Trace Element Status in Patients with Type 2 Diabetes in Norway: The HUNT3 Survey

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Abstract

Several epidemiological studies have indicated that a number of trace elements may play a role in type 2 diabetes (T2D). We investigated the association between prevalent T2D and the concentrations of 25 trace elements in whole blood, and the relationships between T2D duration and blood levels of the trace elements that we found to be related to T2D prevalence. In this population based case-control study, 267 patients with self-reported T2D and 609 controls (frequency matched), were selected from the third Nord-Trøndelag Health Survey. Trace element blood levels were determined by high resolution inductively coupled plasma-mass spectrometry. Multivariable conditional logistic regression and multivariable linear regression were used to estimate associations. The prevalence of T2D was positively associated with boron, calcium and silver, and inversely associated with indium, lead and magnesium ($P_{trend} < 0.05$). We found no statistical evidence for associations between blood levels of arsenic, bromine, cadmium, cesium, chromium, copper, gallium, gold, manganese, mercury, molybdenum, nickel, rubidium, selenium, strontium, tantalum, thallium, tin and zinc and T2D prevalence. After corrections for multiple testing, associations remained significant for calcium and lead ($Q_{trend} < 0.05$), and borderline significant for magnesium, silver and boron. With increasing disease duration, higher calcium levels were observed ($P < 0.05$). This study suggests an association between prevalent T2D and blood levels of boron, calcium, indium, lead, magnesium and silver.

Key words: type 2 diabetes, trace elements, whole blood, HUNT3, case-control study

Introduction

In 2015 the International Diabetes Federation estimated that 415 million people worldwide live with diabetes, about half of them being undiagnosed, and the projected number for 2040 is 642 million [1]. An estimated 85-95% of people with diabetes have type 2 diabetes (T2D), which is a chronic disease characterized by elevated blood glucose levels, resulting from disorders in insulin secretion and/or insulin action [2]. T2D is considered to result from a combination of genetic predispositions and environmental factors. The observed increase in T2D prevalence is probably mainly due to changes in environmental factors [3]. It is well known that unhealthy nutrition habits and sedentary lifestyle are associated with insulin resistance which is typically present in both prediabetes and overt stages of T2D [3]. Exposure to various pollutants has also been suggested to play a role in diabetes onset [3]. Potential pathogenic mechanisms in T2D development involving trace elements include exposure to elevated levels of toxic elements [4] and disruption of essential metal-ion homeostasis [5].

Whether abnormal levels of certain trace elements are the result or a cause of diabetes, or a homeostatic attempt to rectify a parallel condition, is unknown [5]. In spite of a considerable number of studies on trace element levels in diabetic patients, no consistent picture of their involvement in the disease has emerged so far. To address the question on when anomalous levels of trace elements begin to appear in T2D development, one could measure trace elements levels over a longer period in a prospective study, or in the different stages of the disease in a cross-sectional study. We conducted two parallel, population based case-control studies on the association between trace element blood levels and diabetes, one in people with previously undiagnosed, screening detected T2D [6] and the present study in patients with previously diagnosed T2D, within the third Nord-Trøndelag Health (HUNT3) Survey. In

the present study, we also investigated if the trace element levels found to be associated with T2D prevalence vary with disease duration.

Methods

Study population. The participants for this cross-sectional case-control study were selected from the large population-based HUNT3 Survey, in which all residents ≥20 years of age in Nord-Trøndelag County, Norway, were invited to participate. In HUNT3 blood samples for metal/trace element analysis were collected from the residents of 14 out of the total 24 municipalities in the county. Out of the 50 807 adults participating in the HUNT3 Survey (54.1% participation rate) [7], blood samples for trace element analysis were collected from 26 358 subjects (Figure 1). For our study we selected participants from three groups of municipalities: coastal (Nærøy, Vikna, Flatanger, Leka and Fosnes), urban (Levanger and Steinkjer) and inland mountain (Røyrvik, Namsskogan and Grong). Information on diabetes, age at diagnosis and glucose lowering treatment was self-reported, and non-fasting serum glucose was measured in all participants. Participants with known diabetes or high Finnish Diabetes Risk Score (FINDRISC ≥ 15) were invited to an additional examination where those with known diabetes had fasting serum glucose, C-peptide and GAD antibodies (autoantibodies to glutamic acid decarboxylase) measured. Those without known diabetes, but with elevated FINDRISC, underwent an oral glucose tolerance test [8]. The selection criteria for T2D cases and controls are shown in Figure 1. T2D was defined by self-reported diabetes, excluding type 1 diabetes (T1D) as indicated by an index of GAD antibody levels, relative to a standard serum, of ≥ 0.08 or by fasting C-peptide < 150 pmol/L [9]. The self-report of diabetes in the HUNT study population has excellent validity [10]. Controls were selected among participants without known diabetes who had non-fasting glucose < 9.0 mmol/L. For participants with elevated FINDRISC who attended the oral glucose tolerance test, we excluded as eligible controls those who had prevalent, but undiagnosed diabetes

