Trace Elements in Early Phase Type 2 Diabetes Mellitus – a Population-based Study. The HUNT Study in Norway

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Competing financial interests

The authors declare they have no actual or potential competing financial interests.

Abstract

Differences in trace elements levels between individuals with type 2 diabetes and controls have been reported in several studies in various body fluids and tissues, but results have been inconsistent. In order to examine trace element levels in the early phase of type 2 diabetes, we investigated the association between whole blood levels of 26 trace elements and the prevalence of previously undiagnosed, screening-detected type 2 diabetes. The study was conducted as a case-control study nested within the third survey of the population-based Nord-Trøndelag Health Study (HUNT3 Survey). Among participants without previously known diabetes, 128 cases of type 2 diabetes were diagnosed in people with a high diabetes risk score (FINDRISC ≥ 15), and frequency-matched for age and sex with 755 controls. Blood samples were analyzed by high resolution inductively coupled plasma mass spectrometry. Associations between trace element levels and the prevalence of previously undiagnosed type 2 diabetes were evaluated with multivariable conditional logistic regression controlling for age, sex, body mass index, waist-to-hip ratio, education, income, smoking and family history of diabetes. The prevalence of previously undiagnosed type 2 diabetes increased across tertiles/quartiles for cadmium, chromium, iron, nickel, silver and zinc, and decreased with increasing quartiles of bromine ($P_{trend} < 0.05$). After corrections for multiple testing, associations for chromium remained significant ($Q_{trend} < 0.05$), while associations for iron and silver were borderline significant. No associations were found for arsenic, boron, calcium, cesium, copper, gallium, gold, indium, lead, magnesium, manganese, mercury, molybdenum, rubidium, selenium, strontium, tantalum, thallium and tin. Our results suggest a possible role of bromine, cadmium, chromium, iron, nickel, silver and zinc in the development of type 2 diabetes.

Keywords: type 2 diabetes; case-control study; HUNT3; trace elements; HR-ICP-MS; whole blood.

Introduction

Several trace elements have been implicated in the etiology of type 2 diabetes, and their potential roles have been discussed for decades [1]. Trace elements may influence onset or pathogenesis of diabetes in various ways. Early imbalances of specific trace elements may disturb normal glucose and insulin metabolism, or could cause increased oxidative stress that may contribute to insulin resistance and development of diabetes complications [2, 3].

Previous research on the potential roles of trace elements in diabetes has focused particularly on chromium, zinc and iron. Chromium has been shown to modulate insulin response in several ways, including increased binding of insulin to cells, increased number of insulin receptors and insulin receptor kinase activation [4]. Zinc is also an integrated part of insulin and is closely involved in the synthesis, storage and secretion of insulin. Zinc is required as a cofactor for many of the enzymes involved in glucose metabolism, and is an integral component of several antioxidant enzymes [5, 6]. Iron has been proposed to influence the development of diabetes through several mechanisms, notably induction of insulin deficiency and insulin resistance and causing hepatic dysfunction [7]. There is also substantial evidence for the involvement of toxic elements in diabetes, especially arsenic and cadmium [8-10].

Most studies have focused on the association between one single trace element and type 2 diabetes and have been limited to persons with an already established diagnosis. Whether these alterations in trace element status take place prior to disease onset or are a result of the disease or its treatment is still an open question. We therefore sought to investigate trace elements levels at the time of screening-detection of type 2 diabetes in order to probe changes in trace element levels in the early phase of the disease, adjusting for potential confounders often not assessed in previous studies.

Methods

Study population

The population-based Nord-Trøndelag Health Study (The HUNT Study) is one of the largest health studies in Europe. The population in Nord-Trøndelag County is considered to be relatively homogenous with less than 3% non-Caucasians, and to be fairly representative for Norway with regards to geographical, demographic and employment structures [11]. A detailed description of the HUNT Surveys has been given by Krokstad et al. [12]. The last HUNT Survey, HUNT3, was conducted between October 2006 and June 2008. All 93,210 residents in Nord-Trøndelag County ≥ 20 years of age were invited, and the participation rate was 54.5%. Information was obtained through questionnaires, interviews, clinical examinations and collection of blood and urine samples.

