

Lower Toenail Chromium in Men With Diabetes and Cardiovascular Disease Compared With Healthy Men

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OBJECTIVE — Chromium may improve insulin sensitivity, which can modify the risk of diabetes and cardiovascular disease (CVD). Therefore, we evaluated the association between toenail chromium and CVD in diabetic men.

RESEARCH DESIGN AND METHODS — We performed cross-sectional and nested case-control analyses among men aged 40–75 years within the Health Professionals Follow-up Study. The cross-sectional analysis compared men with diabetes only ($n = 688$), diabetes with prevalent CVD ($n = 198$), and healthy control subjects ($n = 361$). The nested case-control study included 202 men with baseline diabetes who developed incident CVD and 361 matched control subjects.

RESULTS — Mean toenail chromium ($\mu\text{g/g}$) was 0.71 in healthy control subjects, 0.61 in diabetes-only subjects, and 0.52 in diabetic subjects with prevalent CVD (P for trend = 0.003). In the cross-sectional analysis, the multivariate odds ratio (OR) between extreme quartiles was 0.74 (95% CI 0.49–1.11; P for trend = 0.18), comparing diabetes only with healthy control subjects. A similar comparison between diabetic subjects with prevalent CVD and healthy control subjects yielded an OR of 0.45 (0.24–0.84; P for trend = 0.003). In the nested case-control study, comparing diabetic men with incident CVD with healthy control subjects, the multivariate OR was 0.65 (0.36–1.17; P for trend = 0.16) between extreme quartiles. When we combined prevalent and incident CVD cases among diabetic men and compared them with healthy control subjects, the OR was 0.62 (0.39–1.01; P for trend = 0.02) between extreme quartiles.

CONCLUSIONS — Our results suggest that diabetic men with CVD have lower toenail chromium than healthy control subjects. However, this study could not distinguish between the effects of chromium on diabetes and those on CVD. Long-term clinical trials are needed to determine whether chromium supplementation is beneficial for preventing CVD among diabetic patients.

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Abbreviations: CABG, coronary artery bypass graft; CVD, cardiovascular disease; PTCA, percutaneous transluminal coronary angioplasty; SRM, standard reference material.

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

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Most of the research on dietary factors affecting the risk of cardiovascular disease (CVD) in people with diabetes has been focused on macronutrients. However, micronutrients, including trace elements such as chromium, may also play a role in the etiology of CVD (1). Although the mechanism of action is largely unknown, chromium can improve insulin sensitivity and therefore may be involved in carbohydrate and lipid metabolism (2,3). Chromium is a transition metal, and its trivalent state is the most prevalent form in organic complexes. Chromium supplements are widely consumed in the U.S. (4), but clinical studies of their efficacy have been inconclusive (5). In addition, epidemiological data on chromium intake and the risk of CVD are limited, partly because of the difficulty in estimating dietary chromium considering its wide variability in food sources. Hence, a sensitive and time-integrated biomarker for chromium intake is required in epidemiological studies. Results from two case-control studies suggest an inverse association between chromium levels in toenails and the risk of myocardial infarction in the general population (6,7). Because the risk of CVD is substantially higher in individuals with diabetes than in those without diabetes (8), we conducted this study to evaluate the association between toenail chromium and CVD among men with diabetes within the Health Professionals Follow-up Study.

RESEARCH DESIGN AND METHODS

The Health Professionals Follow-up Study is a prospective cohort study of 51,529 men aged 40–75 years in 1986 evaluating the role of diet in chronic diseases. At baseline, all participants completed a mailed questionnaire concerning their diet and medical history. A follow-up questionnaire is sent every 2 years to obtain updated information on incident medical conditions including CVD. In 1987, 33,737 of the participants provided samples of toenail clippings; these were stored for analyses of trace el-

ements. Chromium levels were assayed for all men with baseline diabetes and the matched control subjects selected for this study. The study was approved by the institutional review board for the protection of human subjects at the Harvard School of Public Health.

For the cross-sectional analyses, we used data provided in the baseline year of 1986. The study participants were divided into three groups: men with diabetes only ($n = 688$), men with diabetes and prevalent CVD ($n = 198$), and healthy control subjects ($n = 361$). The diabetes-only group consisted of men who had self-reported diabetes but had never been diagnosed with CVD. We defined CVD as fatal or nonfatal myocardial infarction, coronary artery bypass graft (CABG), percutaneous transluminal coronary angioplasty (PTCA), or stroke. The second group included subjects who self-reported both diabetes and CVD at baseline. The healthy control group consisted of the matched control subjects who we selected for the nested case-control study (see below). The diagnosis of diabetes was based on self-reports from the mailed questionnaire at baseline. The validity of self-reported diabetes in this cohort has been verified in a subsample of 71 men (9). A physician blinded to the reported information on the supplementary questionnaire reviewed the medical records according to standard diagnostic criteria. Of the 71 patients, 12 had incomplete records and, among the remaining 59 case subjects, the diagnosis of type 2 diabetes was confirmed in 57 (97%).