(fasting serum glucose ≥ 7.0 mmol/L or 2-h post-load serum glucose ≥ 11.1 mmol/L), impaired glucose tolerance (fasting serum glucose < 7.0 mmol/L and 2-h serum glucose 7.8-11.0 mmol/L) or impaired fasting glucose (fasting serum glucose 6.1-6.9 mmol/L and 2-h serum glucose < 7.8 mmol/L). Among 522 eligible cases, we randomly selected 270 and frequency-matched them by sex and age (5-year intervals) with 615 controls (Figure 1).

Covariates. In addition to sex and age, both T2D prevalence and trace elements blood levels have been previously found to be influenced by numerous factors, including geographic area, body mass index, measures of central obesity, education, economic status, diet and smoking habits [11-18]. Therefore, potential confounding covariates were chosen based on reported associations with both T2D and trace element blood levels. Covariate data were from questionnaires (age, sex, smoking status, alcohol consumption, fat fish and milk intake, and family history of diabetes), interview with participants (ongoing pregnancy) and clinical measurements at the health examination sites (weight, height, waist and hip circumference). Information on education level and income was obtained from Statistics Norway.

Sample collection and storage. In the HUNT3 Survey five blood samples were collected from each participant, and the fifth of these was used for the trace element analysis, to minimize potential contamination from the needles. Blood was drawn using needles for routine blood collection (Vacuette, Greiner Bio-One North America, Inc., Monroe, NC) and collected in 7 mL glass blood collection tubes for trace element sampling, containing sodium heparin (Vacutainer Cat. no. 367735; Becton, Dickinson & Co, Franklin Lakes, NJ). Each trace element blood sample was further divided into seven 0.8 mL aliquots and transferred into 1 mL polypropylene tubes (Thermo Scientific) and stored at –80 °C.

Sample preparation. The blood samples were brought to an ISO 6 clean room and stirred for homogenization after reaching room temperature. Approx. 0.7 mL blood 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). The samples were acidified with 1.0 mL 65% (V/V) ultrapure nitric acid, produced at NTNU from p.a. quality nitric acid (Merck, Darmstadt) using a sub-boiling distillation system (SubPur, Milestone, Shelton, CT). The samples were then digested using a high performance microwave reactor (UltraClave, Milestone). Digested samples were decanted into pre-cleaned 15 mL polypropylene vials (VWR, USA) and diluted with ultrapure water (Purelab Option-Q, Elga) to achieve a final acid concentration of 0.6 M.

Trace element analysis. Trace element levels were measured using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS, Thermo Finnigan Element 2, Bremen). The sample introduction system consisted of an SC2-DX auto-sampler with ULPA filter, a prepFAST system, a concentric PFA-ST nebulizer combined with a quartz micro cyclonic Scott spray chamber with auxiliary gas port, aluminium sample and skimmer cones, and an O-ring-free quartz torch and 2.5 mm injector (Elemental Scientific, Omaha, NE). The radio frequency power was set to 1350 W; nebulizer and T-connection sample gas flow were 0.75 L/min, and 0.55 L/min, respectively. Cooling gas flow was 15.5 L/min, auxiliary gas flow was 1.1 mL/min and additional gas consisted of 10% methane in argon with flow rate of 0.01 L/min.

Two multi-element stock solutions (Elemental Scientific, Omaha, NE) were used for the instrument calibration, one serving as a calibrating solution and the other as a quality control. Four different dilutions of the calibrating solution were prepared to cover wide elemental concentration ranges. Before analysis, the solutions were matrix matched with the blood samples for acid strength (0.6 M ultrapure nitric acid), and by adding 160 mg/L sodium and 115 mg/L potassium (Spectrapure Standards, Oslo). An internal standard of 1 μg/L rhenium was automatically mixed with the sample in the prepFAST system. Elements were

determined in three different resolutions, low (LR 400; cadmium, cesium, gold, indium, lead, mercury, tantalum, thallium and tin), medium (MR 5 000; boron, calcium, chromium, copper, gadolinium, magnesium, manganese, molybdenum, nickel, rubidium, silver, strontium and zinc) and high (HR 10 000; arsenic, bromine and selenium). The elements with blood levels below the limit of detection in more than 33% of study participants were excluded. In addition, we excluded iron from the study because important parameters of iron status, such as ferritin levels and transferrin saturation were not available from the laboratory measurements.

