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Association between direct measurement of active serum calcium and risk of type 2 diabetes mellitus: A prospective study

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Abstract *Background and aims:* Previous prospective studies showing a positive association between serum calcium and incidence of type 2 diabetes mellitus (T2DM) have relied on total calcium or an indirect estimate of active, ionized calcium (iCa). We aimed to assess this relationship using a direct measurement of iCa.
Methods and results: iCa and cardiometabolic risk factors were measured in a population-based sample of 2350 men without a known history of T2DM at baseline. Associations between iCa levels and incident cases of T2DM (self-reported, ascertained with a glucose tolerance test, or determined by record linkage to national registers) were estimated using Cox regression analyses adjusted for potential confounders. At baseline, mean (standard deviation) age was 53 (5) years and mean iCa 1.18 (0.05) mmol/L. During a median follow-up of 23.1 years, 140 new cases of T2DM were recorded. In a multivariable analysis adjusted for age, body mass index, systolic blood pressure, serum HDL-cholesterol, and family history of T2DM, there was no association comparing second (hazard ratio 0.84; 95% confidence interval 0.59–1.18), third (0.77; 0.52–1.14), or fourth (0.98; 0.69–1.39) vs first quartile of iCa (p for trend 0.538); further adjustment for C-reactive protein, physical activity level, and triglycerides did not change the estimates (p for trend 0.389).
Conclusion: In this study, we did not find evidence of an association between direct measurement of active calcium and risk of T2DM. Further studies are needed to confirm our findings and define the relationship between factors influencing indirect calcium estimation and incident T2DM.

Abbreviations: T2DM, type 2 diabetes mellitus; iCa, Ionized calcium; BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; FPG, fasting plasma glucose; CRP, C-reactive protein; SD, standard deviation; HR, hazard ratio; Loge, natural logarithm; CI, confidence interval; SBP, systolic blood pressure; PTH, parathyroid hormone.

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Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder whose major clinical feature is hyperglycemia [1]. Numerous recent studies have highlighted the complexity of its pathophysiology, including abnormal gut/β-cell cross-talk, insufficient insulin secretion, and a reduced insulin action in peripheral tissues [2].

Calcium plays an active role in both insulin secretion and its peripheral effects. Glucose-dependent insulin exocytosis is a calcium-regulated process, being dependent upon β-cell intracellular calcium concentration [3]. Calcium is an established regulator of GLUT4 expression, a passive transporter essential for peripheral glucose uptake [4]. As a consequence, abnormalities in calcium homeostasis could potentially affect glucose metabolism and contribute to the development of T2DM [5].

A possible association between calcium and T2DM risk has been explored in nutritional epidemiology studies: the available evidence, though, is limited because most studies are cross-sectional and did not adjust for important confounders [6]. Similarly, cross-sectional and case-control studies investigating the relationship between calcium homeostasis and glucose metabolism have reported conflicting results [7–15]; however, only a few studies have prospectively evaluated the association between serum calcium and risk of incident T2DM, suggesting that higher levels of serum calcium predict an increased risk of T2DM [16–18]. These studies have relied either on total serum calcium or the indirect, albumin-corrected estimate of serum calcium: these measurements, however, only approximate the levels biological-active serum calcium (iCa) [19]. Interestingly, a previous case-control study did not find an association between directly measured iCa and prevalent diabetes [11]; similarly, in another small study, no association was found between direct iCa measurement and indices of diabetes control [15].

To our knowledge, the relationship between directly measured iCa and the risk of incident T2DM has not been investigated in a prospective study setting. Thus, our aim was to evaluate the risk of T2DM in a population-based study using direct measurement of iCa levels.

Methods

This study was performed following the STROBE guidelines for observational studies [20].

Study population

The Kuopio Ischaemic Heart Disease risk factor study was designed to investigate risk predictors for atherosclerotic cardiovascular outcomes in a population-based sample of men from Eastern Finland. The subjects were a randomly selected sample of men 42–60 years of age resident in the town of Kuopio and its surrounding rural communities obtained from the national population register [21]; baseline examinations were conducted between March 1984 and December 1989.

Of the 3433 invited, 2682 (78.1%) participated. In this study, participants were excluded if they were diagnosed with diabetes at baseline (n = 162), defined as either having regular treatment with an oral hypoglycemic agent, insulin therapy, or having treatment only with diet while also having a fasting plasma glucose level of at least 7.0 mmol/L. We further excluded those subjects with missing baseline information on calcium (n = 170). Therefore, data on 2350 participants were available for the analyses. Incident case of T2DM was defined as a self-reported physician-set diagnosis 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 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.

Assessment of risk factors

Prior to attendance at the baseline appointment, participants were instructed to abstain from drinking alcohol for a minimum of 3 days and from smoking for at least 12 h. Fasting blood samples were taken following a 30-min rest period in the supine position and collected using vacuum tubes (Terumo Venoject; Terumo, Tokyo, Japan).

The resting blood pressure was measured with a random-zero sphygmomanometer (Hawksley, Lancing England) by two trained nurses. A total of six measurements (3 supine, 1 standing, and 2 sitting) were taken following a 5 min supine rest and blood pressure was taken as the mean of all six measurements. Baseline medical history, smoking habits, years of education, family history of T2DM (defined as positive if a first-degree relative of the subject had T2DM history), and drug therapy were assessed by self-administered questionnaires. The diagnosis of chronic diseases was checked during a medical examination by the internist. Detailed descriptions on physical activity estimation have been published previously [22]. Body mass index (BMI) was computed as the ratio of weight in kilograms to the square of height in meters.

The measurement of serum active calcium concentrations [23] was made using ion selective electrodes (Microlyte 6, Kone, Finland; CV, 1.6%; reference interval, 1.16–1.32 mmol/L). Serum insulin level was determined using a radioimmunoassay kit (Novo Biolabs; Novo Nordisk, Bagsvaerd, Denmark). The serum samples were stored frozen at −80 °C for 0.2–2.5 years. The values obtained were immunoreactive insulin as the assay has cross reactivity with proinsulin. A glucose dehydrogenase method (Merck, Darmstadt, Germany) was used to assess blood glucose after precipitation of proteins by trichloroacetic acid. Insulin resistance was estimated as follows: HOMA-IR = fasting plasma insulin (μU/mL) × fasting plasma glucose (FPG) (mmol/L)/22.5. The cholesterol contents of lipoprotein fractions and serum triglycerides were measured enzymatically (Boehringer Mannheim, Mannheim Germany). High-density lipoprotein (HDL) was