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Anica Simic

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Trace Elements in the General Population and Their Possible Role in Type 2 Diabetes - the Third Nord-Trøndelag Health Survey (HUNT3)

Trondheim, October 2017

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Summary

Access to reliable information on the elemental contents of the human body is crucial to understand the roles these elements may have in the maintenance of human health. Establishing trace element blood levels in a general population can help elucidate potential causes of diseases related to natural and anthropogenic sources of these elements; it can also provide a baseline for potential future biomonitoring that could assess and evaluate temporal changes in the trace element status in populations. For the toxic elements such population studies can provide an important source for considering the total exposure of such elements through food, water and air, and may thus warn us on potentially dangerous exposure or contamination.

Several epidemiological studies have indicated that a number of trace elements may play a role in type 2 diabetes (T2D). To address the question on when anomalous levels of trace elements begin to appear in T2D development, one could measure trace elements levels in the different stages of the disease in cross-sectional studies.

In the present dissertation, whole blood samples and data collected in the third wave of the population-based Nord-Trøndelag Health Survey (HUNT3) were used in cross-sectional studies to investigate: 1) the background levels of 28 trace elements in the population of Nord-Trøndelag County and possible regional differences in the trace element levels; 2) potential relations between trace element blood levels and T2D in two case-control studies, one in persons who did not have a T2D diagnosis when blood samples were drawn, and the other in persons with an established T2D diagnosis.

The whole blood concentrations of 28 trace elements in this Norwegian population were found to be well within reference levels, suggesting low exposure to toxic elements in the residents of Nord-Trøndelag County. Our results imply that geographical area, lifestyle, and several socio-demographic characteristics markedly influence the blood concentrations of several trace elements in humans, particularly for the elements arsenic, mercury, bromine, boron and selenium, for which the marine environment may be an important source of exposure.

Our studies on trace element blood levels in T2D showed significant associations of lower indium, lead and magnesium, but higher boron, calcium and silver levels with prevalent T2D, and lower bromine, but higher chromium, iron, nickel, silver and zinc levels in the early phase of T2D. Increasing blood levels of calcium were associated with diabetes duration, which suggest that calcium might be linked to disease progression or antidiabetic treatment.

In future studies of trace elements in the general population, emphasis should be placed on well-characterized prospectively followed population-based cohorts (such as the HUNT population), where detailed information is available on a wide range of socio-demographic and lifestyle characteristics, paying particular attention to nutritional factors. Future studies on trace elements in T2D should focus on changes in trace element levels over longer periods (including samples collected before the disease is manifest); on the speciation of trace elements in different intracellular and extracellular compartments, and on how particular glucose-lowering drugs may affect levels of especially essential trace elements in diabetic patients.

Sammendrag

Pålitelig kunnskap om nivåene av ulike grunnstoffer i menneskekroppen er avgjørende for å forstå hvilke roller disse grunnstoffene kan ha for menneskers helse. Studier av normalnivåer av grunnstoffer i blod i en befolkning kan bidra til å belyse potensielle årsaker til sykdommer knyttet til naturlige og menneskeskapte kilder til disse grunnstoffene, og kan gi en basislinje for fremtidig biomonitorering for å vurdere og evaluere endringer over tid i befolkningers sporelementstatus. For toksiske grunnstoffer kan slike befolkningsstudier være en viktig kilde til å vurdere totaleksponering for slike grunnstoffer gjennom mat, vann og luft, og kan derfor advare oss om potensielt farlig eksponering eller forurensning.

Flere epidemiologiske studier har indikert at ulike sporelementer kan spille en rolle i type 2 diabetes (T2D). Analyser av sporelementnivåer i de ulike stadiene av T2D i tverrsnittsstudier kan være et viktig verktøy for å finne ut når unormale nivåer av sporelementer begynner å opptre i utviklingen av T2D.

I denne avhandlingen ble fullblodsprøver og data innsamlet i den tredje Helseundersøkelsen i Nord-Trøndelag (HUNT3) brukt i tverrsnittsstudier for å undersøke: 1) bakgrunnsnivåene av 28 sporelementer i befolkningen i Nord-Trøndelag fylke og mulige regionale forskjeller i sporelementnivåene; 2) mulige sammenhenger mellom sporelementenes blodnivåer og T2D i to kasus-kontrollstudier, en av personer som ikke hadde en T2D-diagnose da blodprøvene ble tatt, den andre studien av personer med en etablert T2D-diagnose.

Fullblodkonsentrasjonene av 28 sporelementer ble funnet å ligge godt innenfor aksepterte referansenivåer, noe som tyder på lav eksponering for toksiske grunnstoffer blant innbyggerne i Nord-Trøndelag fylke. Våre resultater viser at blodkonsentrasjonen av flere sporelementer varierer med geografisk område (primært kyst/innland), livsstil og ulike sosioøkonomiske forhold, spesielt for arsen, kvikksølv, brom, bor og selen, der det marine miljøet kan være en viktig kilde til eksponering.

Våre studier av sporelementer i blod i T2D viste at nivåene av indium, bly og magnesium var lavere, og nivåene av bor, kalsium og sølv var høyere hos pasienter med en etablert T2D-diagnose enn hos kontrollpersoner. Hos personer i en tidlig fase av T2D fant vi lavere nivåer av brom, men høyere nivåer av krom, jern, nikkel, sølv og sink hos pasienter enn hos kontrollpersoner. Vi fant også økende blodnivåer av kalsium med økende varighet av T2D, noe som tyder på at kalsium kan være knyttet til sykdomsprogresjon eller antidiabetisk behandling.

I fremtidige studier av sporelementer i ulike befolkninger bør det legges vekt på velkarakteriserte prospektivt fulgte populasjonsbaserte kohorter (som HUNT-befolkningen), hvor detaljert informasjon er tilgjengelig for et bredt spekter av sosiodemografiske- og livsstilsvariable, med spesielt fokus på ernæring. Fremtidige studier av sporelementer i T2D bør fokusere på endringer i sporelementnivåer over lengre perioder (inkludert prøver tatt før T2D er manifestert), på spesiering av sporelementer i ulike deler av kroppen, både intracellulært og ekstracellulært, og på hvordan bestemte glukosesenkende legemidler kan påvirke nivåer av spesielt essensielle sporelementer hos diabetespasienter.

Contents

Acknowledgements

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For the original scientific work included in this doctoral thesis, I used data and biological specimens from the third Nord-Trøndelag Health Survey (HUNT3), linked with the National Education Database and Income statistics for persons and families. The Nord-Trøndelag Health Study (HUNT Study) is a collaboration between the HUNT Research Centre (Faculty of Medicine, NTNU), Nord-Trøndelag County Council, Central Norway Health Authority, and the Norwegian Institute of Public Health.

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List of papers

This thesis is based on the following publications:

Paper I

A. Simić, T. Syversen, A.F. Hansen, B.O. Åsvold, T.M. Ciesielski, P.R. Romundstad, K. Midthjell, S. Lierhagen and T.P. Flaten**.** Trace elements in whole blood in the general population in Nord-Trøndelag County, Norway: The HUNT3 Survey. Manuscript in preparation.

Paper II

A. Simić, A.F. Hansen, B.O. Åsvold, P.R. Romundstad, K. Midthjell, T. Syversen, T.P. Flaten. Trace element status in patients with type 2 diabetes in Norway: The HUNT3 Survey. *Journal of Trace Elements in Medicine and Biology* 2017; **41**: 91-98.

Paper III

A.F. Hansen, A. Simić, B.O. Åsvold, P.R. Romundstad, K. Midthjell, T. Syversen, T.P. Flaten. Trace elements in early phase type 2 diabetes mellitus—A population-based study. The HUNT study in Norway. *Journal of Trace Elements in Medicine and Biology* 2017; **40**: 46-53.