The flow chart for selection of cases is shown in figure 1. Whole blood samples were collected in vacutainer tubes designed for trace element analysis (Becton, Dickinson & Co, Cat. no. 367735, Franklin Lakes, NJ) for 26,358 (51.9%) of the 50,807 participants of HUNT3. The samples were collected from the residents of 14 urban and rural municipalities ranging from the coast to the mountain area, out of a total of 24 municipalities in Nord-Trøndelag County. In order to minimize possible contamination of trace elements originating from the syringe, the samples for trace element analysis were collected in the last of a series of five tubes. A leakage test was performed on the vacutainers prior to sampling, and elements shown to contaminate the samples were excluded from the study.

As part of a European multi-center study, Diabetes in Europe – prevention through Lifestyle, Physical Activity and Nutrition (DE-PLAN), participants in the HUNT cohort who had a high (at least 30% in the next ten years) risk for developing diabetes according to the FINnish Diabetes RIsk SCore (FINDRISC ≥ 15), were invited to participate in a diabetes prevention

study. Among the 2,513 participants identified as being at high risk of type 2 diabetes and also sampled for trace element analysis, 1,172 individuals (46.6%) underwent an oral glucose tolerance test (OGTT). In 157 of these individuals (6.2%) results indicated a previously unknown diagnosis of diabetes, using the following criteria for diabetes; fasting serum glucose concentration ≥ 7.0 mmol/L and/or 2-hour glucose concentration ≥ 11.1 mmol/L.

Anti-glutamic acid decarboxylase (anti-GAD), anti-islet antigen 2 (anti-IA2) and fasting C-peptide were analyzed in serum samples at the Hormone Laboratory of Aker University Hospital (Oslo, Norway) as previously described [13]. Anti-GAD measurements were used for classification of diabetes and those who had anti-GAD < 0.08 u/L (43 WHO units/mL) were classified as having type 2 diabetes, in accordance with the Diabetes AutoAntibody Standardization Program (DASP) [14]. Among the 157 individuals, we excluded one individual with GAD ≥ 0.08 u/L and 22 individuals whose GAD levels had not been analyzed. None of the cases had C-peptide levels < 150 pmol/L and for the 119 cases with measurements for anti-IA2, none had anti-IA2 levels ≥ 0.11 u/L (reference value < 0.11 ai). Among the 134 remaining individuals, 128 had provided blood samples and relevant information through questionnaires in HUNT3 and were selected as cases for this study (Figure 1).

Non-diabetic controls were selected from the general population of Nord-Trøndelag County participating in the HUNT3 Survey. Individuals reported being pregnant, or were diagnosed with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) or had established diagnosis of diabetes (both type 1 and 2) were excluded as potential controls. All controls had non-fasting serum glucose < 9.0 mmol/L. Cases and controls where frequency-matched with respect to age (in 5-year intervals) and sex. In total 755 individuals met the selected inclusion criteria for controls and had provided relevant information in the HUNT3 questionnaires.

Trace element analysis

The sample preparation was performed in a clean laboratory (ISO 6) to minimize contamination from the surroundings, and careful attention was paid in all steps of the analysis in order to minimize potential contamination.

Whole blood (approximately 0.7 mL) from each of the study participants was transferred to metal-free 18 mL teflon tubes. The exact weight of each sample was measured and converted back to volume by multiplying with 1.06 g/mL (the average density of whole blood). Ultrapure HNO_3 (conc., 1 mL) was added to each sample. Each run consisted of 80 samples divided into two sample carousels with four blanks, two samples from a healthy volunteer (as an internal control) and one sample of certified reference whole blood (Seronorm Level 1, Sero, Norway) and 73 samples. Blanks and control samples had an alternating position in each run.

The samples were then digested using a high performance microwave reactor (UltraClave, Milestone, Germany). After digestion, samples were decanted into 15 mL tubes (VWR, USA) suitable for HR-ICP-MS analysis and diluted with ultrapure water (approximately 13.5 mL) to achieve a final HNO_3 concentration of approximately 0.6 M.