The case subjects in the nested case-control analysis included all men with baseline diabetes who developed incident CVD during the follow-up period from the date of return of the toenail samples in 1987 until 1998 ($n = 202$). Control subject selection involved 1:2 matching based on age (within 1 calendar year), smoking status (past, never, or current), and date of toenail return (within 1 month). These control subjects were selected from subjects free of chronic disease at the time of case diagnosis (i.e., risk-set sampling) (10). Since the selection criteria for the healthy control subjects were stringent, we obtained only 1 control for 43 case subjects, and hence, the total number of control subjects was 361. The comparison between diabetic men with CVD and healthy control subjects was designed a priori because it

could provide the strongest contrast in toenail chromium levels. Since we did not have a group with CVD without diabetes in either cross-sectional or nested case-control analyses, we were unable to assess the impact of chromium on diabetes separately from that on CVD.

Assessment of outcomes

The primary outcome in the nested case-control study was incident CVD (defined above). Medical records were reviewed by physicians blinded to the questionnaire data. Confirmation of nonfatal myocardial infarction was based on World Health Organization criteria as follows: symptoms plus either diagnostic electrocardiographic changes or elevated cardiac-enzyme levels. Men who were hospitalized for myocardial infarctions but whose medical records were not available were designated as probable case subjects. We included both the confirmed and probable case subjects in our study. Death from CVD was confirmed if it was listed in the hospital records, autopsy reports, or on the death certificate as the underlying cause of death. When medical records were not available or telephone interviews were not feasible, the diagnosis of CVD was corroborated by correspondence with the subject, family member, or personal physician. Information on CABG and PTCA was based on self-reports only. Among the men who reported diabetes at baseline, 198 reported prevalent CVD (myocardial infarction, 131; stroke, 21; and CABG/PTCA, 46). Among the baseline diabetic men who did not report CVD in 1986, we documented 202 cases of CVD (myocardial infarction, 108; stroke, 17; and CABG/PTCA, 77) during the follow-up period.

Assessment of exposure

Toenails incorporate trace elements as they grow, and a sample from each toe reflects their incorporation over a period of ~1 year (11). The level of chromium in the toenails was analyzed by instrumental neutron-activation analysis at the University of Missouri Research Reactor Center and is the most sensitive method for such estimations (12). Initially, the toenail samples were washed in a sonicator with deionized water to remove any contamination. The laboratory personnel were unaware of the disease status of the participant who provided the sample. In a quality-control study, we used organic

standard reference material (SRM) obtained from the National Institutes of Standards and Technology (NIST SRM 1571 Orchard Leaves) with a certified chromium value of $2.6 \pm 0.3 \mu\text{g/g}$. This was analyzed in duplicate in 18 analytic batches. The mean (\pm SD) of the chromium concentration measured in these 36 replicate SRM samples was $2.610 \pm 0.316 \mu\text{g/g}$; this is in good agreement with the certified values. The toenail samples from the case and control subjects in the nested case-control study were analyzed together but in random order. The samples from baseline diabetic men in the cross-sectional analysis were analyzed together but separately from the nested case-control study batches.

Assessment of potential confounders

Anthropometric, lifestyle, and dietary data were obtained from the questionnaires mailed in 1986, which included a semiquantitative food frequency questionnaire. The average nutrient intake from the food frequency questionnaire was computed using the Harvard University food composition database derived from U.S. Department of Agriculture sources, manufacturers' data, and published reports. BMI was calculated as the ratio of self-reported body weight in kilograms to height in meters squared (kg/m^2). Physical activity was expressed as metabolic equivalent hours based on the self-reported types and durations of activities during the previous year. One metabolic equivalent hour is equivalent to the energy expended sitting quietly for 1 h (13). The reproducibility and validity of the dietary and physical activity data have been reported in detail elsewhere (14–16).

Statistical analysis

All statistical analyses were performed with SAS software (SAS, Cary, NC). For all analyses, we categorized chromium levels into quartiles based on the distribution of the reference group. Since the concentration of a trace element is correlated with the weight of the sample, the estimated levels of chromium in toenails are adjusted for their weight and expressed in micrograms per gram of toenail sample ($\mu\text{g/g}$). The reported geometric mean of toenail chromium is the antilog of a log-transformed form of this variable.