1 Introduction

1.1 Trace elements in humans

Access to reliable information on the elemental contents of the human body is crucial to understand the roles these elements have in the maintenance of human health. All the elements present in the human body can be classified in several different ways: according to their physical and chemical properties, their abundance in the body, or their essentiality. The elements may be classified according to their chemical properties as metals, nonmetals and metalloids [1]. In addition, the elements may be grouped with respect to their redox capacity into redox active and redox inactive elements, which is very important for their mode of action in biological systems [2].

1.1.1 Element abundance in the human body

Regarding the elements' abundance in the body, there are several classifications suggested. Frieden (1985) grouped the thirty elements that compose the human body, calling them all essentials, into three categories. Six are bulk or structural, nonmetallic elements, hydrogen (H), oxygen (O), carbon (C), nitrogen (N), phosphorus (P) and sulphur (S), five are macrominerals, the metals sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca), and the nonmetal chlorine (Cl). Frieden subdivided the third category into trace elements: iron (Fe), zink (Zn) and copper (Cu), and ultra-trace elements: the nonmetals fluorine (F), iodine (I), selenium (Se), silicon (Si), arsenic (As) and boron (B), and the metals manganese (Mn), molybdenum (Mo), cobalt (Co), chromium (Cr), vanadium (V), nickel (Ni), cadmium (Cd), tin (Sn), lead (Pb) and lithium (Li). The total content of these ultra-trace elements in the adult human body is less than 10 mg [3].

Another classification divides the elements into major, minor and trace elements [4]. The first group, which comprises 96 % of human body, consists of H, O, C and N, while Na, K, Ca, Mg, P, S and Cl are regarded as minor elements and they together constitute 3.78 % of the body mass. The group of trace elements includes all the other naturally occurring elements, apart from the group of noble gases whose properties eliminate them from the class of potentially biologically active elements [4]. Peereboom ascribes the term “trace” to elements with concentration less than 100 mg/g in the human body [5]. Finally, the World Health Organization (WHO) has defined trace elements as those with concentrations not exceeding 250 μg/g of the matrix [6].

Although it would be more accurate to call the elements Mg and Ca “minor elements”, in this study we included them in the term “trace elements” for simplicity.

1.1.2 Essentiality of the elements

There is no consensus on the definition of essentiality of the elements in biological systems. This pertains particularly to the trace elements. Over time the concept of essentiality has been changing [7]. One of the oldest definitions, actually adopted from protein chemistry, states that an element is essential if it is constantly present in living tissues and its deficiency causes aberrations in the organism’s biological function. These aberrations are reversible and can be prevented or rectified by proper supplementation [4]. Development of advanced analytical techniques in the second part of the 20th century revealed essential function of elements present at part-per-million level and lower, thus increasing the number of essential elements [8]. In the last two decades of the last century many trace elements were suggested to be essential, including fluorine, boron, nickel and silicon, but it was impossible to determine whether some of the observed changes were really the result of low intakes causing malfunctions or the mineral supplements having pharmacological actions in the body [9]. According to the WHO, “an element is considered essential to an organism when reduction of its exposure below a certain limit results consistently in a reduction in a physiologically important function, or when the element is an integral part of an organic structure performing a vital function in that organism” [10]. Currently, the trace elements chromium, cobalt, copper, iron, manganese, molybdenum, and zinc, and the nonmetals selenium and iodine meet this definition [4, 7, 11, 12]. Trace elements like boron, lithium, nickel, vanadium and silicon do not meet WHO’s rigorous definition of essentiality, but are found to have beneficial effects on human health [7, 9, 11]. Recently, bromine has been suggested to be regarded as essential [13]. On the other hand, the essentiality of chromium has been seriously questioned and it has been suggested to be removed from the list of the essentials [14, 15].

1.1.3 Trace element determination in biological material

The analytical method is a crucial factor in the process of establishing trace element levels in different biological and environmental samples, such as tissues, body fluids, air, food and drinking water, due to the wide range of trace element concentrations and many different chemical species present in these matrices [12, 16, 17]. In addition to their concentrations, factors like chemical form, food source or dietary matrix, age, sex, nutritional state and interactions with other elements, influence the effects of trace elements [18]. Inductively

coupled plasma with mass spectrometry detection (ICP-MS) and related techniques for elemental analysis, with its multielement capacity, low detection limits, high sensitivity, wide linear range and capability for isotope and isotope ratio determination, meet the requirements for assessment of environmental or occupational exposures and health effects [19-23]. However, optimal results in trace element analysis do not solely depend on advanced analytical techniques. In particular, sample collection, storage and preparation are critical steps in trace element analysis that are susceptible to sample contamination, which is the most important source of error in trace element determination [24, 25]. For example, stainless steel needles, commonly used for routine blood collection, can contaminate samples with Cr, Co, Mn and Ni [26, 27]. If the samples have been stored in a manner that did not protect against species alteration/degradation, it makes employing even a remarkably powerful analytical technique for trace element determination pointless [17].

1.2 Trace elements in the general population

A number of diseases have been linked to trace element imbalances, deficiencies or metabolic disorders, and to exposure to toxic trace elements [28]. For example, nutritional deficiencies of iron, calcium and iodine are associated with anaemia [29], rickets [30] and endemic goitre [31], respectively. Insufficient dietary intake of copper may lead to neuropathy, impaired immune response and anaemia, while zinc deficiency, common in underdeveloped countries, affects the immune system, impairs DNA synthesis and leads to disorders in normal growth and development in pregnancy, childhood, and adolescence [32].

The major sources of exposure to trace elements for humans are air, food and water, and the exposure routes for entering a human body are by inhalation, ingestion or through the skin [33]. The natural background has a huge impact on the health of humans and other living organisms. Rapid economic development radically changes the environment [34], but also the lifestyle of the inhabitants. Establishing trace element blood levels in a general population can indicate potential causes of diseases related to natural and anthropogenic sources of these elements; it also provides a baseline for potential future biomonitoring that could assess and evaluate temporal changes in the trace element status in populations [35]. As stated by Underwood and Mertz, the common property of essential elements is that they normally occur and function in living tissues in low concentrations [36]. Studying the relationships between the levels of a high number of essential trace elements and numerous factors potentially influencing these levels in a single study poses several challenges. Including non-

essential elements makes it even more complicated as each of the elements has its specific mode of action, and can originate from different sources. Trace element status depends on numerous factors, such as age, sex, food intake, smoking habits, alcohol consumption, physical activity, occupational exposures, socioeconomic and health status [37-55]. Therefore, it is crucial in such surveys to provide detailed data on these parameters. It is also potentially important to have access to relevant regional geochemical data, such as the elemental composition of soil, air, and water [23, 35]. Biomonitoring of a total population-based cohort within a large health survey can provide reliable information on trace elements content and can help in quantifying the effects of several different factors within the general population, population groups and individuals [56].

Recently, studies on trace elements in different segments of the population have been conducted in several countries [40, 56-66], including Norway [67-70].

The Health Study in Nord-Trøndelag (HUNT), launched in 1984, is Norway's largest collection of health data about a population [71]. This total population-based cohort addresses a broad range of determinants related to lifestyle, prevalence and incidence of illness and disease and other health-related factors. Three so far completed health surveys, HUNT1, HUNT2, and HUNT3 include data from questionnaires, interviews, clinical measurements and biological samples (blood and urine). The HUNT3 Survey, which was carried out in 2006-2008, includes blood samples collected from survey participants suitable for analysis for a wide range of environmental chemicals [72].