Trace element concentrations were measured using a HR-ICP-MS instrument (Thermo Finnigan model Element 2, Bremen, Germany), the best multi-element method for determination of trace element levels commercially available due to low detection limits and high sample throughput. The radio frequency power was set to 1350 W. The samples were introduced using a prepFAST sample injection system. The instrument was equipped with a concentric PFA-ST nebulizer, coupled to a quartz cyclonic micro mist spray chamber, aluminium skimmer and sample cones and a demountable quartz torch with a guard electrode. The instrument was calibrated using a solution of a multielement standard from

ESI, matrix matched with regard to acid strength (0.6 M), sodium (160 mg/L) and potassium (115 mg/L). Calibration curves were made using four different concentrations of a multielement standard to cover the concentration ranges necessary for the analysis. Corrections for instrumental drift were done by repeated measurements of one of the multielement standards. The stability of the instrument was checked by inspection of the argon signal and measurements of 1 μg/L rhenium added as an internal standard through the prepFAST system. In order to minimize the influence of analytical interferences, trace elements were determined employing different resolution levels; low resolution: cadmium, cesium, gold, indium, lead, mercury, tantalum, thallium and tin; medium resolution: boron, calcium, chromium, copper, gadolinium, iron, magnesium, manganese, molybdenum, nickel, rubidium, silver, strontium and zinc; high resolution: arsenic, bromine and selenium.

The accuracy of the trace element determinations was evaluated by analysis of the certified reference material Seronorm Level 1 (Sero, Norway). In addition a sample from a healthy volunteer was repeatedly analyzed to evaluate the precision of the method over time. Contamination from pipet tips, flasks and the ultrapure acid were checked prior to analysis and found to be negligible.

Statistical analysis

Trace element concentrations lower than the limit of detection (LOD, set equal to 3 times the standard deviation of blank samples) were replaced with a value equal to LOD/2. Trace elements where more than 33% of the study population had levels lower than LOD were not included in this study. Trace elements known to be associated with contamination e.g. from the sample tubes were also excluded. This left 26 trace elements to be investigated. Trace element concentrations were analyzed as quartile categories (defined by the distribution among the controls), except for chromium and tantalum, where 29 and 26% of the samples

had values lower than the detection limit and were analyzed as tertile categories. For each trace element, we used conditional logistic regression analysis to estimate odds ratios (OR) of diabetes in each quartile/tertile of the trace element distribution, using the lowest quartile/tertile as the reference category. Tests for trend across categories were used to assess any relation of increasing trace element levels with the odds of being newly diagnosed with type 2 diabetes. P_{trend} values were corrected for multiple testing using the Benjamini-Hochberg procedure. The initial models were adjusted for sex and age (known to be related to diabetes and levels of some trace elements). Second, the model (hereafter termed the multivariable model), was further adjusted for education, body mass index (BMI), waist-to-hip ratio, self-reported family history of diabetes (defined as diabetes among siblings, children or parents) and daily smoking.

The participants in HUNT3 answered a questionnaire which included information on sex, age, smoking, family history of diabetes, seafood intake and alcohol consumption and intake of various foods. Smoking and family history of diabetes was treated as binary variables, i.e. daily smoking (yes/no), and mother/father or siblings with diabetes (yes/no).

BMI (measured weight in kilograms divided by measured height in meters squared) was treated as a categorical variable with three categories based on WHO's BMI classification: underweight and normal weight (< 25 kg/m^2), overweight (25-30 kg/m^2) and obese (≥ 30 kg/m^2). Waist-to-hip ratio was included in our model as a measure of body fat distribution, and treated as a categorical variable with three categories based on the tertile distribution among the controls: ≤ 0.88, > 0.88 - < 0.94 and ≥ 0.94.

