain symptoms suggestive for angina pectoris were recorded by specially trained nurses using the Rose cardiovascular questionnaire (7). 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 possible myocardial infarction (MI) based on chest pain symptoms, electrocardiographic changes, and enzyme determinations were used to define previous MI (8). Electrocardiographic abnormalities were clas-

Table 1—*Serum zinc level (unadjusted), cardiovascular risk factors, and other clinical characteristics of type 2 diabetic men and women at baseline in relation to future CHD events*

	CHD death					
	Men			Women		
	No	Yes	*P*	No	Yes	*P*
n	480	96		414	60	
Zinc (μmol/l)	15.8 ± 2.5	15.1 ± 2.5	0.016	15.7 ± 2.4	15.0 ± 2.2	0.022
Mean age (years)	57.1 ± 5.1	58.6 ± 4.6	0.009	58.7 ± 4.9	61.5 ± 2.8	<0.001
Duration of diabetes (years)	7.9 ± 4.1	8.8 ± 4.8	0.063	7.8 ± 3.8	9.3 ± 4.5	0.008
Total cholesterol (mmol/l)	6.3 ± 1.4	7.1 ± 1.5	<0.001	7.0 ± 1.9	7.4 ± 2.1	0.207
HDL cholesterol (mmol/l)	1.18 ± 0.34	1.10 ± 0.31	0.033	1.27 ± 0.37	1.20 ± 0.37	0.141
Triglycerides (mmol/l)	2.26 ± 2.03	3.03 ± 2.12	<0.001	2.71 ± 3.01	3.97 ± 5.28	0.014
HbA_1 (%)	9.6 ± 2.3	10.0 ± 1.9	0.100	10.1 ± 2.2	10.5 ± 2.0	0.188
BMI (kg/m^2)	28.2 ± 4.3	28.7 ± 4.7	NS	30.4 ± 5.7	30.2 ± 5.5	NS
Current smokers (%)	24.6	25.0	NS	7.2	1.7	0.102
Hypertension (%)	56.3	59.4	NS	70.5	73.3	NS
	Fatal or nonfatal MI					
	Men			Women		
	No	Yes	*P*	No	Yes	*P*
n	421	155		375	99	
Zinc (μmol/l)	15.8 ± 2.5	15.3 ± 2.7	0.022	15.8 ± 2.4	15.0 ± 2.2	0.004
Mean age (years)	57.0 ± 5.2	58.2 ± 4.6	0.009	58.7 ± 4.9	60.6 ± 3.9	<0.001
Duration of diabetes (years)	8.0 ± 4.1	8.4 ± 4.6	NS	7.8 ± 3.8	9.0 ± 4.2	0.004
Total cholesterol (mmol/l)	6.3 ± 1.4	7.0 ± 1.4	<0.001	7.0 ± 1.7	7.3 ± 2.6	0.235
HDL cholesterol (mmol/l)	1.20 ± 0.35	1.09 ± 0.28	<0.001	1.28 ± 0.38	1.20 ± 0.34	0.084
Triglycerides (mmol/l)	2.22 ± 2.07	2.85 ± 1.98	<0.001	2.63 ± 2.65	3.79 ± 5.29	0.025
HbA_1 (%)	9.6 ± 2.3	9.8 ± 1.9	NS	10.1 ± 2.3	10.4 ± 1.9	0.244
BMI (kg/m^2)	28.1 ± 4.4	28.6 ± 4.3	NS	30.4 ± 5.8	30.1 ± 5.1	NS
Current smokers (%)	24.0	26.5	NS	6.9	5.1	NS
Hypertension (%)	55.6	60.0	NS	69.9	74.7	NS

Data are means ± SD unless otherwise indicated.

sified according to the Minnesota code (7).

BMI was calculated as the weight in kilograms per the square of height in meters, and blood pressure measured with the person in the sitting position after a 5-min rest. A patient was defined as having hypertension if systolic blood pressure was ≥160 mmHg, if diastolic pressure was ≥95 mmHg, or if the patient was receiving drug treatment for hypertension.

All blood specimens were drawn at 8:00 A.M. after a 12-h fast. Samples were centrifuged within 1 h and the sera frozen immediately at −20°C. Fasting plasma glucose was determined by the glucose oxidase method (Boehringer Mannheim, Mannheim, Germany). HbA_1 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 $MgCl_2$ (9). Serum zinc was analyzed by direct atomic absorption spectrophotometry ~10 years after the baseline study from stored samples (10). In our analyses, the interassay coefficient of variation was 3.03% at mean serum zinc level of 13.91 μmol/l ($n = 20$).