1.3 Trace elements in diabetes (partly based on Simić & Flaten [73])

Diabetes mellitus, or simply diabetes is a chronic, highly prevalent non-communicable disease characterized by elevated glucose blood levels, resulting from disorders in insulin secretion and/or insulin action [74]. The disease is strongly linked to serious health complications, affecting the eyes, kidneys, nerves, heart and blood vessels, which result in reduced quality of life and premature mortality [75, 76]. According to the WHO, diabetes is diagnosed if the fasting plasma glucose is $\geq$ 7.0 mmol/L and/or the glucose concentration two hours after a 75 g oral glucose load is $\geq$ 11.1 mmol/L [6]. Diagnosing a subtype of diabetes in an individual usually depends on the circumstances present at the time of diagnosis and sometimes a single type of diabetes is not easily assigned to diabetic patients [76]. According to the current classification of the WHO, the main subtypes of diabetes are [6]:

- Type 1 diabetes (T1D), previously known as juvenile-onset diabetes or insulin-dependent diabetes, results from a cellular-mediated autoimmune destruction of the insulin-producing β-cells of Langerhans islets in the pancreas;
- Type 2 diabetes (T2D), formerly known as adult onset diabetes or non-insulin-dependent diabetes, encompasses individuals who have insulin resistance and usually have relative or, rarely, absolute insulin deficiency;
- Gestational diabetes (GD) is defined as diabetes or impaired glucose tolerance occurring for the first time in pregnancy;
- Other specific types [77]

The growing global epidemic of diabetes mellitus has become a very serious issue. In 2015 the International Diabetes Federation (IDF) estimated that 415 million adults worldwide live with diabetes, half of them being undiagnosed, and the projected number for 2040 is 642 million [78]. Statistics for the occurrence of diabetes in Norway are uncertain, the IDF's prevalence estimate for 2015 is 7.8% [78]. The Norwegian Institute of Public Health reports a prevalence of 4.3% of diagnosed diabetes in 2014; in addition, there is a considerable number of cases of undiagnosed diabetes [79]. It is estimated that 85-95% of people having diabetes have type 2 diabetes (T2D). In Norway, at least eight of ten cases are T2D [80]. There is a general consensus that a combination of genetic predispositions and environmental factors can lead to the onset of the disease, and changes in environmental factors are probable explanations for the observed alarming increase in the incidence of T2D [81]. 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 T2D stages of the disease [81]. In addition, exposure to various pollutants has been suggested to play a role in diabetes onset [81]. All these factors may also disturb the levels of essential and non-essential trace elements in the human organism and thus provoke harm. A considerable number of published studies on associations between essential and non-essential trace elements and T2D suggest that several elements may play important roles, either detrimental or beneficial.

Potential pathogenic mechanisms in T2D development involving trace elements include exposure to elevated levels of toxic elements [82] and disruption of essential metal-ion homeostasis [83]. On one hand, redox-active metals like iron, copper, chromium and cobalt may stimulate generation of reactive oxygen species (ROS), which subsequently may influence the disease through oxidative stress pathways. On the other hand, some redox-inactive elements, like arsenic, cadmium and lead, may indirectly contribute to the harmful

effects of ROS by preventing antioxidants' protective activity [2]. Alterations in levels of trace elements that have the potential to reduce oxidative stress, either by improving glycaemic control (chromium, copper, magnesium, selenium, zinc and vanadium) or by exhibiting effective antioxidant properties (manganese, selenium and zinc) could also be associated with disease prevalence [83].

1.3.1 **Essential minor and trace elements in type 2 diabetes**

Of special concern are essential elements that are necessary in central biochemical processes in the organism, but can be toxic if their levels are elevated. In human physiology, these elements can have structural, catalytic, regulatory or signalling functions. Contents of essential elements in the organism are under tight homeostatic control governed by the action of a variety of hormones and metal sensing and transporter proteins.

Magnesium and calcium are two minor essential elements. Calcium, together with sodium, is the major extracellular cation, while magnesium, along with potassium and zinc, is the major cation in the intracellular fluid compartment [84]. Both calcium and magnesium have been reported to be associated with T2D prevalence, but there are differences in the type and mechanisms of the reported associations. It has been hypothesized that T2D is associated with inversely correlated levels of calcium and magnesium, namely elevated calcium and supressed magnesium [85-87]. This can be because magnesium is a physiological calcium channel blocker. Reduced levels of magnesium can induce increased intracellular calcium concentrations [88]. Some studies have reported an inverse association between T2D and both calcium and magnesium [89, 90].

Magnesium, engaged in a number of biochemical processes in the organism, is predominantly an intracellular ion; 99% is located in the intracellular compartments, while only 1% is found in the extracellular fluid. Therefore, serum magnesium content is a poor indicator of total magnesium body load [91]. Magnesium is a cofactor in enzymes involved in carbohydrate metabolism. It also plays an important role in the regulation of insulin actions, including insulin-mediated glucose uptake by controlling insulin receptor affinity in the target tissues. As an essential cofactor in reactions involving phosphorylation, magnesium deficiency could impair the insulin signal transduction pathway [92]. A number of studies have reported a negative association between magnesium and diabetes, but explanations for these findings are not clear. One of the central questions is whether the hypomagnesemia, hypermagnesuria and low magnesium tissue levels predispose to T2D, or if such status is a consequence of the

disease. Some authors have linked magnesium depletion in T2D to a low magnesium intake in the Western diet [90, 93]. Specifically, foods rich in phosphate and calcium, alcohol consumption and high fat and high protein food decrease magnesium absorption, which subsequently may lead to impaired insulin regulation [94]. An association between magnesium homeostasis misbalance 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. In addition, low serum magnesium was 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 [95]. In turn, pro-inflammatory changes supress insulin signal transduction [96]. Some findings suggest hypomagnesemia in T2D to be an epiphenomenon. As the disease progresses the levels of insulin production is decreasing gradually. In light of the fact that insulin is a promoter of magnesium tubular absorption, one of the key sites for maintenance of Mg homeostasis, insulin deficiency may be the reason for lower magnesium blood levels as the disease develops [97].

Calcium has a cardinal role in cellular life. It exists in three basic forms in the organism: ionized, complexed to organic compounds and precipitated in inorganic salts. The balance among these forms is maintained by hormones and diet [84]. Calcium is a second messenger ion in many signal transduction pathways, including insulin signalling, thanks to the ability of the cells easily to detect changes in free calcium ion concentrations. Investigations on the role of calcium in T2D have reported associations between calcium imbalance and pancreatic β-cell malfunction, insulin sensitivity reduction and systemic inflammation, all conditions central to T2D pathogenesis [98]. However, studies of the associations between T2D and blood levels of calcium have produced 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 [98]. Calcium as a second messenger has an important signalling role for insulin action in insulin-responsive tissues, like skeletal muscles and adipose tissue [99], and some studies have reported an inverse association between insulin resistance and intracellular cytosolic calcium levels in insulin target tissues [98]. Insulin, in turn, can suppress calcium tubular reabsorption [88] and thus reduce calcium levels.