Data for income and education were provided by Statistics Norway. Specifically, we used the after-tax income per consumption unit (IES), which was treated as a categorical variable with four categories based on the control population distribution of income. The variable for

education was also treated as a categorical variable with three categories (primary (< 10 years), secondary (10-12 years) and tertiary (≥ 13 years).

The statistical analyses were performed with Stata 13 (StataCorp, USA), and corrections for multiple testing were performed using R 3.2.2 (Foundation for Statistical Computing, Vienna, Austria).

Ethical approvals

The study was approved by the Regional Committee for Medical and Health Research Ethics, Region Central (reference no. 2010/2947) and by the Norwegian Data Protection Authority. All participants signed an informed consent for participation and use of data and blood samples for research purposes.

Results

Table 1 shows key characteristics for the 128 cases and 755 controls included in the study. Table 2 shows the median values and 10th and 90th percentiles for trace element concentrations for controls and cases for the 26 investigated trace elements.

Table 3 shows the results from the conditional logistic regression analysis for each of the 26 investigated trace elements. We found a significantly ($P_{trend} < 0.05$) increasing prevalence of diabetes across tertiles/quartiles for cadmium, chromium, iron, nickel, silver and zinc and a decreasing prevalence across quartiles for bromine after adjustment for the aforementioned confounders (Figure 2). Additionally, adjusted for age and sex only, gold was positively associated with the prevalence of type 2 diabetes, however the association was borderline insignificant after further adjustment. Seven trace elements showed a $P_{trend} < 0.05$ (Figure 2). The multivariable adjusted odds ratios (OR) comparing the highest tertile/quartile to the lowest tertile/quartile were 0.52 (95% CI: 0.27-0.44) for bromine, 2.78 (95% CI: 1.55-4.99)

for chromium, 2.97 (95% CI: 1.34-6.60) for iron, 2.24 (95% CI: 1.18-4.26) for nickel, 2.32 (95% CI: 1.20-4.48) for silver and 2.19 (1.05-4.59) for zinc. Although the multivariable adjusted OR comparing the highest quartile to the lowest quartile was 1.99 (95% CI: 0.92-4.28) for cadmium, an increasing prevalence of diabetes across quartiles was detected ($P_{trend} < 0.05$).

No associations were found for arsenic, boron, cesium, copper, gallium, indium, lead, magnesium, manganese, mercury, molybdenum, rubidium, selenium, strontium, tantalum, tin and thallium (all $P_{trend} > 0.05$).

The results were corrected for multiple testing using the Benjamini-Hochberg procedure, and for bromine, chromium, iron, nickel and zinc the associations remained significant adjusted for age and sex. Adjusted for additional confounders, only chromium remained significant after correction for multiple testing, while the associations for iron and silver showed borderline significance (Table 3).

We further adjusted our results for other possible confounding factors, including alcohol consumption, physical activity and the use of vitamin and mineral supplements. For arsenic and mercury, we also adjusted for seafood intake. In addition, adjustments were performed including highly correlated trace elements in the model. Specifically, if two trace elements had a Pearson correlation coefficient higher than 0.5 ($|r| > 0.5$), correlated trace elements were included individually, one at a time, in the multivariable model. However, adjustment for these factors did not substantially change the estimates, and they were not included in our final models.

Discussion

The main objective of this study was to investigate the association between levels of selected trace elements and the prevalence of previously undiagnosed, screening-detected type 2

diabetes in order to probe disturbances of trace element status before diagnosis. In summary, we found significant positive associations between levels of cadmium, chromium, iron, nickel, silver and zinc and type 2 diabetes, and a negative association between levels of bromine and type 2 diabetes. Corrected for multiple testing, chromium remained significant, while iron and silver were borderline significant.

We found a significant trend across quartiles between increasing levels of cadmium and the prevalence of previously undiagnosed type 2 diabetes. This is in line with previous studies reporting higher cadmium levels in urine among persons with diabetes [15, 16]. A recent study from Sweden did not find any significant association between cadmium levels in blood and the incidence of type 2 diabetes [17]. However, they reported slightly lower levels of cadmium (medians 0.24 and 0.27 μg/L for men and women in their cohort) compared to our study (median 0.35 μg/L in controls). These cadmium levels are all quite low, being below the average blood concentration reported for European non-smoking populations, 0.5-1.0 μg/L [18]. On the other hand, a study from South Korea [19] did not find any significant association between cadmium and the prevalence of type 2 diabetes in spite of geometric mean cadmium blood levels in diabetic patients and controls as high as 1.16 and 1.10 μg/L, respectively.