Analytical quality control. Blood collection tubes, pipet tips, polypropylene vials, flasks and the ultrapure acid were checked for possible elemental contamination prior to the analysis. Ten blood collection tubes were tested by soaking them for eight days in room temperature with a 0.9% NaCl (suprapure grade) solution, and elements shown to contaminate the samples were excluded from the study. In order to check for instrumental drift, one of the multi-element standards was analysed for every 20 samples. Repeated analysis of a certified reference material (Seronorm Level 1, Sero, Norway), and of blood collected from a healthy volunteer, were used to verify the accuracy of the instrument.

Statistical analysis. In the analysis, trace elements levels were categorized into quartiles (tertiles for chromium and tantalum since 25-33% of the samples had levels below the detection limit). The cut points were based on the distribution in the controls. Element concentrations less than the detection limit were replaced with half the detection limit. Conditional logistic regression analysis for matched case-control studies was used to assess associations between the trace elements and T2D. In the first model, odds ratios (ORs) stratified by sex and age (5-year categories) were calculated. Then, multivariable analysis was performed adjusting for the potential confounders: body mass index (BMI, categorized according to WHO recommendations as < 25.0, 25.0-29.9, and ≥ 30 kg/m^2) waist-to-hip ratio

(≤ 0.88, 0.89-0.93, and ≥ 0.94), smoking status (current daily smoking), first-degree family history of diabetes (parents, siblings or children with diabetes), education (< 10, 10-12 and ≥ 13 years), income level (given as after-tax equivalent income – EU-equivalent scale, divided into quartiles), residence area (mountain, urban and coastal). In addition, some elements were adjusted for element specific factors: arsenic and mercury levels were adjusted for fat fish intake (< 4 meals/month,1-3 meals/week, and ≥ 4 meals/week); calcium, lead and magnesium levels were adjusted for alcohol consumption (≤ 3 and 4-7 times/week) and calcium blood levels additionally for magnesium levels and milk intake (≤ 1 and > 1 glass/day), while lead and magnesium levels were additionally adjusted for calcium blood levels. Tests for trend across categories were used to assess any relationship of increasing trace element levels with the odds of having T2D. P_{trend} values were corrected for multiple testing using the Benjamini-Hochberg procedure.

For the trace elements we found to be associated with T2D prevalence, we examined whether disease duration (continuous, years) was associated with trace element concentrations using multivariable linear regression analysis among the T2D cases. First we adjusted for age (10-year intervals) and sex (model 1), then further (model 2) for the same variables used in the conditional logistic regression analysis (see previous paragraph). In model 3, the models were additionally adjusted for type of glucose-lowering treatment to examine whether the associations might be mediated by type of treatment. Diabetes treatment was categorized as only lifestyle treatment, only insulin, only oral glucose-lowering drugs, and both insulin and oral glucose-lowering drugs. Boron, indium, lead and silver blood levels were non-normally distributed; therefore, log-transformed data were used in the linear regression models for these four elements. Then the regression coefficients were back-transformed and relationships expressed as percentage change in elements blood levels per year increase in diabetes duration.

The data were analysed using Stata 13 (StataCorp, TX). All statistical tests were two-sided and $P < 0.05$ was considered statistically significant.

Ethics. Both the HUNT3 Survey and our study were approved by the Regional Committee for Medical and Health Research Ethics, Region Central 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

Excluding participants with missing blood sample, low blood volume and/or without necessary data, a total of 267 diabetic patients and 609 controls were included in the study (Figure 1). There were 244 diabetic patients with valid information on disease duration (mean 8.4 ± 7.3 years) and 190 diabetic patients with valid information on glucose-lowering treatment. Characteristics of the participants are shown in Table 1. Trace element blood levels (median values with 10-90 percentile ranges) for the diabetic patients and controls are shown in Table 2.