Iron, an integral component of many biomolecules, including cytochromes, oxygen-binding molecules and enzymes, can cause serious damage to the organism if not well balanced within the homeostatic range. What makes iron so useful is its capability to accept or donate electrons, and hence readily interchange between Fe^{2+} and Fe^{3+} forms [100]. The association between excess iron and diabetes is well established and might be explained by the oxidative action of iron. Haemochromatosis, a genetic disorder manifested as body iron overload, has been linked to diabetes in that as many as 60% of individuals with hereditary haemochromatosis develop T2D [101]. Elevated levels of ferritin, the major iron storage protein in the body, are reported in diabetic patients [102, 103], but also in metabolic syndrome and insulin resistance, both known to frequently precede T2D [104]. In addition, clinically elevated transferrin saturation, a measure of actual serum iron binding, is strongly associated with higher risk of T2D [105]. Some recent studies revealed an increased risk of diabetes associated with heme iron, which is the organic form of iron in the diet. This was explained by the higher bioavailability of heme iron and stronger effects on the body iron stores [106, 107]. However, if sufficient levels of iron are not acquired, metallation of proteins involved in glucose oxidation and glucose sensing will be affected. Iron deficiency has been clearly linked to obesity and overweight, major risk factors for T2D. This might be explained by higher demands for iron to support higher rates of lipid oxidation [108]. A recent Australian population-based study reported an association between diabetes and increased ferritin levels, but not increased transferrin saturation or serum iron levels; in nondiabetic adults with insulin resistance, elevated ferritin was found [109]. It is important to be aware of the very wide “normal” range of serum ferritin, commonly used in the studies on the association between iron and T2D [109, 110]. Within boundaries with 10-fold difference between lowest and highest “normal” level, there may be iron concentrations with health risks of which we are not currently aware [108]. These conflicting findings clearly show the need for more thorough investigation on the potential role and the mechanism of action of all iron-related parameters in T2D pathogenesis.

Zinc is incorporated in a myriad of proteins, some of which are enzymes that employ the catalytic properties of zinc, and the rest are proteins that utilize the structural or regulatory properties of zinc in interactions with other proteins, lipids and nucleic acids [111]. In humans, altered zinc levels have been linked to diverse health disorders. In the 1930s it was discovered that insulin crystals contain zinc [112]. Since then, possible roles of zinc in diabetes aetiology and progression has been widely studied, confirming links between the

disease and different aspects of zinc involvement in insulin storage, secretion and action, as well as its antioxidative function [113]. Inactive insulin is stored as a hexamer surrounding zinc ions in the secretory granules vesicles, located in the β cells of the Langerhans islets in the pancreas. Each hexamer binds two zinc ions [83], but vesicles contain, in addition, a 10-fold molar excess of zinc that is not coordinated to insulin [114]. Zinc transporters, like ZnT8, maintain uptake and high zinc concentrations in vesicles [83]. The family of metallothionein (MT) redox proteins is also involved in cellular zinc homeostasis, regulating its release into the cytoplasm [114]. Polymorphisms in the genes encoding for ZnT8 and MT-1 have been reported to be associated with T2D [113]. Zinc exerts insulin-mimetic activity, stimulating lipogenesis in adipose tissue and enhancing tissue sensitivity to insulin. By mediating oxidative stress which causes insulin resistance, zinc has been linked indirectly to regulation of insulin resistance [113].

Copper is the third most abundant transition trace element in humans, following iron and zinc. It is a strong Lewis base and because of its property to fluctuate readily between reduced (Cu (I)) and oxidized (Cu (II)) states, copper is an important cofactor at the catalytic sites of many enzymes [115]. Important Cu-containing enzymes are cytochrome c oxidase, involved in energy metabolism, ceruloplasmin that has a role in iron homeostasis, and copper/zinc superoxide dismutase that acts as an antioxidant [116]. Copper exerts both a pro-oxidant (following the Fenton reaction in ROS production) and an antioxidant action (through ceruloplasmin activity) [117]. Impaired copper homeostasis, either deficiency or overload, have been associated with altered glucose metabolism and diabetes. Copper deficiency impairs glucose utilization [118], while both higher ceruloplasmin and copper plasma levels have been found in diabetic subjects [116].

Chromium was among the last added to the list of elements that are essential for humans. Its essentiality has been ascribed to the role of trivalent chromium in carbohydrate and lipid metabolism [119]. Chromium deficiency associated with diabetes and insulin resistance has been reported [120, 121]. Some of the suggested metabolic pathways of chromium action are: increased number of insulin receptors; enhanced insulin binding to the target sites; receptor signalling increase by linking to chromodulin (a low-weight binding protein that potentiates the activity of insulin); and mediation of increased insulin sensitivity [122]. However, the mechanisms of action of chromium remain unclear. Furthermore, its status as an essential element has been questioned [119]. The studies that supported chromium being essential were of the following types: rodents with chromium-deficient diet; chromium absorption as a

function of diet; patients on total parenteral nutrition, since they develop impaired glucose utilization or intolerance; and studies on associations between chromium body transport and insulin action [119]. A recent systematic review and meta-analysis of the efficiency and safety of chromium supplements in diabetes concluded that chromium supplements improve glycaemic control by reducing glycated haemoglobin HbA1c, an indicator showing if diabetes is under control, and fasting plasma glucose [122]. Lately, it has been suggested that chromium does not satisfy either a nutritional or biochemical definition of essentiality and hence should be removed from the list of essential elements and rather be classified as "possibly essential" or "pharmacologically beneficial" [7, 15].

Selenium is a trace element essential for human health. As selenocysteine, it is a constituent of 25 selenoproteins that can be grouped into housekeeping and stress-related proteins. As enzymes, these proteins are mostly involved in antioxidative responses in the organism (glutathione peroxidases, thioredoxin reductases and methionine sulfoxide reductases), in thyroid hormone regulation (deiodinases), and selenium transport to organs (e.g. selenoprotein P) [123]. Increased oxidative stress linked to excess levels of ROS has been reported in diabetic patients, possibly related to hyperglycaemia. Selenium has shown anti-inflammatory, insulin-mimetic and antidiabetic properties. The capability of selenium to scavenge reactive oxygen species led to implementation of selenate supplements as protective against T2D in the 1990s [124]. However, a fierce debate on a potentially detrimental role of selenium in T2D onset took place after the Nutritional Prevention Cancer trial revealed, in a posthoc analysis, a two-fold increase in T2D incidence in the group consuming selenium supplements compared to the placebo group [125]. A recent study in mice has reported that both selenoprotein deficiency and overexpression of selenoproteins are associated with T2D development [126]. Rayman and Stranges argued for a U-shape relationship between selenium and T2D, suggesting possible harmful effects of selenium both below and above the homeostatic range [124].

1.3.2 **Non-essential trace elements in type 2 diabetes**

Arsenic is a redox inactive element that may indirectly contribute to the harmful effects of ROS by preventing the protective activity of antioxidants [2]. Some mechanisms of arsenic action associated with diabetes pathogenesis have been suggested, including oxidative stress, effects on calcium signalling, glucose uptake and transport, and gluconeogenesis [127]. Two groups of arsenic species are important in human exposure; organic (e.g. in seafood) and

inorganic (e.g. in drinking water) species [128]. Early studies reporting associations between arsenic and T2D were primarily conducted in high-exposure regions like Taiwan, Bangladesh and Mexico, with drinking water arsenic content above 100 µg/L [129-131], while studies on populations in lower-exposure regions [132-134] showed no association between arsenic and T2D. However, two recent Danish and Serbian studies on the effects of long-term exposure to low-levels of arsenic, originating from the drinking water, revealed a weak, but still significant association with T2D development [82, 135].