A significant and strong increase in OR was also found for previously undiagnosed type 2 diabetes with increasing quartiles of chromium. Although several studies suggest an improved glycemic control with chromium supplements [20], there is only a limited number of studies reporting differences in chromium level among persons with type 2 diabetes, and these report in general a lower chromium concentration in blood plasma [21-23].

Iron showed the strongest association with the prevalence of previously undiagnosed type 2 diabetes in the present study. After corrections for multiple testing, the association was

borderline significant. Few studies have reported iron levels among persons with type 2 diabetes: Kazi et al. [24] found higher iron levels in hair and blood among persons with type 2 diabetes, but the differences were non-significant. Similarly, Ekmekcioglu et al. [21] did not detect any differences between persons with type 2 diabetes and healthy controls in neither whole blood, blood plasma or erythrocytes. However, iron levels in whole blood is not a very reliable measure of the iron status of an individual, for this purpose, ferritin and/or transferrin should be used. Increased ferritin levelshave been reported to be associated with increased risk of type 2 diabetes [25, 26], and a recent study found a significant association between ferritin levels and type 2 diabetes, but not for serum iron [27]. Although poorly understood, several plausible mechanisms underlying these associations have been suggested [7, 28-30], although three mechanisms have been prominent: 1) insulin deficiency, 2) insulin resistance and 3) hepatic dysfunction. Iron may also act as a pro-oxidant molecule, and by catalyzing the formation of hydroxyl radicals, iron may contribute in the pathogenesis of diabetes through destruction of cell membranes, lipids, proteins and DNA [31].

A positive association was found between nickel and the prevalence of previously undiagnosed type 2 diabetes. Only a limited number of studies report nickel concentrations in persons with type 2 diabetes: Kazi et al. [24] reported higher nickel concentrations in hair samples from persons with type 2 diabetes, but did not find any significant difference for blood concentrations of nickel. A recent study in China showed an association between urinary nickel concentration and the prevalence of type 2 diabetes [32].

Our results suggest a possible relationship between increasing levels of zinc and the prevalence of previously undiagnosed type 2 diabetes. However, several studies have reported decreased serum/plasma and urinary zinc levels among persons with established type 2 diabetes [24, 33-37]. It has been hypothesized that zinc deficiency may be of

importance in the etiology of type 2 diabetes, as zinc deficiency in many countries seem to be related to a high increase in the prevalence of type 2 diabetes [38]. Low zinc levels among persons with type 2 diabetes may also be linked to an enhanced urinary excretion of zinc, a phenomenon known as hyperzincuria which has been known for decades [39]. However, a recent prospective study found an increased risk for type 2 diabetes with increased serum zinc levels [40], supporting our findings. Yary et al. [40] proposed plausible mechanisms linking high zinc levels and insulin resistance, including interference with hormonal homeostasis trough e.g. leptin or modulation of β-cell function and secretion of insulin. In addition, our analysis was performed on whole blood samples, not plasma/serum as often assessed in previous studies. It has also been hypothesized that an up-regulation of zinc transporters as a response to zinc depletion may lead to increased zinc uptake in order to maintain intracellular zinc homeostasis, and that the relation between intracellular and serum/plasma zinc may change over time [41].

We also found an association between increasing levels of silver and decreasing levels of bromine and the prevalence of previously undiagnosed type 2 diabetes. The association between silver and the prevalence of type 2 diabetes remained borderline significant after corrections for multiple testing, while the association for bromine was insignificant after corrections. To our knowledge no previous studies have reported relations between bromine or silver and type 2 diabetes.