To examine the association between the quartiles of chromium and the risk of

Table 1—Age-standardized baseline characteristics of 361 healthy control subjects from the Health Professionals Follow-up Study according to quartiles of chromium levels

| Variables | Quartiles of chromium levels | | | |
|--|------------------------------|------------------|------------------|------------------|
| | Q1 | Q2 | Q3 | Q4 |
| Median chromium level (range) ($\mu\text{g/g}$) | 0.23 (0.04–0.35) | 0.48 (0.36–0.68) | 0.97 (0.69–1.35) | 2.08 (1.36–26.3) |
| Men (n) | 90 | 90 | 90 | 91 |
| Mean age (years) | 61.7 | 62.5 | 61.3 | 60.4 |
| Mean physical activity, (METs/ week) | 14.6 | 17.2 | 20.7 | 23.5 |
| Mean BMI (kg/m^2) | 24.8 | 25.4 | 25.0 | 24.8 |
| Current smokers | 8.3 | 9.1 | 11.1 | 6.1 |
| Family history of myocardial infarction | 9.6 | 15.9 | 13.1 | 7.2 |
| High blood pressure | 21.0 | 15.1 | 25.9 | 24.1 |
| High cholesterol | 11.3 | 9.7 | 14.5 | 10.2 |
| Mean alcohol intake (g/day) | 13.6 | 14.8 | 15.9 | 12.3 |
| Mean total calories (kcal/day) | 1,975 | 2,013 | 2,080 | 2,024 |
| Calories from carbohydrates | 47.1 | 46.1 | 45.3 | 48.4 |
| Calories from total fat | 31.9 | 31.9 | 33.1 | 30.7 |
| Mean cereal fiber intake (g/day) | 5.7 | 5.4 | 5.5 | 6.5 |
| Mean glycemic load | 118 | 120 | 114 | 127 |
| Mean folate intake ($\mu\text{g/day}$) | 491 | 468 | 455 | 505 |
| Mean dietary composite score* | 8.7 | 8.4 | 8.8 | 9.4 |

Data are percent, unless otherwise indicated. *Dietary composite score was created with quartile scores of six variables (marine ω -3, *trans* fatty acids, folate, cereal fiber, polyunsaturated/saturated fat ratio, and glycemic load). MET, metabolic equivalent hour.

CVD, we used unconditional logistic regression adjusted for the matching factors (i.e., age, smoking, and date of toenail return) and other potential confounders such as BMI, physical activity, alcohol intake, hypertension, hypercholesterolemia, and family history of myocardial infarction. We estimated the odds ratios (ORs) and 95% CIs using the lowest quartile as the reference category. We also adjusted for nutrients as quartiles of a dietary composite score created with quartile scores of six variables (marine ω -3, *trans* fatty acids, folate, cereal fiber, polyunsaturated/saturated fat ratio, and glycemic load) (17). In addition, we controlled for mercury and selenium levels in toenails, as these elements have been associated with the risk of CVD in previous studies (18,19). Tests for trend were conducted by assigning an ordinal score to each quartile. All *P* values reported are two tailed, and values below 0.05 were considered statistically significant.

RESULTS— Table 1 shows age-standardized characteristics at baseline among healthy control subjects according to the quartiles of chromium levels.

Cross-sectional analyses

At baseline, the mean toenail chromium level ($\mu\text{g/g}$) was 0.71 in healthy control subjects, 0.61 in diabetes-only subjects, and 0.52 in diabetic men with prevalent CVD (*P* for trend = 0.003), and controlling for potential risk factors for CVD had little impact on these levels. After adjustment of potential confounders (smoking, alcohol, physical activity, age, BMI, myocardial infarction, family history of myocardial infarction, high cholesterol, hypertension, toenail levels of selenium and mercury, and dietary score), the OR between extreme quartiles was 0.74 (95% CI 0.49–1.11; *P* for trend = 0.18), comparing men with diabetes only with healthy control subjects (Table 2). A similar comparison between diabetic men with prevalent CVD and healthy control subjects yielded an OR of 0.45 (0.24–0.84; *P* for trend = 0.003). Comparison between diabetes-only subjects and diabetic men with prevalent CVD yielded an OR of 0.68 (0.42–1.10; *P* for trend = 0.06) between extreme quartiles. In this analysis, additional adjustment for duration and treatment of diabetes did not have significant impact on the results. Ex-

cluding men with stroke (*n* = 21) in all analyses did not alter the results.