Vanadium has been classified by WHO as a probably essential trace element for humans [10], based on its demonstrated essential function in certain organisms. However, a specific functional role of vanadium in humans has not been proven so far. Vanadium is a bioactive transition metal with properties resembling phosphorus in biological systems [136]. The oxyanion vanadate is a phosphate analogue thought to bind to phosphoryl transfer enzymes inhibiting their action [137]. Vanadium compounds exhibit insulin-mimetic properties, including mitogenic and metabolic effects as well as stimulatory and inhibitory responses in cell differentiation [136]. A recent Chinese study on vanadium plasma levels in newly diagnosed T2D revealed significantly lower vanadium levels in diabetic subjects compared with controls [138]. Therapeutic effects of vanadium compounds have been tested in both type 1 and type 2 diabetes. Treatment with metavanadate improved insulin sensitivity in T2D and lowered the requirement for insulin in T1D [139]. However, a recent systematic review of the literature on vanadium sulphate supplements could not confirm the effectiveness of oral vanadium supplementation in improving glycaemic control in T2D [140]. The results were explained by poor quality of the studies considered in the review, including small sample size and short duration of treatment. Therefore, more rigorous and substantial future studies are necessary to evaluate the therapeutic value of vanadium in diabetes [140].

The question of whether abnormal levels of certain trace elements are the result of diabetes, a cause, or perhaps even a homeostatic attempt by the cell, tissue, organelle, or subject to rectify a parallel condition associated with the disease is debated [83]. In spite of an increasing number of studies on trace elements levels in diabetic patients, no consistent picture of their involvement in the disease has emerged so far. Some of the ways to address the question on when anomalous levels of these metals begin to appear in T2D development are either to measure trace elements levels over a longer period in a prospective study, or in the different stages of the disease in cross-sectional studies.

2 Research aims

The main aims of this thesis were:

1. Based on the HUNT3 Survey, to establish the background levels of 28 trace elements in whole blood samples collected in the general population of Nord-Trøndelag, and to evaluate possible associations between regional and other socio-demographic and lifestyle characteristics with the trace element concentrations (Paper I).
2. To investigate potential associations between trace element blood levels and prevalent type 2 diabetes, and possible effects of disease duration on trace element levels found to be associated with diabetes prevalence in the study (Paper II).
3. To investigate potential associations between trace element blood levels and prevalent type 2 diabetes in the early phase of diabetes, in previously undiagnosed, screening-detected type 2 diabetes (Paper III).

3 Methods

3.1 Study population

The participants for these three cross-sectional studies were selected from the HUNT3 Survey, one of the largest population health studies in Norway so far. From 1984-2008, all inhabitants aged ≥ 20 years in Nord-Trøndelag County, Norway, were invited to participate in three consecutive cross-sectional surveys: HUNT1 (1984-86), HUNT2 (1995-97) and HUNT3 (2006-08). For HUNT3, one important difference relative to HUNT1 and HUNT2 was collecting blood samples intended for studies of compounds that may indicate environmental exposure, including heavy metals, trace elements and persistent organic pollutants. These blood samples were collected from the residents of 14 urban and rural municipalities ranging from the coast to the inland-mountain area, out of 24 municipalities in Nord-Trøndelag County. Out of 50 807 adults participating in the HUNT3 Survey (54.1% attendance rate) [72], blood samples for trace element analysis were collected from 27 962 subjects.

3.1.1 Subject selection in the study on trace element blood levels in general population

Initially, we divided Nord-Trøndelag County into three geographical regions, inland-mountain, urban and coastal area (Figure 1), and we selected participants from the municipalities: coastal (Nærøy, Vikna, Flatanger, Leka and Fosnes), urban (Levanger and Steinkjer) and inland mountain (Røyrvik, Namsskogan and Grong).

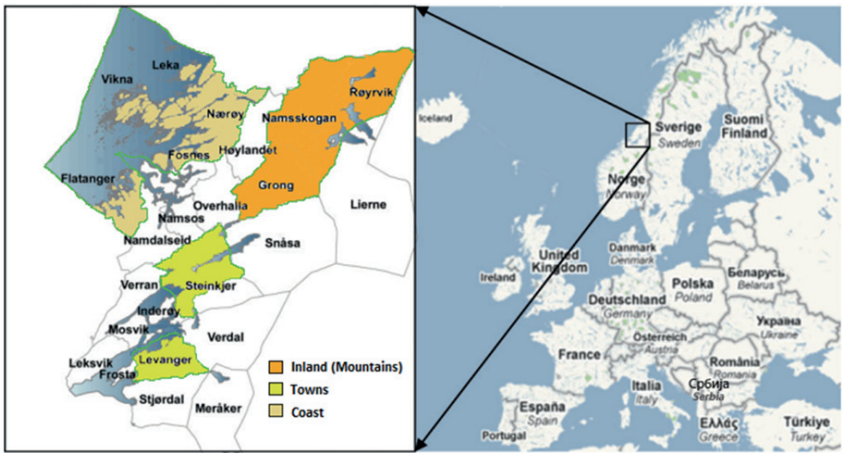

Figure 1. Map of Nord-Trøndelag County and the selected geographical regions in the study (Modified and reproduced with permission of the author Dr. Steinar Krokstad from [72]).

We applied region, sex and age stratified probability sampling design, oversampling participants from the mountain and coastal regions with smaller population, to provide reliable statistical estimates for these subpopulations. We randomly selected equal number of males and non-pregnant females from each of the three regions among those older than 19. Of the 16 808 adults aged 20-91 from the selected municipalities, who provided blood sample for the trace element analysis in the HUNT3 Survey, we selected 1 016 participants for the present study.

3.1.2 Population in the study on diagnosed T2D

For this study we selected participants from the same three groups of municipalities used in the general population study (section 3.1.1). Information on diabetes, age at diagnosis and glucose lowering treatment was self-reported in questionnaires, 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 measured. Those without known diabetes, but with elevated FINDRISC, underwent an oral glucose tolerance test [141]. T2D was defined as self-reported diabetes, excluding type 1 diabetes (T1D) as indicated by an index of GAD antibody levels (autoantibodies to glutamic acid decarboxylase) relative to a standard serum of ≥ 0.08, or by fasting C-peptide < 150 pmol/L [142]. The self-report of diabetes in the HUNT Study population has excellent validity [143]. Controls were selected among participants without known diabetes who had non-fasting glucose < 9.0 mmol/L. For participants with elevated FINDRISC who underwent the oral glucose tolerance test, we excluded as eligible controls those who had prevalent, but undiagnosed diabetes, impaired glucose tolerance or impaired fasting glucose. Among 522 eligible cases, we randomly selected 270 and frequency-matched them by sex and age (5-year intervals) with 615 controls.

3.1.3 Population in the study on undiagnosed, screening detected T2D

Participants in the HUNT3 Survey who had a high risk (at least 30% in the next ten years) for developing diabetes (FINDRISC ≥ 15) were invited to participate in a study of diabetes prevention, a part of a European multi-centre study "Diabetes in Europe – prevention through Lifestyle, Physical Activity and Nutrition" (DE-PLAN). As many as 5 428 (10.7%) of all participants were identified as being at high risk of developing diabetes and for 2 513 of them blood sample was drawn for the trace element analysis. Among these individuals 1 172

(46.6%) underwent an oral glucose tolerance test (OGTT) and in 157 of them diabetes diagnosis was established. Further, patients with newly diagnosed T1D, with no data on anti-GAD, and/or not available blood sample were excluded. The same criteria for control selection as in the study on diagnosed T2D were applied.

3.2 Potential confounders

The potential confounding factors that we used were chosen based on previously published associations with trace elements blood levels, regional distribution or T2D prevalence. The data were collected from the first questionnaire, which was filled out by the participants at home and delivered when they attended the basic health examination (residential area, age, sex, self-reported diabetes, smoking status, alcohol consumption, fatty-fish and milk intake and family history of diabetes), from interview with the participants at the health examination sites (ongoing pregnancy), from the basic clinical measurements at the health examination sites (weight, height, waist and hip circumferences, serum non-fasting glucose), and from the sub-study on diabetes [144] where all the participants with known diabetes or having FINDRISC ≥ 15 were invited to participate (fasting serum glucose, oral glucose tolerance test, C-peptide and anti-GAD). Information on education level and income was obtained from Statistics Norway after selected participants were linked to the National Education Database and Income statistics for persons and families.