Several studies suggest an association between arsenic and type 2 diabetes, and the National Institute of Environmental Health Sciences has argued that this association is plausible, but may only be valid for inorganic arsenic and in areas with high levels of arsenic in drinking water [9, 10]. In our study, we found no association between arsenic and type 2 diabetes. Levels of arsenic in drinking water in Nord-Trøndelag (median 0.05 µg/L, maximum 3.2

μg/L) [42], are much lower than levels where associations between arsenic and type 2 diabetes have been reported [43, 44].

Strengths of this study include the detailed characterization of diabetes, including the measurement of anti-GAD, anti-IA2 and C-peptide to distinguish between type 1 and type 2 diabetes, and the availability of information on potential confounders often not assessed in previous studies. Notably, because the database contains data on antibodies associated with type 1 diabetes and LADA, it was possible in the present study to exclude individuals with other types of diabetes that may mask or lead to false associations between trace elements and type 2 diabetes. The cases are in an early phase of their disease, as emphasized by the fact that their HbA_{1c} values are low (Table 1, HbA_{1c} values tend to increase during disease progression without treatment). Nonetheless, our cases had developed diabetes prior to the blood sampling for trace element analysis, and we cannot exclude that trace element levels may have been influenced by diabetes. Nord-Trøndelag county is dominated by rural areas and does not contain any larger cities, which may result in low exposure to trace elements associated with anthropogenic pollution. Exposure to higher levels of trace elements could possibly reveal associations with diabetes, and a dose-response relationship between trace elements and type 2 diabetes should also be addressed in future research. Associations involving nickel and chromium should be interpreted with caution as syringes may leak chromium and nickel ions to the samples. Since the blood samples were collected through HUNT3, standardized sampling devices and procedures were used, and no special precautions could be paid to e.g. pre-cleaning of sampling devices. However, we attempted to minimize this contamination, as the trace element samples were collected after flushing the syringes with ~20 mL of blood prior to sampling. Our results suggest that the trace element concentrations are comparable to previously published values, although chromium values seem slightly higher [45]. We were interested in the association between each trace element

and diabetes, as opposed to a joint hypothesis for all trace elements, and we emphasized the P_{trend} values in our interpretation of the results. Nonetheless, multiple testing increases the possibility for false positive findings, which is why we additionally presented Q_{trend} values corrected for multiple testing.

Conclusions

Our study suggests associations between bromine, cadmium, chromium, iron, nickel, silver and zinc and early type 2 diabetes, but the causality of the associations remains unclear. This is the first study of trace element levels in previously undiagnosed type 2 diabetes, and the early phase of the disease makes it is more likely that the alterations in trace element levels are not solely due to changes associated with the disease, but may be of a causal nature.

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Figure/table legends

Figure 1: **Flow chart for selection of cases from the HUNT3 cohort.**

FINDRISC: Finnish Diabetes Risk Score. HUNT3: the third survey of the Nord-Trøndelag Health Study. Anti-GAD: Glutamic acid decarboxylase antibodies.

Figure 2: **Trace elements with significant associations for previously undiagnosed, screening detected type 2 diabetes**. Multivariable adjusted odds ratios (OR) for previously undiagnosed, screening-detected type 2 diabetes with increasing concentrations of trace elements with significant associations over quartiles (tertiles) of trace element concentrations (P_{trend}).

Table 1: **Key characteristics of cases and controls**

Key characteristics of cases and controls with mean values (± SD) or medians (25 percentile, 75 percentile) or %-distribution if indicated.

