Journal of Trace Elements in Medicine and Biology 29 (2015) 145–150

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.de/jtemb

Epidemiology

Toenail selenium and risk of type 2 diabetes: the ORDET cohort study

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ARTICLE INFO

Article history:
Received 3 June 2014
Accepted 21 July 2014

Keywords:
Cohort study
Diabetes
Risk assessment
Selenium
Toenails

ABSTRACT

Epidemiologic studies, particularly randomized controlled trials, have shown a direct relation between dietary and environmental exposure to the metalloid selenium and risk of type 2 diabetes. We investigated the association between baseline toenail selenium levels and diabetes occurrence in a case–control study nested in ORDET, a population-based female cohort in Northern Italy. After a median follow-up of 16 years, we identified 226 cases of type 2 diabetes cases and 395 age-matched control women with available toenail samples at baseline. The multivariate odds ratios of diabetes in increasing a priori defined categories of toenail selenium exposure were 1.09 (95% confidence interval 0.61, 1.96), 0.71 (0.38, 1.34) and 1.14 (0.46, 2.80) compared with the lowest category. The results were not substantially altered when quartile distribution of toenail selenium in controls was used to define exposure categories. Spline regression analysis did not show homogeneous risk trends. Overall, we did not find an association between toenail selenium and subsequent development of diabetes. Since the diabetogenic activity of selenium is strongly supported by experimental studies and some observational investigations, our null results might be explained by the limitations of overall selenium toenail content to assess environmental exposure to selenium species of etiologic relevance in the study population.

Introduction

The metalloid selenium has been linked to various human chronic diseases such as cancer and cardiovascular disease, under the hypothesis that, in line with its Janus-faced nature, it may have both adverse and a beneficial health effects [1–3]. These potential relations have been investigated by a number of epidemiologic and laboratory studies, the evidence of which was only recently brought together under a more homogeneous and defined pattern, in some cases raising concern about its potentially harmful effects [3–8].

Diabetes in particular is among those diseases the risk of which has been suspected of being influenced by abnormal exposure to selenium, based not only on anecdotal reports and 'old' case–control investigations [9,10], but on consistent and systematic reporting of an excess risk in trials using selenium as a method of intervention [11–15], an observation that has caused considerable concern and also contributed to the interruption of the largest of these trials, 'SELECT' [1].

We therefore decided to investigate the relation between selenium and diabetes within the a cohort established in 1987 in Varese, Northern Italy, for the investigation of the role of dietary factors in the etiology of breast cancer in females. Our aim was to assess the potential relation between selenium exposure and diabetes risk in this population, which was characterized, as are Italian populations more generally, by lower ranges of selenium intake than other Western populations and, on the basis of a few studies, was expected to have a very limited consumption of dietary supplements [16,17]. An initial analysis based on dietary selenium confirmed a direct association between selenium intake at baseline and subsequent development of diabetes, even at unexpectedly low levels [18]. In this study, we assessed this association using overall toenail selenium content, a frequently used peripheral biomarker of exposure [19,20].

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http://dx.doi.org/10.1016/j.jtemb.2014.07.017
0946-672X/

Materials and methods

Study population

ORDET (Hormones and Diet in the Etiology of Breast Cancer) is a prospective cohort study of 10,786 healthy women residing in the Province of Varese, in northern Italy, recruited between June 1987 and June 1992, the methodology of which has been described in detail elsewhere [21]. Briefly, the women, aged between 35 and 70, were volunteers from the general population. Women with a history of cancer, bilateral ovariectomy, chronic or acute liver disease or who had received hormone therapy in the 3 months before recruitment were excluded. After the exclusion of women who were lost immediately after baseline, 10,633 participants remained, who were contacted by phone during the follow-up. In this study we also excluded women who reported type 2 diabetes already diagnosed at the baseline assessment ($N = 203$), as well as women who died ($N = 336$), could not be contacted during the follow up for other reasons. On recruitment, all women donated a blood sample and most of them (95%) also provided toenails clippings. The study was approved by the Ethical Review Board of the Italian National Cancer Institute based in Milan.

Each participant completed a lifestyle questionnaire providing information on their reproductive history, education, alcohol and coffee consumption, smoking habits and personal medical history. Weight and height were measured by trained nurses according to a standardized protocol; body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Diet over the previous year was assessed using a semi-quantitative food frequency questionnaire specifically designed to determine local dietary habits in the population of Northern Italy [22].