Plasma creatinine level was determined by the Jaffe method. The estimated glomerular filtration rate (GFR) was calculated by using the following formula described by Cockcroft and Gault: GFR (ml/s) = (1/60) × [(140 − y) × kg × K]/serum creatinine, where K is a constant factor (that is, 1.23 for men and 1.05 for women), y is age, and kg is body weight. These values were then corrected for body surface area.

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 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 similarly as 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.

Table 2—*Relative risks for CHD events in type 2 diabetic patients with serum zinc level of 14.1 μmol/l or less compared with patients with serum-zinc level above 14.1 μmol/l*

	CHD mortality		CHD mortality or nonfatal MI	
	RR (95% CI)	*P*	RR (95% CI)	*P*
Unadjusted	1.80 (1.30–2.49)	<0.001	1.40 (1.06–1.84)	0.019
Adjusted for age and sex	1.81 (1.30–2.51)	<0.001	1.40 (1.06–1.85)	0.017
Adjusted for multiple factors*	1.70 (1.21–2.38)	0.002	1.37 (1.03–1.82)	0.033

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

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 by using SPSS for Windows, version 10.0 (SPSS, Chicago, IL). Data of continuous variables are expressed as means ± SD. Differences between groups were assessed by the Student's *t* test for independent samples. Triglycerides were analyzed after logarithmic transformation because of skewed distribution. The χ^2 test was used to compare categorical variables with variables divided in quartiles. 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 period, of the whole cohort (1,050 patients), 156 died from CHD. Of these, 64 were from West Finland (43 men and 21 women) and 92 from East Finland (53 men and 39 women). A total of 254 patients had a fatal or nonfatal MI; of these, 111 were from West Finland (71 men and 40 women) and 143 from East Finland (84 men and 59 women).

The mean unadjusted serum zinc level was similar in insulin-treated subjects (fasting serum zinc = 15.3 μmol/l) compared with those subjects who did not have insulin included in their treatment (fasting serum zinc = 15.7 μmol/l) ($P = 0.074$); in final analyses, the study subjects were combined. In men and women the mean unadjusted serum zinc level was higher in diabetic patients who lived in East Finland (fasting serum zinc = 15.8 μmol/l) than in those who lived in West Finland (fasting serum zinc = 15.5 μmol/l) ($P = 0.038$). While in final analyses patients from the two areas were combined, in multivariate analyses the area of residence was included as a covariate.

Baseline characteristics and unadjusted serum zinc levels of the study sample in relation to CHD mortality during the 7-year follow-up are presented in Table 1. The mean serum zinc level was statistically significantly lower in men and women who died from CHD than in those who did not ($P = 0.016$ and $P = 0.022$, respectively). The total cholesterol level was significantly higher in men, and HDL cholesterol was lower in men who died from CHD than in those who did not ($P < 0.001$ and $P = 0.033$, respectively). In both men and women, those who died from CHD were older ($P = 0.009$ and $P < 0.001$, respectively) and had higher triglyceride levels than those who did not ($P < 0.001$ and $P = 0.014$, respectively). In women, those whose duration of diabetes was longer had a greater risk of death from CHD than those who had had diabetes a shorter period of time ($P = 0.008$).

The mean unadjusted serum zinc level was also lower in those men and women who had a fatal or nonfatal MI than in those who did not ($P = 0.022$ and $P = 0.004$, respectively).

With respect to CHD death and serum zinc level, there was a step-function effect, whereas with respect to all CHD events the effect was linear when we divided serum zinc levels into quartiles (<14.1, 14.1–15.6, 15.6–17.1, and >17.1 μmol/l). Unadjusted CHD mortality rates were 20.8, 13.9, 12.4, and 12.1%, respectively (overall $P = 0.015$) (Fig. 1). Corresponding values for fatal or nonfatal MI were 30.5, 25.1, 22.1, and 18.8%, respectively ($P = 0.014$) (Fig. 1).

We then compared the CHD mortality in subjects in the lowest quartile (serum zinc level ≤14.1 μmol/l) with those