	Controls	Cases
n	755	128
Sex		
%women (n)	48.2% (364)	46.1% (59)
Age (years)	61.4±14.1	65.2±10.3
Weight (kg)	79.0±14.0	89.8±13.9
Waist-to-hip ratio	0.91±0.07	0.97±0.07
BMI (kg/m^2)	27.4±4.1	31.2±3.6
S-glucose (mmol/L)	5.5±0.9	7.9±2.1
HbA_{1c} (%)	Not measured	6.4 (5.9, 6.7)
Family history of diabetes, first degree[a] (%)	141 (18.7%)	71 (55.0%)
Daily smoking	149 (19.7%)	22 (17.2%)
Economic status, level		
1 (lowest)	189 (25.0%)	30 (23.4%)
2	187 (24.8%)	35 (27.3%)
3	190 (25.2%)	39 (30.5%)
4 (highest)	189 (25.0%)	24 (18.8%)
Education, years (%)		
Primary, <10 years	204 (27.0%)	33 (25.8%)
Secondary, 10-12 years	399 (52.9%)	77 (60.2%)
Tertiary, ≥13 years	152 (20.1%)	18 (14.1%)

[a]Family history of diabetes defined as diabetes among siblings, children or parents (first degree).

Table 2: **Trace element concentrations in whole blood in cases and controls**

Median values and 10 and 90 percentiles for trace element concentrations in whole blood in cases and controls, and percentage of subjects with lower values than the limit of detection (LOD).

			Controls		Cases	
Trace element	LOD	<LOD (%)	Median	Percentiles 10, 90	Median	Percentiles 10, 90
Arsenic (µg/L)	0.46	1.9	2.90	0.90, 11.5	3.38	1.02, 12.1
Boron (µg/L)	2.2	0	27.5	15.3, 45.5	27.9	16.5, 41.7
Bromine (mg/L)	0.11	0	1.56	1.03, 2.18	1.36	0.58, 2.02
Calcium (mg/L)	0.14	0	58.7	53.9, 63.8	58.8	52.3, 64.2
Cadmium (µg/L)	0.016	0.1	0.35	0.16, 1.31	0.40	0.19, 1.24
Cesium (µg/L)	0.0039	0	4.60	3.13, 7.06	4.77	3.47, 7.25
Chromium (µg/L)	0.40	29.2	0.59	< LOD, 1.78	0.79	< LOD, 2.28
Copper (mg/L)	0.0013	0	1.01	0.865, 1.17	1.01	0.890, 1.20
Indium (µg/L)	0.002	0.1	0.028	0.019, 0.052	0.029	0.020, 0.057
Iron (mg/L)	0.133	0	542	477, 601	560	499, 621
Gallium (µg/L)	0.010	0	0.075	0.050, 0.105	0.071	0.052, 0.112
Gold (µg/L)	0.0057	19.4	0.009	< LOD, 0.030	0.011	< LOD, 0.033
Lead (µg/L)	0.41	0	19.9	10.8, 38.0	19.4	11.0, 37.2
Magnesium (mg/L)	0.015	0	39.5	35.2, 44.4	40.2	35.9, 45.6
Manganese (µg/L)	0.40	0	9.1	6.6, 13.3	8.9	6.5, 13.7
Mercury (µg/L)	0.036	0	3.18	1.36, 8.47	3.47	1.86, 7.27
Molybdenum (µg/L)	0.43	5.0	0.81	0.51, 1.49	0.88	0.50, 1.59
Nickel (µg/L)	0.22	11.3	0.49	< LOD, 1.47	0.61	0.23, 1.50
Rubidium (µg/L)	0.16	0	2271	1807, 2774	2242	1742, 2697
Selenium (µg/L)	6.5	0	101.4	80.3, 125.4	101.2	80.3, 124.4
Silver (µg/L)	0.039	14.7	0.102	< LOD, 0.301	0.116	< LOD, 0.325
Strontium (µg/L)	0.13	0	18.0	13.3, 25.9	19.0	13.4, 27.9
Tantalum (µg/L)	0.0018	25.9	0.0026	< LOD, 0.0048	0.0028	< LOD, 0.0053
Thallium (µg/L)	0.0011	0	0.026	0.017, 0.043	0.025	0.017, 0.039
Tin (µg/L)	0.10	16.8	0.19	< LOD, 1.01	0.20	< LOD, 2.96
Zinc (mg/L)	3.7	0	7.54	6.28, 8.85	7.99	6.75, 8.81

Table 3: **Associations between individual trace elements and previously undiagnosed, screening detected type 2 diabetes**

Associations between individual trace elements and the prevalence of previously undiagnosed, screening-detected type 2 diabetes, comparing the highest quartile (tertile for chromium and tantalum) to the reference (lowest) quartile (tertile).