In the conditional logistic regression analysis (Table 3, Supplementary Table 1), magnesium and lead were significantly negatively associated with T2D prevalence. The crude (age- and sex-adjusted) ORs comparing the highest tertile/quartile to the lowest were 0.46 for magnesium and 0.31 for lead, and the ORs remained similar after adjustment for confounding variables (Table 3). For indium, the crude model showed a negative, but non-significant association (OR 0.75), while additional adjustment resulted in a stronger (OR 0.49) and significant association.

Boron, calcium and silver were significantly positively associated with T2D in both the crude and multivariable models. For boron (OR 2.24) and silver (OR 2.61), the crude association

was attenuated after adjustment for the potential confounders, slightly for boron (OR 2.08) and stronger for silver (OR 1.92); while the OR for calcium was 3.11 in the crude model and even higher in the multivariable model (OR 3.51).

In the crude model bromine, cadmium, rubidium and thallium were significantly negatively associated with T2D with ORs 0.56, 0.52, 0.45, and 0.45, respectively, but in the multivariable model the association was attenuated and non-significant. For the remaining trace elements, we found no statistical evidence for associations with prevalent T2D in either the crude or the multivariable model.

After correction for multiple testing, the associations for boron, bromine, cadmium, calcium, lead, magnesium, rubidium, silver and thallium remained significant adjusted for age and sex. Adjusted for additional confounders, calcium and lead were still significant after correction for multiple testing ($Q_{trend} < 0.05$), and the associations for magnesium, silver and boron showed borderline significance (Q_{trend}: 0.052 and 0.068, respectively).

Three multivariable linear regression models were applied for diabetes duration as a predictor for the trace elements that were significantly associated with T2D prevalence (Table 4). In both the age- and sex-adjusted and multivariable models, the association with increasing diabetes duration was significantly positive for calcium blood concentration (Figure 2). We further adjusted for glucose-lowering treatment to examine whether type of treatment could mediate the association, and after this adjustment, the association of diabetes duration with calcium was modestly attenuated. We found no statistical evidence of associations between magnesium, boron, indium, lead or silver, and diabetes duration.

Discussion

In this case-control study we investigated the association between whole blood levels of 25 trace elements and T2D prevalence in participants from the HUNT3 Survey. Our results indicate that several trace elements may play a role in T2D; we found positive associations for boron, calcium and silver, and negative for lead and magnesium.

We found a strong association between increased calcium levels and T2D prevalence. Calcium imbalance has been reported to be associated with pancreatic β-cell malfunction, insulin sensitivity reduction and systemic inflammation, all conditions central to T2D pathogenesis. However, studies of associations between T2D and blood levels of calcium have shown conflicting results. There are some indications that insufficient calcium and vitamin D intake may alter the balance between intracellular and extracellular calcium pools in β-cells, leading to reduction in insulin secretion [19]. Calcium as a second messenger has an important signalling role for insulin action in insulin-responsive tissues [20], and some studies have reported an inverse association between insulin resistance and intracellular cytoplasmic calcium levels in insulin target tissues [19]. Insulin, in turn, may suppress calcium tubular reabsorption [21] and thus reduce calcium levels.

We found low blood levels of magnesium to be associated with increased prevalence of T2D, consistent with studies reporting suppressed magnesium levels in T2D [22-24]. Magnesium plays an important role in the regulation of insulin actions, including insulin-mediated glucose uptake by controlling insulin receptor affinity in the target tissues and vascular tone [25, 26]. Being an essential cofactor in reactions involving phosphorylation, magnesium deficiency could impair the insulin signal transduction pathway [25]. Some authors have linked magnesium depletion in T2D to a low magnesium intake in the Western diet [27, 28]. An association between magnesium homeostasis imbalance and decreased tyrosine kinase activity at insulin receptors has been reported, leading to insulin resistance, decrease of glucose-stimulated insulin secretion and affecting β-cell insulin secretion, thus supporting the

hypothesis that magnesium deficiency is associated with T2D onset [29]. In addition, low serum magnesium has been reported to be related to increased levels of tumour necrosis factor α and high sensitive C-reactive protein, both characteristically present in obesity and chronic inflammation, which usually precede T2D [30]. However, some findings suggest hypomagnesemia in T2D to be an epiphenomenon. β-cell dysfunction starts long before diabetes diagnosis and by the time of diagnosis its function may already be 50% reduced [31]. In the light of the fact that insulin can promote magnesium tubular absorption, one of the key sites for maintenance of magnesium homeostasis, insulin deficiency may be causing lower magnesium blood levels as the disease develops [32].