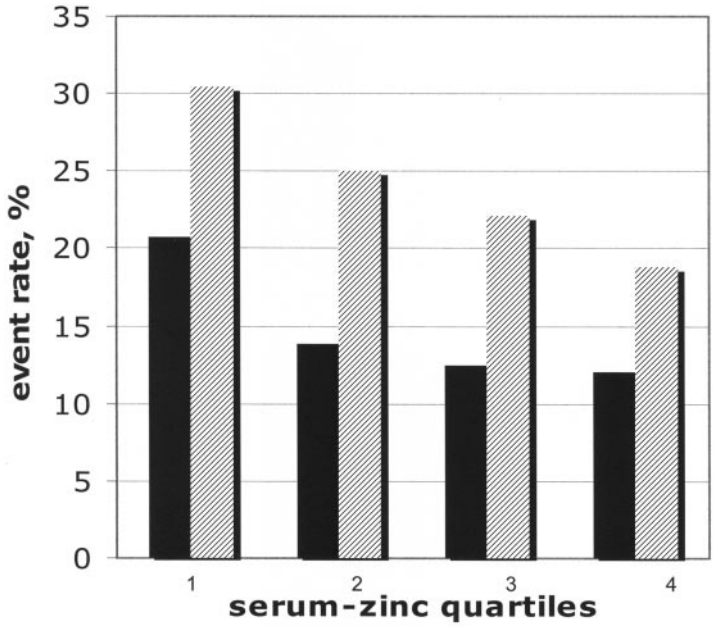

Figure 1—*CHD event rates (unadjusted) in type 2 diabetic patients according to their baseline fasting serum zinc levels divided in quartiles. ■, CHD death (P = 0.015); ▨, nonfatal or fatal MI (P = 0.014).*

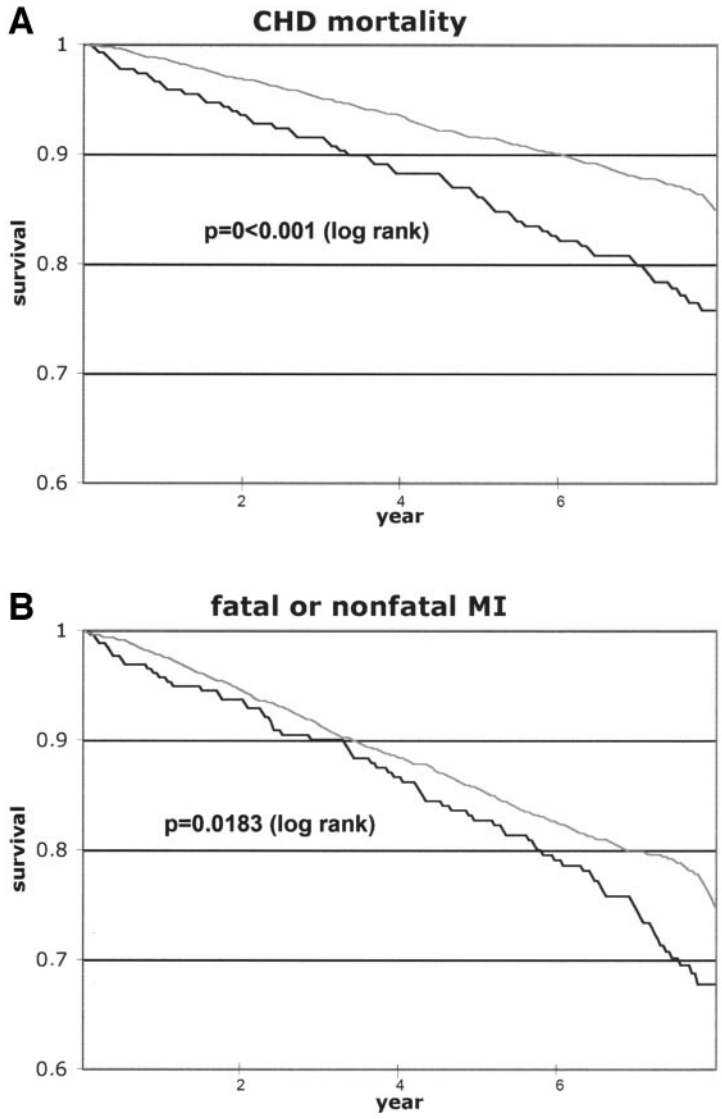

Figure 2—*Kaplan-Meier survival curves (unadjusted) for CHD mortality* (A) *and for fatal or nonfatal MI* (B) *in men and women with type 2 diabetes with fS-zinc level >14.1 μmol/l (grey line) or ≤14.1 μmol/l (black line).*

in the upper three quartiles (serum zinc >14.1 μmol/l). The risk for death from CHD was higher in subjects in the lowest quartile than in those in the upper three quartiles (20.8 and 12.8%, respectively; $P = 0.001$). Nonfatal or fatal MI rates in corresponding groups were 30.5 and 22.0%, respectively ($P = 0.005$).

In Cox regression analyses (men and women combined), subjects with type 2 diabetes whose serum zinc levels were ≤14.1 μmol/l had a 1.81-fold higher CHD mortality rate and a 1.4-fold higher risk for fatal or nonfatal MI than patients with serum zinc level >14.1 μmol/l when adjusted for age and sex (Table2). Further adjustment for total cholesterol level, HDL cholesterol level, triglyceride level, duration of diabetes, HbA_1, hypertension, estimated GFR, smoking, BMI, type of treatment, and area of residence did not alter the association (relative risk [RR] for CHD death 1.7, RR for all CHD events 1.37) (Table 2). Adding an inflammation marker, high-sensitivity CRP, in the model did not change the results; those whose serum zinc levels were ≤14.1 μmol/l still had more CHD deaths (RR 1.67) and fatal or nonfatal MI (RR 1.35) than those whose serum zinc levels were >14.1 μmol/l ($P = 0.003$ and $P = 0.041$, respectively).