T2D was defined as fasting serum glucose ≥7.0 mmol/L and/or 2-h oral glucose tolerance test (OGTT) serum glucose ≥11.1 mmol/L. Impaired glucose tolerance (IGT) was defined as fasting serum glucose <7.0 mmol/L and 2-h oral glucose tolerance test serum glucose OGTT ≥7.8, but <11.1 mmol/L. Impaired fasting glucose (IFG) was defined as fasting serum glucose <7.0 mmol/L and/or 2-h OGTT serum glucose ≥7.1, but <7.8 mmol/L. Serum glucose was analysed by hexokinase/G-G-PDH methodology [145], by Hemocue at the Central laboratory of Levanger Hospital (Levanger, Norway). T1D was distinguished from T2D based on the index value of autoantibodies to glutamic acid decarboxylase relative to a standard serum (anti-GAD) and C-peptide serum level. T1D was diagnosed if anti-GAD ≥ 0.08 or anti-GAD < 0.08 and C-peptide < 150 pmol/L [146]. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Height and weight were measured with the participants wearing light clothes without shoes: height to the nearest centimetre and weight to the nearest half kilogram. Waist-to-hip ratio, a measure of abdominal obesity, was calculated as waist circumference divided by hip circumference, both in cm. The circumferences were measured

with a tape measure to the nearest centimetre, with the participant standing and with arms hanging relaxed. The waist circumference was measured horizontally at the height of the umbilicus, and the hip circumference was measured likewise at the thickest part of the hip [72]. Regarding family history of diabetes, participants answered the question: Do your parents, siblings or children have, or have they had diabetes? (yes/no). The disease duration in years was determined from to the participants' answers to the question on their age when the disease was diagnosed and the calculated age when the blood sample was drawn. Information on treatment of T2D was retrieved from the self-administered questionnaire Q3 where diabetic patients answered the questions on current use of insulin (yes/no) and/or oral glucose-lowering medications – tablets (yes/no).

3.3 Sample collection and storage

In the HUNT3 Survey biological material was collected at the health examination stations and transported daily by courier to the biobank. Blood sampling followed a strict quality protocol [72]. Blood samples for the trace element analysis were collected during the period November 2006 – November 2007. Five blood samples were collected from each participant in Vacutainer tubes. In order to minimize possible contamination of trace elements originating from the needle and tubing, the samples used for trace element analysis were the last of these five tubes. Blood was drawn by use of needles for routine blood collection (Vacuette, Greiner Bio-One North America, Inc., Monroe, NC, USA) and collected in 7 mL glass blood collection tubes specially designed for trace element sampling, containing 158 USP units sodium heparin (Vacutainer; Becton, Dickinson & Co, Franklin Lakes, NJ, USA). Each trace element blood sample was further divided into seven 0.8 mL aliquots and transferred into 1 mL polypropylene Matrix 2D barcoded screw top storage tubes (Thermo Scientific); six of them were stored at -80 °C and one in liquid nitrogen. In our study the samples stored at -80 °C were analysed. The selected samples were sent on dry ice to the Department of Chemistry, Norwegian University of Science and Technology (NTNU), where they were stored at -20 °C until the analysis.

3.4 Sample preparation

The sample preparation was performed in a clean laboratory (ISO 6) to minimize contamination from the surroundings, paying strict attention to contamination control in all steps. One hour before the preparation, the blood samples were brought to room temperature and then stirred for homogenization. Approx. 0.7 mL blood sample was taken out with

pipette (Rainin E-Man Hybride, Mettler Toledo, Oakland, CA, USA) into 20 mL teflon vessels (TFM PTFE UC). Pipette tips (Bioclean) were washed with ultrapure water (PURELAB Option-Q, ELGA, UK) before use and the purity was checked by analysis of the pipette blanks. Precise weight was measured on an analytical balance (Sartorius, with Sartorius SartoCollect Software, Krugersdorp, South Africa). Then 1.0 mL 65% (V/V) ultrapure nitric acid was added using 5 mL bottle-top dispenser (Seastar Chemicals INC, Sidney, BC, Canada). The ultrapure nitric acid was produced at NTNU from nitric acid in pro analysis quality (Merck, Darmstadt, Germany) by use of a sub-boiling distillation system (SubPur, Milestone, Shelton, Connecticut, USA). Then samples were transferred to the digestion unit, and digested (UltraClave, Milestone). Samples were heated gradually up to 240 °C then left to cool down to room temperature. Digested samples were transferred into pre-cleaned 15 mL polypropylene vials (VWR, European Catalogue no. 525-0461, batch no. 142CB) and diluted with 13.5 mL ultrapure water (1:20) to achieve a final acid concentration of 0.6 M. The final weight was controlled by balance.

3.5 Trace element analysis

Trace element levels were measured using high resolution inductively coupled plasma – mass spectrometry (HR-ICP-MS) on a Thermo Finnigan Element 2 (Thermo Finnigan, Bremen, Germany). The sample introduction system consisted of an SC2-DX auto-sampler with ULPA filter, a prepFAST system, concentric PFA-ST nebulizer combined with a quartz micro cyclonic Scott spray chamber with auxiliary gas port, aluminium sample and skimmer cones, and O-ring-free quartz torch and 2.5 mm injector (Elemental Scientific, Omaha, NE, USA). 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, PS-ClBrI, made by different producers and delivered by Elemental Scientific were used for the instrument calibration, one serving as a calibrating solution (CS) and the other as a quality control (QC). Four different dilutions of calibrating solution PS-70 were prepared to cover the concentration ranges of the elements. They were all matrix matched with samples for acid strength (0.6 M nitric acid) and main elements by adding 160 mg/L sodium and 115 mg/L potassium. Sodium and potassium solutions were prepared from single element standard solutions (10 000 ppm, Spectrapure Standards AS,

Oslo, Norway). The internal standard (IS) containing 1 ppb of rhenium was automatically mixed with the sample in the prepFAST system. Elements were determined in three different resolutions, low (LR 400; beryllium, cadmium, cesium, gold, indium, lead, mercury, thallium, tin, and tungsten), medium (MR 5 000; boron, calcium, chromium, copper, gallium, iron, magnesium, manganese, molybdenum, nickel, rubidium, scandium, silver, strontium and zinc) and high (HR 10 000; arsenic, bromine, and selenium). The elements proved to be present as contaminants in the blood collection tubes were excluded from the analysis. That left 28 elements for establishing trace elements blood levels in the general population study. In addition, the elements with blood levels below the limit of detection in 33% or more of the study participants were excluded from the statistical analyses in all three studies. That left 25 elements for statistical analyses. In the study on diagnosed T2D, we excluded iron from the study results, because important parameters of iron status, such as ferritin levels and transferrin saturation were not available from the laboratory measurements.

3.6 Analytical quality control

To test for possible elements leaching and contamination, blood collection tubes, pipet tips, polypropylene vials, flasks and ultrapure acid used in the samples manipulation were checked prior to the analysis. Ten blood collection tubes were tested by holding them for eight days at room temperature with 0.9% NaCl water solution of supra pure grade. Prior to the analysis the NaCl solution was diluted three times and preserved with 0.1 M HNO_3. This matrix was equal to the calibration solution used in the samples analysis. In each sample batch, three blanks were prepared by adding 0.9% NaCl water solution directly to the polypropylene vials. In order to check for instrumental drift, one of the multi-element standards was analysed for every 20 samples. In each analysis batch, one sample of the certified reference material Seronorm Level 1 (Sero, Norway, Table 1) and two samples of one healthy volunteer blood specimen were analysed to verify the accuracy of the instrument. Blanks and control samples had an alternating position in each batch. The stability of the instrument was controlled by checking the internal standard concentrations and argon signals.