Nested case-control analyses

On comparing the baseline characteristics between diabetic men with incident CVD (case subjects) and healthy control subjects, we found that a higher proportion of case subjects had a history of hypertension (*P* < 0.001) and hypercholesterolemia (*P* < 0.001) compared with healthy control subjects. The healthy control group had a significantly lower BMI (*P* < 0.001) and lower alcohol intake (*P* < 0.001). The mean toenail chromium was lower in diabetic men with incident CVD than in healthy control subjects (0.60 vs. 0.71 $\mu\text{g/g}$; *P* = 0.08). After adjusting for potential confounders, the OR between extreme quartiles was 0.65 (95% CI 0.36–1.17; *P* = 0.16) (Table 3). A total of 17 subjects (8 case and 9 control subjects) reported using chromium supplements in 1986; excluding these subjects from the analyses had virtually no impact on the results. Also, excluding men with stroke (*n* = 17) in this analysis did not change our results. In a secondary analysis, we compared the nail chromium of diabetic

Table 2—Age-adjusted and multivariate ORs and 95% CIs from cross-sectional comparisons by quartiles of toenail chromium: Health Professional Follow-up Study

| | Q1* | Q2 | Q3 | Q4 | P/TD |
|---|-----|------------------|------------------|------------------|-------|
| Diabetes-only versus healthy control subjects | | | | | |
| Age adjusted | 1 | 0.84 (0.59–1.21) | 0.89 (0.62–1.27) | 0.68 (0.47–0.99) | 0.07 |
| Multivariate† | 1 | 0.94 (0.63–1.40) | 0.94 (0.63–1.40) | 0.74 (0.49–1.11) | 0.18 |
| Diabetic men with prevalent CVD versus healthy control subjects | | | | | |
| Age adjusted | 1 | 0.75 (0.47–1.19) | 0.54 (0.33–0.89) | 0.46 (0.28–0.77) | 0.001 |
| Multivariate | 1 | 0.83 (0.47–1.47) | 0.43 (0.23–0.81) | 0.45 (0.24–0.84) | 0.003 |
| Diabetic men with prevalent CVD versus diabetes-only subjects | | | | | |
| Age adjusted | 1 | 0.76 (0.50–1.18) | 0.64 (0.41–0.99) | 0.63 (0.40–0.99) | 0.03 |
| Multivariate | 1 | 0.85 (0.54–1.33) | 0.64 (0.40–1.03) | 0.68 (0.42–1.10) | 0.06 |

*Quartiles based on distribution of 361 healthy control subjects. Q1 is the reference category. †Adjusted for age (five categories: ≤ 50 , 51–54, 55–59, 60–64, and >65 years), BMI (three categories: ≤ 25 , 25.1–30.0, and >30.0 kg/m²), alcohol intake (five categories: 0.0 [non-drinkers], 0.1–5.0, 5.1–10.0, 10.1–15.0, and >15.0 g/day), smoking status (three categories: never smoked, former smoker, and current smoker), family history of myocardial infarction (binary), physical activity (tertiles of metabolic equivalent hours/week), high cholesterol (binary), hypertension (binary), quartiles of dietary score, and toenail levels of selenium and mercury.

men who developed incident CVD ($n = 202$) with those who did not ($n = 447$). After adjusting for potential confounders, the OR between extreme quartiles was 1.12 (0.68–1.83; $P = 0.55$). However, this analysis was limited by the fact that toenail chromium levels of the case and control subjects were analyzed in different laboratory batches.

Pooled analyses of incident and prevalent CVD cases among diabetic men

We also performed a pooled analysis combining prevalent and incident CVD cases in diabetic men ($n = 400$) and compared them with healthy control subjects (Table 3). The multivariate OR in this

analysis was 0.62 (95% CI 0.39–1.01) after comparing the highest to the lowest quartile of toenail chromium ($P = 0.02$).

CONCLUSIONS— Overall, we found lower levels of toenail chromium among men with diabetes and CVD compared with healthy control subjects. The results from the nested case-control analyses were consistent with those from the cross-sectional analyses.

Trivalent chromium is a cofactor for insulin action. When patients receiving total parenteral therapy were supplemented with chromium, their diabetes symptoms reversed and they required smaller doses of exogenous insulin (20). In atherosclerotic rabbits, an injection of

chromium chloride results in a marked reduction in the plaques covering the aortic intimal surface, in aortic weight, and in cholesterol content (21). In the last decade, numerous supplementation studies in humans have examined the role of chromium in glucose intolerance, lipid levels, and type 2 diabetes. A recent meta-analysis of clinical trials concluded that there was no appreciable effect of chromium supplementation on glucose or insulin concentrations in nondiabetic subjects, and the results in studies among people with diabetes were inconclusive (5). The equivocal evidence has led to a substantial controversy about the role of chromium in human nutrition.