Magnesium and calcium may potentially antagonize each other in many physiologic processes, such as inflammation, oxidative stress and insulin resistance, which are all involved in the progress of T2D [33]. Concurring with our results of calcium being positively and magnesium negatively associated with T2D, it has been shown that T2D is associated with inversely correlated serum levels of calcium and magnesium [34], and intracellular, cytoplasmic free calcium and magnesium ions [22, 23], and also in different organs [35]. Depleted levels of magnesium can exacerbate intracellular calcium accumulation by modulating calcium ion flux across cell membranes [21]. Some studies have, however, reported negative association between T2D and both calcium and magnesium [28, 36, 37].

We found blood levels of boron to be positively associated with T2D prevalence in the multivariable analysis. Recent evidence suggests that boron may be under homeostatic control in humans, but the potential mechanism is unclear [38]. Hunt hypothesized an essential role of boron in insulin metabolism since both circulating insulin concentrations and peak insulin pancreas release increases in a condition of boron deficiency [38], and suggested that this was due to boron inhibiting glucose-6-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase, both of which are key enzymes in the pentose phosphate

pathway (PPP). Boron deprivation seems to increase PPP activity which leads to increased insulin secretion, suggesting a possible role of boron in ion transport across the cell membrane [38]. As T2D progresses, the insulin production by the pancreas β-cells decreases [31] and we may speculate that higher levels of boron in diabetic patients might be connected with lower levels of circulating insulin. However, our analysis of the association between disease duration and boron blood levels revealed no effect.

Lead blood levels were negatively associated with T2D prevalence. To our knowledge, three other studies have reported slightly lower blood levels in diabetic patients than in controls [34, 39, 40]. It is well known that simultaneous intake of lead and calcium may cause a reduced gastrointestinal lead absorption [41, 42]. Also, alcohol intake is associated with increased blood levels of lead [42], but we did not find any changes in the association after adjusting for calcium blood levels and alcohol intake. In contrast with our study, Babalola et al. reported elevated levels of lead in male and female diabetic patients compared to control subjects [43]. Increased levels of lead has been reported in plasma samples of non-smoking patients with T2D, impaired fasting glucose, and impaired glucose tolerance, in addition to a positive correlation between plasma levels of glycated haemoglobin (HbA_{1c}) and lead [44]. Afridi et al. reported higher lead levels in hair and whole blood samples and elevated lead urine excretion in diabetic patients [45]. Moon reported slightly but non-significantly increased lead blood levels in diabetic patients in a large population-based Korean study [46].

We found silver to be positively, and indium to be negatively associated with prevalent T2D. We are not aware of any other studies reporting associations between these two metals and T2D. At present, neither metal is known to exhibit any essential metabolic function [47, 48].

No association was found in our study for arsenic, chromium, selenium and zinc, for which ample evidence suggests roles in T2D [4, 5].

For the six trace elements significantly associated with prevalent T2D, disease duration was significantly related only to calcium. After adjustment for four categories of glucose-lowering treatment the association was moderately attenuated. This may suggest that the association could in part be mediated by treatment type. Metformin is a known metal chelator, able to combine with many transition metals including zinc, copper and iron, and also with magnesium [49, 50]. Logie et al. suggest that the cellular effects of metformin and other biguanides depend on their metal-binding properties [51]. Some studies suggest that metformin, sulfonylurea, and glitazones may influence magnesium status in T2D patients [52-55]. Since we do not have information on the specific medications used by the individual patients in our study, the mechanism(s) behind our findings on the relationship between T2D treatment and calcium is not easily interpreted. We cannot exclude that other types of medication often used by people with T2D, such as antihypertensives, may have influenced the associations.

Major strengths of our study are that it is population-based, with high attendance and with strict diagnostic criteria, enabling us to distinguish between T2D and other types of diabetes and pre-diabetes. Emphasis was placed on optimal handling and storage of blood samples, as part of the establishment of a new state-of-the-art biobank. Finally, the access to a wide range of variables allowed us to control for a variety of potential confounding factors. All elemental blood levels found in our study in both cases and controls were within generally accepted reference ranges [17, 18].

We acknowledge some limitations to our study. The use of whole blood is not optimal for all trace elements, for which the blood levels may be not representative for the total body burden and actual intracellular concentrations in important target tissues [56]. Information on trace element speciation, location in specific blood cells and cell compartments is important for a full evaluation of the body burden and the element's potential biological effects [5].