We actively collected follow-up information for diagnosis of type 2 diabetes to cohort members by conducting telephone interviews in 2007, using criteria complying with the protocol of the InterAct Study, an EU-funded large scale collaboration between nine European Countries and India designed to investigate how genes and lifestyle may interact to lead to diabetes [23]. We considered as affected by diabetes those women who both self-reported a medical diagnosis of type 2 diabetes and/or therapy with antidiabetic drugs at interview, and were included in the Lombardia Region databases of hospital discharges for diabetes and/or of drug prescriptions for hypoglycemic drugs [18]. For each case with available toenail clippings and whenever possible, we randomly drew two controls matched by age (±3 years) from the cohort members also having available toenail clippings.

Exposure assessment

We determined selenium concentrations in toenails though instrumental neutron activation analysis (INAA) carried out at the Helmholtz-Centre for Materials and Energy in Berlin, to which we shipped toenails samples we had previously stored in special non-reactive plastic envelopes at room temperature. All the tools that came into contact with the samples had been pre-washed for 20 min in 5% nitric acid (high-purity water and 65% nitric acid, Merck suprapur) and in high-purity water using an ultrasonic bath. The complete sample preparation was carried out in a laminar flow hood and talc-free gloves were worn.

The original toenail samples were split into approximately 5 mm long clippings using a pair of titan tweezers. We subsequently washed the nail first in 10 mL high-purity water, 10 mL acetone (Sigma–Aldrich p.a.), 1% Triton X100 (Sigma), and then three times in 25 mL high-purity water and 10 mL acetone (Sigma–Aldrich p.a.) using an ultrasonic bath for 10 min, choosing not to add nitric acid in this procedure to avoid any dissolution of the sample [24]. After each cleaning step we dried the clippings overnight at room temperature in a desiccator and continued the last drying step until the sample mass was constant. We stored the samples in a desiccator before packaging them and then transferred them into pre-washed Suprasil quartz ampoules. Sample masses were determined via a Sartorius model MC210P microbalance (precision 0.01 mg), and ranged from 10 to 30 mg.

We performed INAA analyses using the multi-elemental Standards M183a (Merck, charge number 10090c183a) and 1472 (Spex XGLEN-1472, Lot number: 2-222JB) for method of comparison and the Bovine Liver (SRM 1577b) standard reference material for quality control. The 71 determinations carried out with SRM 1577b (Bovine Liver) yielded a mean value of 0.71 mg/kg (standard deviation 0.03), while the certified value was 0.73 mg/kg (standard deviation 0.06).

We sealed the quartz ampoules by means of an automatic quartz fusing unit and then irradiated the samples and standards for 3–6 days at the Research Reactor (BER II) of the Berlin Helmholtz-Centre. Irradiation took place inside the DBVK irradiation device with a thermal neutron flux of about 1.2×10^{14} cm^{-2} s^{-1}. Before gamma-ray measurement after a decay time of approximately one month, the quartz ampoules (standards and samples) were subsequently washed in HF, triply water, ethanol and acetone. HPGe (high purity germanium) detectors were used for taking measurements for up to 12 h.

In one study subject (a control) we identified an anomalous selenium value of 1.18 μg g^{-1}, which we considered as an outlier and thus removed for further analysis.

Data analysis

We used multivariate conditional logistic regression to compute the odds ratios (OR) with related 95% confidence intervals (CIs) for developing type 2 diabetes based on toenail selenium levels. Two different analyses were carried out in this study: first, toenail selenium was categorized in four categories using a priori cutpoints (0.50, 0.60, and 0.70 μg g^{-1}), we then repeated the analysis by categorizing exposure status according to quartile distribution in the controls, using the bottom category as the reference category. For both analyses we provide a crude model, a reduced model adjusted for BMI as a linear term and a fully adjusted model further adjusted for education (<8 years, 8–13 years, >13 years), smoking status (never, former, current), alcohol intake (servings/day) and coffee intake (cups/day). We performed trend analysis by entering continuous toenail selenium levels in the regression model, and computed the Spearman's correlation coefficient to analyze the association between toenail selenium and dietary selenium in subjects for whom both data were available. We used the statistical package STATA (version 13.1, Stata Corp., College Station, TX 2014) for data analysis. We eventually modeled the relation between the odds of being a case and toenail selenium level with a restricted cubic smoothing spline with knots at 10th, 25th, 50th, 75th and 90th percentile, using the Stata 13.1 'xblc' command after computing an unconditional no-intercept logistic regression model [25].

Results

The characteristics of the 226 women who developed type 2 diabetes during the follow-up and of the 395 age-matched controls are shown in Table 1. Distribution of toenail selenium ranged from 0.34 to 0.93 μg g^{-1} (after removing the single outlier of 1.18 μg g^{-1}). Toenail and dietary selenium were uncorrelated, since in the overall population the Spearman coefficient between these two variables was 0.02 (95% CI −0.07, 0.10; $P = 0.721$), and the results were substantially confirmed after restricting the analysis to cases and controls (Table 2). Toenail selenium was correlated with body mass