3.7 Statistical analyses

3.7.1 Study on trace element levels in the general population

The data were analysed using Stata 13 (StataCorp, TX,) and SPSS 24 (SPSS, Inc., Chicago, IL). All statistical tests were two-sided. Sampling weights based on sex, age and geographic

areas were calculated and used in all analyses to provide accurate estimates reflecting the population in the three regions of Nord-Trøndelag. We examined the distribution for all the elements and determined ranges, means, geometric means (GM), medians, and percentiles (5th, 25th, 75th, and 95th percentiles). A descriptive analysis was conducted to show the distribution of general characteristics in the study population, total and stratified by sex. The Spearman's rank-correlation test was used to assess the correlations between concentrations of the trace elements. In the further statistical analyses, natural logarithm transformation was used for the elements that were not normally distributed. We applied three multiple linear regression models to identify associations between trace element blood concentrations and three geographical areas (coastal – the reference, urban and inland-mountains). In the first model, we adjusted for sex and age (10-year categories). Then, multivariable analysis was performed adjusting for potential socio-demographic factors previously known to be associated with trace elements blood levels: body mass index (BMI, categorized according to WHO recommendations as < 25.0, 25.0-29.9, and ≥ 30 kg/m^2), education (< 10, 10-12 and ≥ 13 years), and income level (given as after-tax equivalent income – EU-equivalent scale, divided into quartiles). Finally, we further adjusted for intercorrelated trace element levels based on the Spearman's rank correlation coefficient $|r_s|> 0.5$. The levels of significance were corrected using the Bonferroni multiple-comparisons procedure. Considering a total of 50 tests performed (for each of 25 elements and two area categories in contrast to the reference category), we set the level of significance α at 0.05/50 = 0.001. Additionally, we applied a univariate general linear model to compare trace elements blood levels at different demographic and life-style categories. In the first model, we presented crude estimates, then we adjusted for sex and age, while in the third model we adjusted further each of the variables for all the others, as fixed at the mean level: geographical region, waist-to-hip ratio (divided into tertiles, ≤ 0.88, 0.89-0.93, and ≥ 0.94), BMI, education, income, smoking status (never-smokers, former smokers and smokers), fatty-fish consumption (< 4 meals monthly, 1-3 meals weekly and ≥ 4 meals weekly), and alcohol intake (divided into quartiles of daily amount of grams of alcohol consumption, 0 - abstainers, 0.2 – 2.6, 2.7 - 6.0 and > 6.0), and moderately/highly correlated trace elements, based on the Spearman's correlation coefficient, $|r_s| > 0.5$. *P*-values were corrected for multiple testing using the Dunn–Šidák correction procedure, and $P < 0.05$ was considered statistically significant. Daily amount of alcohol consumption in grams was calculated based on the participants answers to the following question: "How many drinks of beer, wine or spirits do you usually drink in the course of 2 weeks?". To calculate daily amount of alcohol for each type of beverage, the reported

consumed amount was multiplied by the alcohol content of the specified beverage (16 g for one can/bottle/glass of beer, 12 g for one glass of wine and 12 g for one standard drink of spirits) and the numbers were summed up to give total average alcohol intake per day [147]. The analyses were also performed after excluding outliers. For the essential elements we determined the minimum and maximum outliers (1st quartile – 1.5 * interquartile range and 3rd quartile + 1.5 * interquartile range, respectively), and for the non-essential maximum outliers (3rd quartile + 1.5 * interquartile range).

3.7.2 Studies on type 2 diabetes

In the studies on T2D, trace element levels were categorized into quartiles (tertiles for chromium and tantalum since 25-33% of the samples had levels below the detection limit).

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: BMI (categorized according to WHO recommendations as < 25.0, 25.0-29.9, and ≥ 30 kg/m^2) waist-to-hip ratio (categories based on the tertile distribution among controls, ≤ 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), and 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 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 in the study on diagnosed T2D we found to be associated with T2D prevalence, we examined whether disease duration (in years, analysed as a continuous variable) 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, namely BMI, waist-to-hip ratio, first-degree family history of diabetes, smoking habits, living area, education, and economic status. Finally, 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,), and corrections for multiple testing were performed using R 3.2.2 (Foundation for Statistical Computing, Vienna, Austria). All statistical tests were two-sided and $P < 0.05$ was considered statistically significant.

3.8 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.

4 Results

4.1 Paper I – Trace elements in whole blood in the general population in Nord-Trøndelag County, Norway: The HUNT3 Survey

Using whole blood samples and data from the HUNT3 Survey, we determined the concentrations of 28 elements in blood samples in the general population of Nord-Trøndelag County, Norway. We assessed the associations between 25 trace element concentrations and three geographical areas, coast, urban and inland-mountain, and estimated differences among estimated trace element levels at different socio-demographic and lifestyle categories. Excluding five participants (samples were missing or contained low blood volume), a total of 1011 subjects, 505 women and 506 men, were included in the study.

The multiple linear regression model adjusted for sex and age showed that people living in the urban and inland-mountain areas had significantly ($P< 0.0001$) lower levels of arsenic (-47.2% and -55.5%, respectively), mercury (-29.5% and -32.5%), and bromine (-11.8% and -11.7%), and higher levels of gallium (10.5% and 21.8%), than people living in the coastal area. These associations were not substantially changed after further adjustment for BMI, education and income in the second model. Adjusting arsenic for correlated levels of mercury, the associations were slightly attenuated, but remained significant in both the urban (-33.1%) and inland-mountains (-41.0%) populations. Adjusting mercury for correlated levels of arsenic and selenium, the association was attenuated, but still significant in inland-mountains population (-14.7%, $P < 0.001$) and borderline significant in the urban population (-13.1%, $P = 0.0012$).

Comparing to the coast, iron and zinc blood concentrations were significantly higher in the urban population. Levels of calcium, silver and tin were lower, while rubidium and thallium levels were higher in the population living in the inland-mountain region. Lead concentrations were 15% higher in the inland-mountains population in both models. Selenium concentrations in the urban population, in the first two models were 4.6% and 5.8% lower, respectively, but after selenium concentrations were further adjusted for mercury, no significant association was found. For the remaining elements, cadmium, chromium, copper, gold, indium, magnesium, manganese, nickel, and strontium, we found no statistical evidence for associations with geographical area.

We estimated geometric/arithmetic means (GM/AM) of 25 trace element blood concentrations at different variables' categories, employng three models, crude, adjusted for sex and age, and then further adjusted for general demographic and lifestyle factors, geographical area, body mass index, waist-to-hip ratio, income, education level, smoking status and alcohol and fatty-fish consumption.

In the age-adjusted model, estimated concentrations of boron, bromine, cadmium, calcium, copper, manganese, nickel, silver, strontium, and tin were higher in women, and the concentrations of cesium, gallium, iron, lead, magnesium, mercury, rubidium and zinc were higher in men.

There was a trend for increasing concentrations with increasing age for arsenic (although the differences were not significant in the fully adjusted model), boron, cadmium, cesium, gold, lead, mercury and silver. For bromine, calcium, chromium, copper, gallium, indium, iron, magnesium, manganese, molybdenum, nickel, rubidium, selenium, strontium, thallium, tin and zinc, there were only small variations with age, variations that were significant in some age groups for calcium, iron, rubidium, selenium, strontium and zinc.