Accordingly, in spite of that we measured iron in samples of whole blood, as data on blood storage parameters (e.g. ferritin) was not available to be measured in these samples, the total iron measurements are not included in the present study.

Due to the cross sectional study design, we cannot separate between effects of trace elements on diabetes, and effects of diabetes or its treatment on the trace element levels. Differences in trace element associations between this study and a previous study of newly diagnosed T2D within the same HUNT3 cohort [6] may suggest that some of our observed associations have occurred after the clinical onset of T2D. Although we controlled for many potentially confounding factors we cannot exclude the possibility of residual confounding. For example, association between calcium blood levels and diabetes still may be confounded by dairy products intake due to the fact that we have information only on "glasses of milk", while our data set includes no information on other dairy products intake. Further, the variable on seafood intake provides only information on "fat fish" intake. Likewise, the accuracy of self-reported exposures, such as food frequency is uncertain. We were interested in the association between each trace element and diabetes, as opposed to a joint hypothesis for all trace elements, so 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

In summary, our study shows that lower whole blood levels of indium, lead, and magnesium and higher levels of boron, calcium, and silver are significantly associated with prevalent T2D. These elements may play a role in the development of the disease, be linked to effects of the disease or to antidiabetic treatment. We found increasing calcium blood levels to be associated with diabetes duration, suggesting that calcium may be linked to disease

progression or to antidiabetic treatment. Future studies should focus on changes in trace elements levels over longer periods, on speciation of specific metals in different intracellular and extracellular compartments, and on how particular glucose-lowering drugs affect levels of trace elements, especially essential ones, in diabetic patients.

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Table 1. Characteristics of the diabetic persons and controls included in the study.

	Controls	Cases
Number of subjects	609	267
Females (%)	313 (51)	129 (48)
Mean age in years (SD)	59.2 (12.2)	65.4 (10.6)
Mean waist-to-hip ratio (SD)	0.91 (0.07)	0.97 (0.07)
Mean body mass index in kg/m^2 (SD)	27.5 (4.2)	30.6 (4.9)
Mean non-fasting serum glucose in mmol/L (SD)	5.4 (0.8)	8.8 (3.5)
Family history of diabetes[a] (%)	120 (19.5)	156 (57.8)
Daily smoking (%)	137 (22.7)	46 (17.8)
Region		
Mountain (%)	207 (33.7)	20 (7.4)
Urban (%)	205 (33.3)	174 (64.4)
Coastal (%)	203 (33.0)	76 (28.1)
Education (years)		
< 10 (%)	175 (28.5)	83 (30.7)
10-12 (%)	317 (51.6)	156 (57.8)
≥ 13 (%)	122 (19.9)	31 (11.5)
Economic status level		
Quartile 1 (%) (lowest)	150 (24.4)	66 (24.4)
Quartile 2 (%)	139 (22.6)	84 (31.1)
Quartile 3 (%)	163 (26.5)	64 (23.7)
Quartile 4 (%) (highest)	163 (26.5)	56 (20.7)
Fat fish intake[b]		
< 4 meals monthly (%)	189 (31.0)	74 (27.7)
1-3 meals weekly (%)	357 (58.6)	150 (56.2)
≥ 4 meals weekly (%)	63 (10.3)	43 (16.1)
Alcohol intake ≥ 4 glasses weekly (%)	13 (2.1)	5 (1.9)
Milk intake > 1 glass daily (%)	183 (30.9)[c]	41 (16.4)[d]
Mean diabetes duration in years (SD)	-	8.36 (7.3)[e]
Glucose-lowering treatment		
Lifestyle only (%) (reference category)	-	40 (20.6)
Insulin (%)	-	17 (8.8)
Oral antidiabetic drugs (%)	-	118 (60.8)
Insulin and oral antidiabetic drugs (%)	-	19 (9.8)

[a]Family history of diabetes defined as diabetes among siblings, children or parents (first degree).
[b]Fat fish includes salmon, trout, herring, mackerel and redfish
[c]Data available for 593 (93.6%) control subjects
[d]Data available for 250 (88.2%) diabetic persons
[e]Data available for 244 (92.4%) diabetic persons

Table 2. Median, 10 and 90 percentiles for whole blood concentrations of trace elements in cases and controls, and percentage of subjects with lower values than the limit of detection.