Epidemiological studies assessing the

Table 3—Age-adjusted and multivariate ORs and 95% CIs from the nested case-control study and pooled analysis by quartiles of toenail chromium levels: Health Professional Follow-up Study

| | Quartile of chromium levels | | | | P |
|--|-----------------------------|------------------|------------------|------------------|-------|
| | Q1* | Q2 | Q3 | Q4 | |
| Nested case-control analysis with incident CVD cases | | | | | |
| n | 67 | 42 | 50 | 43 | |
| Age adjusted | 1 | 0.62 (0.38–1.01) | 0.75 (0.47–1.19) | 0.64 (0.39–1.04) | 0.11 |
| Multivariate† | 1 | 0.75 (0.42–1.36) | 0.75 (0.42–1.33) | 0.65 (0.36–1.17) | 0.16 |
| Pooled analysis of incident and prevalent CVD cases | | | | | |
| n | 139 | 97 | 89 | 75 | |
| Age adjusted | 1 | 0.69 (0.46–1.02) | 0.64 (0.43–0.95) | 0.55 (0.36–0.82) | 0.003 |
| Multivariate | 1 | 0.82 (0.51–1.30) | 0.66 (0.41–1.05) | 0.62 (0.39–1.01) | 0.02 |

*Quartiles based on distribution of 361 healthy control subjects. Q1 is the reference category. †Adjusted for age (five categories: ≤ 50 , 51–54, 55–59, 60–64, and >65 years), BMI (three categories: ≤ 25.0 , 25.1–30.0, and >30.0 kg/m²), alcohol intake (five categories: 0.0 [non-drinkers], 0.1–5.0, 5.1–10.0, 10.1–15.0, and >15.0 g/day), smoking status (three categories: never smoked, former smoker, and current smoker), family history of myocardial infarction (binary), physical activity (tertiles of metabolic equivalent hours/week), high cholesterol (binary), hypertension (binary), quartiles of dietary score, and toenail levels of selenium and mercury.

role of chromium in CVD are limited. Rimm et al. (6) reported a nested case-control study in which higher levels of chromium in toenails were associated with lower risk of myocardial infarction in men. After controlling for potential confounders, the OR for myocardial infarction comparing the extreme quintiles was 0.69 (95% CI 0.44–1.08; P for trend = 0.01). Another case-control study in Europe by Guallar et al. (7) found a similar inverse association between chromium in toenails and the risk of first myocardial infarction; the multivariate OR for the highest quintile was 0.65 (0.42–0.99; P for trend = 0.04). Both of these studies included subjects who were apparently healthy at baseline.

Diet is the main source of chromium in humans. Absorption of chromium is poor and can be affected by other dietary components (22). In self-selected western diets, the average daily intake is lower (men 33 μg , women 25 μg) than the estimated adequate intake of 50–200 $\mu\text{g}/\text{day}$ (23). The foods high in chromium are whole grains and most fruits and vegetables, but they vary widely (24). In contrast, polished rice, fish, dairy products, and refined flour are poor sources. Chromium in the diet is affected by many factors such as source, processing, and method of preparation. Thus, data on food composition are unlikely to provide a valid measure of the chromium status. This problem in exposure assessment makes it difficult to conduct epidemiological studies with chromium. Few epidemiological studies have used toenails to evaluate the role of trace elements in chronic disease, e.g., in cancer (25,26) and cardiovascular disease (27,28).

Our study has several limitations. Contamination of samples may be a problem in the measurement of trace elements and can produce erroneously high levels of chromium in toenails. However, we washed the samples in a sonicator with deionized water before the analysis to ensure minimal contamination. Because a single measurement is prone to random errors, the effects are likely to be underestimated. The cross-sectional study is prone to the bias of “reverse causation” and chronic medical disorders such as diabetes or CVD could lower the levels of chromium in toenails, although there are no data in this area. We conducted a prospective nested case-control analysis simultaneously to examine the consistency

of our findings. The prospective design has an advantage because the toenail samples were collected before the diagnosis of CVD; hence, the levels of chromium in toenails are unlikely to be affected by CVD status. The comparison between diabetic men with CVD and healthy control subjects cannot distinguish the effects of chromium on diabetes from those on CVD. Future studies should be designed to specifically address the role of chromium in diabetes separately from its role in CVD among people with diabetes. Furthermore, it is not yet established whether toenail levels of chromium adequately reflect dietary intake.

In conclusion, our results suggest that levels of toenail chromium are lower among men with diabetes and CVD than in healthy control subjects. Whether chromium supplementation is beneficial for preventing CVD among people with diabetes needs to be determined in long-term clinical trials.

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