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# Serum zinc and risk of type 2 diabetes incidence in men: The Kuopio Ischaemic Heart Disease Risk Factor Study

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ABSTRACT

*Objectives:* Zinc may play a role in the development of type 2 diabetes (T2D), because it is involved in antioxidant and anti-inflammatory activities. However, the role of zinc in the etiology of T2D has been poorly investigated. This study was conducted to study the association of serum zinc on T2D risk in middle-aged and older Finnish men.

*Methods:* This was a 20-year prospective follow-up study on 2220 Finnish men from the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) who were 42 to 60 years old at baseline in 1984–1989. The main outcome was incident T2D. Serum zinc, body mass index (BMI), fasting blood glucose (FBG), serum insulin, C-reactive protein (CRP) and, in a subset of 751 participants, insulin-like growth factor-binding protein-1 (IGFBP-1), were measured. Also, the homeostatic model assessment (HOMA) was used to quantify insulin resistance (HOMA-IR), beta-cell function (HOMA-β) and insulin sensitivity (HOMA-IS).

*Results:* At baseline, serum zinc was associated with higher BMI, serum insulin, HOMA-IR, HOMA-β and IGFBP-1 and lower HOMA-IS. During the average follow-up of 19.3 years, 416 men developed T2D. Men in the highest quartile of serum zinc had 60% higher risk (95% CI 20–113%; *P*-trend < 0.001) for incident T2D compared with the men in the lowest quartile, after multivariate adjustments. This association was attenuated after adjustment for BMI (HR = 1.39, 95% CI 1.04–1.85; *P*-trend = 0.013) or HOMA-IS (HR = 1.38, 95% CI 1.04–1.83; *P*-trend = 0.015), whereas adjustment for the other factors had only modest impact on the association.

*Conclusion:* Higher serum zinc was associated with higher risk of T2D; effects of zinc on BMI and insulin sensitivity may partly explain the association. Further prospective studies are warranted to confirm our results and explore potential mechanisms.



## 1. Introduction

Type 2 diabetes (T2D) is characterized by hyperglycemia because of insulin resistance or relative lack of insulin in advanced patients [1]. During the last decades a number of studies have been conducted on T2D, and there has been significant improvement in the prevention and treatment of this disease. Nevertheless, T2D remains a major cause of morbidity and mortality in both developed and developing countries [2]. Assessment and controlling of the risk factors of T2D need to be addressed in order to prevent or decrease mortality and co-morbidity associated with T2D. Advancing age, physical inactivity, poor dietary habits and other factors increase the incidence of T2D [3].

T2D patients have been found to have lower levels of blood zinc compared to healthy controls [4], and this may have an adverse effect on the performance of zinc-dependent hormones and enzymes in body [5,6] and possibly will result in unwanted complications among T2D patients. For example, it has been shown that higher levels of insulin-like growth factor-binding protein-1 (IGFBP-1) are associated with lower incidence of T2D or its risk factors [7]. Insulin plays an important role in the management of IGFBP-1 [8], and zinc is involved in the secretion of insulin [9]. In addition, inflammatory and oxidative stress markers are associated with higher risk of T2D [10], and zinc has been shown to have antioxidative and anti-inflammatory activities [11]. However, cross-sectional studies have rather shown a direct association between higher levels of serum zinc and the metabolic syndrome

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[12,13]. No prospective studies have been conducted on the association between serum zinc and T2D incidence; therefore, we investigated the relationship between serum zinc and risk of incident T2D in a long-term prospective cohort study in middle-aged and older Finnish men.

## 2. Subjects and methods

### 2.1. Study design

The Kuopio Ischemic Heart Disease Risk Factor (KIHD) study was designed to investigate risk factors for cardiovascular disease, atherosclerosis, and related outcomes in a population-based, randomly selected sample of men from Eastern Finland [14]. The baseline examinations were carried out from 1984 to 1989. A total of 2682 men who were 42, 48, 54, or 60 years old at baseline (82.9% of those eligible) were recruited in two cohorts. The first cohort consisted of 1166 men who were 54 years old, enrolled from 1984 to 1986, and the second cohort included 1516 men who were 42, 48, 54, or 60 years old, enrolled from 1986 to 1989. The baseline examinations were followed by the 4-year examination round (1991–1993) in which 1038 men from the second cohort (88% of those eligible) participated. At the 11-year examination round (1998–2001), all men from the second cohort were invited, and 854 men (95% of the eligible) participated. During the 20-year examination round, all eligible participants from the first and second cohorts were invited to the study site. A total of 1241 men (80% of the eligible) participated. The baseline characteristics of the entire study population have been described [14]. The KIHD study protocol was approved by the Research Ethics Committee of the University of Kuopio. All subjects gave written informed consent for participation. Subjects with T2D ($n = 167$), impaired fasting glucose ($n = 127$) or unknown diabetes status ($n = 38$) at baseline, or with missing data on serum zinc ($n = 105$), or who had the serum CRP >10 mg/L ($n = 46$), were excluded, leaving 2220 men. The data for serum IGFBP-1 was available for 751 men.