			Controls (n = 607)		Cases (n = 267)	
Trace element	LOD[a] (µg/L)	< LOD (%)	Median (µg/L)	10%; 90% (µg/L)	Median (µg/L)	10%; 90% (µg/L)
Arsenic	0.46	2.1	3.02	0.90; 11.91	3.36	1.12; 11.76
Boron	2.2	-	26.1	14.9; 45.4	30.7	17.8; 50.7
Bromine	0.11	-	1585	1094; 2203	1471	938; 2161
Cadmium	0.14	-	0.35	0.15; 1.42	0.32	0.14; 0.93
Calcium	0.016	-	58665	53990; 63736	60227	53990; 65252
Cesium	0.0039	-	4.64	3.26; 7.17	4.51	2.29; 6.84
Chromium	0.40	32.7	0.58	< LOD; 1.78	0.67	< LOD; 2.05
Copper	0.0013	-	1005	866; 1174	997	866; 1177
Gallium	0.002	-	0.075	0.050; 0.104	0.071	0.049; 0.102
Gold	0.13	19.0	0.0092	< LOD; 0.0232	0.0099	< LOD; 0.0252
Indium	0.010	-	0.028	0.019; 0.050	0.027	0.018; 0.055
Lead	0.41	-	20.2	11.2; 37.9	16.4	9.7; 35.2
Magnesium	0.015	-	39463	35116; 44251	38739	33914; 43810
Manganese	0.40	-	9.0	6.5; 13.2	9.1	6.5; 14.2
Mercury	0.036	-	3.19	1.43; 8.42	3.60	1.51; 8.00
Molybdenum	0.43	4.5	0.80	0.51; 1.48	0.82	0.49; 1.45
Nickel	0.22	12.7	0.46	< LOD; 1.47	0.58	< LOD; 1.70
Rubidium	0.16	-	2305	1822; 2824	2115	1697; 2803
Selenium	6.5	-	102.3	81.9; 125.8	102.3	77.7; 127.1
Silver	0.039	12.2	0.117	< LOD; 0.307	0.129	< LOD; 0.461
Strontium	0.13	-	18.0	13.3; 25.7	18.8	13.3; 30.5
Tantalum	0.0018	27.2	0.0025	< LOD; 0.0048	0.0026	< LOD; 0.0046
Thallium	0.0011	-	0.026	0.018; 0.044	0.024	0.016; 0.037
Tin	0.10	15.7	0.19	< LOD; 0.87	0.20	< LOD; 1.01
Zinc	3.7	-	7512	6235; 8782	7643	6436; 8933

[a]LOD: limit of detection, 3 times the standard deviation of blank samples

Table 3. Crude and adjusted odds ratios (OR) and 95% CI of diagnosed type 2 diabetes comparing the highest to the lowest tertiles/quartiles of trace element concentrations.

Trace element	Age- and sex-adjusted model			Multivariable model[a]		
	OR (95% CI)	P_{trend}	Q_{trend}[b]	OR (95% CI)	P_{trend}	Q_{trend}[b]
Arsenic	0.83 (0.53, 1.30)	0.351	0.480	0.73 (0.40, 1.31)	0.312	0.312
Boron	2.24 (1.41, 3.56)	<0.001	0.002	2.08 (1.14, 3.80)	0.013	0.068
Bromine	0.56 (0.36, 0.87)	0.007	0.020	0.76 (0.43, 1.33)	0.503	0.657
Cadmium	0.52 (0.37, 0.84)	0.006	0.020	0.61 (0.30, 1.23)	0.324	0.509
Calcium	3.11 (1.95, 4.96)	<0.001	<0.001	3.51 (1.87, 6.60)	<0.001	<0.001
Cesium	0.73 (0.46, 1.14)	0.114	0.269	1.08 (0.61, 1.92)	0.853	0.853
Chromium	1.23 (0.86, 1.77)	0.182	0.315	1.40 (0.89, 2.21)	0.102	0.295
Copper	0.78 (0.48, 1.26)	0.215	0.349	0.97 (0.53, 1.78)	0.745	0.778
Gallium	0.71 (0.46, 1.10)	0.075	0.195	0.88 (0.50, 1.53)	0.505	0.657
Gold	1.14 (0.74, 1.75)	0.711	0.804	0.95 (0.55, 1.66)	0.658	0.744
Indium	0.75 (0.49, 1.14)	0.369	0.480	0.49 (0.28, 0.84)	0.025	0.100
Lead	0.31 (0.20, 0.49)	<0.001	<0.001	0.24 (0.13, 0.47)	<0.001	0.002
Magnesium	0.46 (0.30, 0.71)	<0.001	0.001	0.53 (0.30, 0.94)	0.033	0.055
Manganese	1.16 (0.77, 1.75)	0.283	0.409	1.03 (0.61, 1.73)	0.571	0.707
Mercury	0.83 (0.53, 1.29)	0.609	0.720	0.61 (0.34, 1.10)	0.259	0.312
Molybdenum	1.07 (0.68, 1.66)	0.953	0.953	1.42 (0.81, 2.52)	0.327	0.509
Nickel	1.21 (0.79, 1.85)	0.248	0.379	1.56 (0.91, 2.67)	0.089	0.289
Rubidium	0.45 (0.30, 0.70)	<0.001	<0.001	0.87 (0.50, 1.50)	0.277	0.509
Selenium	0.88 (0.57, 1.36)	0.765	0.829	1.13 (0.65, 1.96)	0.367	0.530
Silver	2.61 (1.70, 4.01)	<0.001	<0.001	1.92 (1.10, 3.32)	0.008	0.052
Strontium	1.18 (0.77, 1.81)	0.179	0.315	1.04 (0.61, 1.79)	0.656	0.744
Tantalum	1.20 (0.77, 1.87)	0.571	0.707	1.62 (0.92, 2.85)	0.206	0.446
Thallium	0.45 (0.29, 0.71)	0.004	0.015	0.58 (0.33, 1.03)	0.162	0.421
Tin	0.98 (0.64, 1.56)	0.801	0.833	0.66 (0.38, 1.13)	0.206	0.446
Zinc	1.59 (1.00, 2.54)	0.143	0.310	1.08 (0.59, 1.97)	0.748	0.778