Fig. 2 shows Kaplan-Meier estimates for the probability of CHD death and all CHD events in men and women with type 2 diabetes whose serum zinc levels were ≤14.1 and >14.1 μmol/l. Those patients with serum zinc levels ≤14.1 μmol/l at baseline had a poorer prognosis than those with serum zinc levels >14.1 μmol/l. In CHD mortality the difference was seen shortly after the baseline, and in all CHD events the difference was substantially seen after 2 years.

CONCLUSIONS — In our prospective study with a large cohort of type 2 diabetic patients, we found that low serum zinc level is an independent risk factor for CHD mortality and also fatal or nonfatal MI in middle-aged patients with type 2 diabetes.

In nondiabetic subjects, low serum zinc has been associated with increased cardiovascular mortality (3). In a population in North India, the prevalence of coronary artery disease, diabetes, and impaired glucose tolerance was higher among subjects with lower dietary zinc intake (4). Among alcohol drinkers who consumed ≥10 g alcohol/day, higher dietary zinc intake was inversely associated with cardiovascular mortality (5).

Previous studies have shown that serum zinc level is lower in diabetic patients than in nondiabetic subjects, increased urinary zinc excretion being the main reason (2,11–12). There are studies where zinc supplementation in diabetic patients shows antioxidant properties (13,14). In one study in diabetic patients, zinc supplementation decreased lipid peroxidation (15). However, there are no prospective studies in type 2 diabetic patients on the association between serum zinc level and CHD. In one intervention study in a general population, a substudy of the SU.VI.MAX (Supplémentation en Vitamines et Minéraux Antioxydants) Study, the investigators found no difference in cardiovascular events between the subjects who received 20 mg zinc or placebo (16), although the proportion of diabetic patients in this study was rather small.

Theoretically there are several mechanisms by which zinc might be capable of inhibiting atherogenesis. Zinc is an important component of biomembranes and an essential cofactor in a variety of enzymes (17). Zinc has antioxidant-like properties; thus, it can stabilize macromolecules against radical-induced oxidation in vitro as well as limit excess radical production (18,19). Zinc plays an important role in the synthesis and function of insulin, it is capable of modulating insulin action, and it improves hepatic binding of insulin (20). As an antioxidant, zinc has membrane-stabilizing properties and is said to preserve endothelial function be-

cause of its ability to inhibit the pathways of processes leading to apoptosis, probably by upregulating caspase genes (21,22).

Oxidative stress plays an important role in the formation of vascular complications in diabetes (23). In diabetes, there is overproduction of reactive oxygen radicals but also decreased efficiency of antioxidant defense systems (24,25). Hyperglycemia and hyperinsulinemia increase the production of free radicals (26). Oxidatively modified LDL seems to have the main role in the initiation of atherogenesis (27,28), and there is evidence that lipid peroxidation is increased in type 2 diabetic patients (29,30). Diets rich in antioxidants have been associated with antiatherogenic properties (28). There are two studies in diabetic patients where low serum levels of zinc are associated with increased oxidative stress (31,32).

At the time of the baseline examination of our study, zinc was added in vitro to insulin preparations to prolong the duration of insulin action. In the present study there were 149 type 2 diabetic subjects using insulin as a treatment for diabetes. However, it is unlikely that the zinc in the insulin would have affected our results, since serum zinc level did not differ significantly between insulin-treated and non–insulin-treated subjects. Moreover, low serum zinc predicted CHD events in analyses adjusted for mode of treatment. We cannot judge whether zinc in insulin preparations affected serum zinc levels. Supposing that insulin treatment had increased serum zinc level, these patients with obviously more severe diabetes would have had lower serum zinc without insulin treatment. Thus, if anything, the low serum zinc as CHD risk factor would have been even stronger than observed.

Our study was performed before the statin era. Today ~50% of type 2 diabetic patients use statins, which are known to prevent CHD events effectively (33,34). It would be interesting to study whether low serum zinc level also predicts CHD events in the statin era.

Theoretically, our results are in favor of the possibility that zinc supplementation might be useful in preventing atherosclerotic complications in patients with type 2 diabetes. However, large studies in general populations have failed to demonstrate prevention of cardiovascular disease by antioxidant supplements other than zinc (35). As for possible zinc supplementation in patients with type 2 diabetes, intervention studies in this high–CHD risk population is needed.

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