Trace element	Age- and sex-adjusted model			Multivariable model[1]		
	OR (95% CI)[4]	P_{trend}[2]	Q_{trend}[3]	OR (95% CI)[4]	P_{trend}[2]	Q_{trend}[3]
Arsenic	0.80 (0.45–1.42)	0.484	0.662	0.63 (0.32–1.24)	0.215	0.430
Boron	0.74 (0.41–1.33)	0.392	0.600	0.87 (0.43–1.75)	0.998	0.998
Bromine	0.39 (0.22–0.68)	<0.001	0.003	0.52 (0.27–0.99)	0.032	0.139
Cadmium	1.15 (0.63–2.08)	0.575	0.698	1.99 (0.92–4.28)	0.027	0.139
Calcium	0.94 (0.52–1.71)	0.966	0.966	1.33 (0.66–2.69)	0.289	0.537
Cesium	1.34 (0.75–2.41)	0.385	0.600	1.76 (0.89–3.49)	0.157	0.371
Chromium	2.36 (1.44–3.88)	0.001	0.007	2.78 (1.55–4.99)	0.001	0.026
Copper	0.91 (0.48–1.71)	0.684	0.741	0.95 (0.46–1.96)	0.774	0.875
Gallium	0.91 (0.53–1.55)	0.478	0.662	0.63 (0.34–1.19)	0.095	0.274
Gold	1.66 (0.97–2.86)	0.029	0.108	1.70 (0.91–3.16)	0.068	0.221
Indium	1.15 (0.68–1.93)	0.316	0.587	1.23 (0.66–2.28)	0.327	0.567
Iron	4.27 (2.11–8.62)	<0.001	<0.001	2.97 (1.34–6.60)	0.009	0.078
Lead	0.68 (0.39–1.19)	0.204	0.442	1.12 (0.58–2.16)	0.755	0.875
Magnesium	1.73 (0.99–3.03)	0.063	0.205	1.26 (0.66–2.40)	0.706	0.875
Manganese	1.18 (0.70–2.00)	0.591	0.698	1.13 (0.61–2.11)	0.694	0.875
Mercury	1.65 (0.87–3.11)	0.554	0.698	1.66 (0.79–3.47)	0.924	0.961
Molybdenum	1.09 (0.62–1.93)	0.678	0.741	1.10 (0.57–2.14)	0.719	0.875
Nickel	2.11 (1.21–3.70)	0.004	0.021	2.24 (1.18–4.26)	0.016	0.104
Rubidium	0.57 (0.33–1.01)	0.078	0.225	0.74 (0.38–1.43)	0.349	0.567
Selenium	0.92 (0.54–1.59)	0.738	0.768	0.93 (0.50–1.74)	0.837	0.907
Silver	1.68 (0.98–2.89)	0.024	0.104	2.32 (1.20–4.48)	0.006	0.078
Strontium	1.15 (0.67–1.97)	0.372	0.600	1.12 (0.59–2.11)	0.400	0.612
Tantalum	1.57 (0.88–2.78)	0.139	0.361	1.74 (0.89–3.41)	0.173	0.375
Thallium	0.65 (0.37–1.13)	0.171	0.404	0.53 (0.28–1.02)	0.151	0.371
Tin	1.15 (0.68–1.94)	0.242	0.484	0.91 (0.50–1.68)	0.709	0.875
Zinc	3.49 (1.82–6.72)	<0.001	<0.001	2.19 (1.05–4.59)	0.038	0.141

[1] Multivariable model: age, sex, body mass index, waist-to-hip ratio, education, income, smoking and family history of diabetes.
[2] P_{trend}: Associations over quartiles/tertiles of trace element concentrations.
[3] Q_{trend}: P_{trend} values corrected for multiple testing using the Benjamini-Hochberg procedure.
[4] OR (95% CI): Odds ratio with 95 % confidence interval.