### 2.2. Measurements

Fasting venous blood were collected between 8:00 and 10:00 a.m. at the baseline examinations. Subjects were instructed to abstain from ingesting alcohol for 3 days and from smoking and eating for 12 h prior to giving the sample. Detailed descriptions of the determination of serum lipids and lipoproteins [15], assessment of medical history and medications [15], family history of diseases [15], smoking [15], alcohol consumption [15], blood pressure [15], and physical activity [16] have been earlier published. Plasma glucose was measured using a glucose dehydrogenase method after precipitation of proteins by trichloroacetic acid. The serum samples for insulin determination were stored frozen at −80 °C. Serum insulin was determined with a Novo Biolabs radioimmunoassay kit (Novo Nordisk, Bagsvaerd, Denmark). Insulin resistance and sensitivity and beta cell function were estimated by HOMA computer algorithm [17].

Serum zinc concentrations were determined from frozen samples stored at −20°C for 1–5 years prior to analyses. The PerkinElmer 306 atomic absorption spectrophotometer (Norwalk, Connecticut, USA) was used for measurements. Seronorm (Nycomed, Oslo, Norway) control serum samples were included in all daily batches [15,18]. IGFBP-1 concentration was determined by immunofluorometric assay using two monoclonal antibodies, F34-15C9 and F36-9G3 as previously described [19]. Serum CRP was measured with an immunometric assay (Immulite High Sensitivity CRP Assay, DPC, Los Angeles, Calif., USA). Education was assessed in years by using a self-administered questionnaire. The family history of diabetes was defined as positive if a first-degree relative of the subject had diabetes history.

### 2.3. Diagnostic criteria for type 2 diabetes

T2D was defined as a self-reported physician-set diagnosis of T2D and/or fasting plasma glucose ≥7.0 mmol/L or 2-h oral glucose tolerance test plasma glucose ≥11.1 mmol/L at re-examination rounds 4, 11, and 20 years after the baseline and by record linkage to the national hospital discharge registry and to the Social Insurance Institution of Finland register for reimbursement of medicine expenses used for T2D for the entire study period until the end of the follow-up. Impaired fasting glucose at baseline was defined using World Health Organization criteria, fasting plasma glucose 6.1–6.9 mmol/L.

### 2.4. Statistical analysis

ANOVA (for continuous variables) and Chi-square (for categorical variables) tests were used to examine the univariate associations between serum zinc and baseline characteristics. Analysis of covariance (ANCOVA) was used to examine the associations between serum zinc and BMI and markers of glucose homeostasis and inflammation at baseline. Cox proportional hazards regression models were used to estimate hazard ratios of incidence of T2D in quartiles of serum zinc. The model 1 included age (years) and examination year. The multivariable model 2 was further adjusted for potential confounders, family history of T2D (yes/no), smoking (never smoker, previous smoker, current smoker <20 cigarettes/day, and current smoker ≥20 cigarettes/day), education (years), leisure-time physical activity (kcal/day), intake of alcohol (g/week), and total energy intake (kcal/day). Cohort means were used to replace missing values in covariates (<0.5%). All P values were two-tailed ($\alpha = 0.05$). Data were analyzed using SPSS 21.0 for Windows (Armonk, NY: IBM Crop.).

## 3. Results

Table 1 shows the baseline characteristics of the study participants based on the quartiles of serum zinc. Men with higher serum zinc were younger, smoked less, had a higher intake of fruits, berries and vegetables and fiber and had lower alcohol consumption. Higher serum zinc concentration was also associated with higher education.

Table 2 presents the association of serum zinc with BMI and markers of glucose metabolism and inflammation at baseline. Serum zinc was not associated with plasma glucose concentration (Table 2). However, serum zinc was associated with higher serum insulin, HOMA-IR and HOMA-β, and lower HOMA-IS, even after multivariate adjustments (Table 2). Serum zinc was also associated with higher BMI (Table 2) and lower CRP after adjusting for age and examination year (model 1, Table 2). However, the association with CRP was weaker and statistically nonsignificant after further adjustment for confounders (model 2, Table 2). In the subset of participants ($n = 751$) higher serum zinc was also associated with lower IGFBP-1 concentration (Table 2). During the average follow-up of 19.3 years (SD 6.5 years), 416 men (18.7%) developed T2D (42,846 person-years). After adjustment for age and examination year (model 1), serum zinc was associated with 52% higher risk of incident T2D (HR 1.52; 95% CI 1.15–2.01, $P$-trend < 0.001). This association remained after further adjustment for potential confounders (HR 1.60; 95% CI 1.20–2.13, $P$-trend < 0.001). When the potential effect mediators from Table 2 were added to the Model 2 one by one, addition of BMI or HOMA-IS attenuated the association between zinc and risk of T2D for >10%, whereas the addition of the other factors had only modest impact on the association (Table 3).