[a]Adjusted for BMI, waist-to-hip ratio, first-degree family history of diabetes, smoking habits, area, education and economic status. In addition, arsenic and mercury were adjusted for fat fish intake; calcium for milk and alcohol consumption and magnesium blood levels; lead and magnesium for alcohol consumption and calcium blood levels.

[b]P_{trend} values corrected for multiple testing using the Benjamini-Hochberg procedure.

Table 4. Relationships between diabetes duration and trace elements given as the regression coefficient β (μg/L) for the normally distributed calcium and magnesium blood levels, and as the percentage of change ("Effect") in the non-normally distributed boron, indium, lead, and silver blood levels (log-transformed) with 95% confidence intervals (CI), per year of diabetes duration.

	Model 1[a] (n = 244)		Model 2[b] (n = 244)		Model 3[c] (n = 190)	
Element	β (95% CI) [μg/L]	*P* value	β (95% CI) [μg/L]	*P* value	β (95% CI) [μg/L]	*P* value
Calcium	84.8 (11.7, 158.0)	0.023	86.7 (11.8, 161.7)	0.023	68.9 (-25.1, 163.0)	0.150
Magnesium	-31.1 (-95.3, 35.6)	0.342	-30.7 (-97.1, 35.6)	0.362	-25.4 (-104.1, 53.2)	0.524
	Effect[d] (95% CI) [%]		Effect[d] (95% CI) [%]		Effect[d] (95% CI) [%]	
Boron	-0.3 (-1.1, 0.5)	0.450	-0.4 (-1.2, 0.3)	0.270	-0.4 (-1.3, 0.6)	0.469
Indium	0.1 (-0.8, 0.9)	0.899	-0.1 (-0.9, 0.7)	0.765	0.1 (-1.1, 1.1)	0.983
Lead	-0.4 (-1.3, 0.5)	0.361	-0.4 (-1.3, 0.5)	0.422	-0.7 (-1.9, 0.4)	0.209
Silver	0.6 (-1.2, 2.4)	0.522	0.4 (-1.4, 2.2)	0.658	-0.04 (-2.1, 2.0)	0.964

[a]Adjusted for sex and age

[b]Adjusted for sex, age, BMI, waist-to-hip ratio, smoking status, first-degree family history of diabetes, education, income level and residence area

[c]Adjusted for sex, age, BMI, waist-to-hip ratio, smoking status, first-degree family history of diabetes, education, income level, residence area and glucose-lowering treatment

[d] Effect: percentages of change, representing 1 subtracted from the antilogs of the β regression coefficients for log-transformed trace element blood levels

Figure legends

Figure 1. Flow chart for selection of diagnosed type 2 diabetes cases and control subjects from the HUNT3 cohort.

Figure 2. Calcium whole blood levels as a function of diabetes duration.