In current smokers, the concentration of cadmium was more than fourfold higher than in never-smokers in the sex and age adjusted model, and in former smokers, it was about 50% higher. The concentration of gold was slightly higher in both current and former smokers than in never-smokers. The concentrations of copper, lead, magnesium, rubidium, and silver were slightly higher only in current smokers. Boron, bromine, manganese, and selenium levels were slightly lower in current smokers than in never-smokers. We found the same associations in all statistical models, but for selenium in the crude model the association with current smoking was not significant. Cesium levels were significantly lower in current smokers than in never-smokers only in the fully adjusted model.

Clear positive associations with alcohol intake were found for boron, cesium, lead, mercury, and silver. In addition, the concentrations of chromium, selenium and strontium were slightly higher for the highest quartile of alcohol intake, comparing to the abstainers.

Regarding fatty-fish consumption, the blood concentrations of especially arsenic and mercury, but also of bromine, cesium, selenium and tin, increased with increasing intake. For arsenic and mercury, the differences in concentrations with increasing fatty-fish intake were smaller in the fully adjusted model (when arsenic was adjusted for blood mercury, and mercury for arsenic and selenium) than in the sex and age adjusted model.

For BMI and waist-to-hip ratio, we found a negative association for bromine and calcium, and a positive association for iron. For magnesium, strontium and zinc, there was a positive relationship with BMI, but not with waist-to-hip ratio. Differences between BMI groups, either in the crude or in the adjusted models, were also found for boron, cesium, copper, gallium, lead, mercury, molybdenum, and tin.

We found decreasing cadmium and increasing selenium and boron concentrations across the increasing education and economic status categories. Differences between some of the education and/or economic status strata were also found for cesium, chromium, copper, lead, magnesium and rubidium.

The results of the statistical analyses additionally performed after excluding outliers did not appreciably change the results (data not shown).

4.2 Paper II – Trace element status in patients with type 2 diabetes in Norway: The HUNT3 Survey

Using whole blood samples and data from the HUNT3 Survey, we examined the association between prevalent T2D and the concentrations of 25 trace elements, and the relationships between T2D duration and blood levels of the trace elements that we found to be related to T2D prevalence. Excluding nine participants (samples were missing or contained low blood volume and/or were without necessary covariate data), a total of 267 diabetic patients and 609 controls were included in the study. 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.

In the conditional logistic regression analysis, 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. 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 associations were 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. In both the age- and sex-adjusted and multivariable models, the association with increasing diabetes duration was significantly positive for calcium blood concentration. 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.

4.3 Paper III – Trace elements in early phase type 2 diabetes mellitus – A population-based study. The HUNT Study in Norway

Using whole blood samples and data from the HUNT3 Survey, we examined associations between trace element blood levels and undiagnosed, screening-detected T2D. Excluding 29 participants (samples were missing or contained low blood volume and/or were without necessary covariate data), a total of 128 cases and 755 controls were included in the study.

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. 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 $P_{trend} < 0.05$. The multivariable adjusted

odds ratios (OR) comparing the highest tertile/quartile to the lowest tertile/quartile were (95% CI in parentheses) 0.52 (0.27-0.44) for bromine, 2.78 (1.55-4.99) for chromium, 2.97 (1.34-6.60) for iron, 2.24 (1.18-4.26) for nickel, 2.32 (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 (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, corrected for multiple testing using the Benjamini-Hochberg procedure and adjusted for age and sex, persisted as statistically significant associations, for bromine, chromium, iron, nickel and zinc the associations. Adjusted for additional confounders, only chromium remained significant after correction for multiple testing, while the associations for iron and silver showed borderline significance. 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.

5 Conclusions and future perspectives

The findings from this thesis are presented as three original epidemiological studies assessing environmental exposures and health effects of trace elements in a general population. The first study is based on trace element status in blood samples as the outcome of interest and different socio-demographic and lifestyle characteristics as the exposures of interest, while the two other studies are based on trace element blood levels as the exposures of interest and type 2 diabetes as the outcome of interest. Diabetes duration was an additional key element in the study on diagnosed type 2 diabetes.

5.1 Paper I

Our study provides information on 28 trace element whole blood levels in the general population of Nord-Trøndelag County, and assesses regional differences between populations living on the coast, in urban and in inland-mountain areas. For the first time, whole blood concentrations of a large number of trace elements were examined in such a large, total-population based Norwegian cohort. Our results demonstrate a considerable influence of regional geochemical characteristics on the blood concentrations of several elements, particularly of elements attributed to the marine environment, namely As, Hg, Br, B and Se. For many elements, we also found differences in blood concentrations according to the participants' lifestyle and socio-demographic indices, which shows the importance of considering these characteristics in evaluation of exposure to trace elements. Our results suggest low exposure to toxic trace elements in the residents of Nord-Trøndelag County, Norway.

5.2 Paper II

Our study on trace element blood levels in diagnosed type 2 diabetes 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.

5.3 Paper III

Our study on trace element blood levels in the early phase of type 2 diabetes 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.

5.4 Final comments

In future studies on trace elements in the general population, emphasis should be placed on well-characterized prospectively followed population-based cohorts (such as the HUNT population), where detailed information is available on a wide range of socio-demographic and lifestyle characteristics, paying particular attention to nutritional factors and regional geochemical data, using state-of-the-art analytical techniques and methods. Future studies on trace element associations with type 2 diabetes should focus on changes in trace element levels over longer periods and in different phases of the disease, 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.

All our data on trace element levels in more than 1500 participants from the HUNT3 population is made openly available in the HUNT database, and we hope that this can benefit future research on trace elements, including environmental and occupational exposures and their effects on human health.

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Paper I

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Trace elements in whole blood in the general population in Nord-Trøndelag County, Norway: The HUNT3 Survey

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Short title: Trace Element Status in general population of Nord-Trøndelag County in Norway

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Abstract

Objective. This study aimed to determine the concentrations of 28 elements in whole blood samples in the general population of Nord-Trøndelag County, Norway, collected in the third Nord-Trøndelag Health Survey (HUNT3), and to investigate how the element concentrations vary with geographical area as well as demographic and lifestyle factors.

Methods. In this cross-sectional study, we applied region, sex and age stratified probability sampling design. Among adults aged 20-91, we randomly selected equal number of men and women from each of three geographical regions: coastal, urban, and inland-mountain; and from each of six age categories. We used multivariable linear regression to estimate associations between geographical regions and trace element blood concentrations, and a univariate general linear model to estimate element concentrations in subjects in different demographic and lifestyle categories. In total, blood samples from 1011 individuals were analyzed by high-resolution inductively coupled plasma-mass spectrometry.

Results. The concentrations ranges were generally similar to those observed in other recent surveys. People living on the coast had significantly ($P < 0.0001$) higher blood levels of As, Br, and Hg, and lower levels of Ga, compared to those living in the urban and inland-mountain regions. Compared with the coastal population, Fe and Zn blood concentrations were higher, and Se lower in the urban population. Levels of B, Ca, Ag, and Sn were lower, while Cs, Pb, Mo, Rb, and Tl levels were higher in the population living in the inland-mountain region, comparing to the coast. The most significant ($P < 0.001$) associations between trace elements and socio-demographic and lifestyle characteristics were found for: sex (age adjusted) for B, Br, Cd, Ca, Cu, Ga, Fe, Pb, Mg, Mn, Hg, Rb, Ag, Sn and Zn; age (sex adjusted) for As, B, Cd, Cs, Au, Pb, Hg, Rb, Ag and Sr; education and economic status for B, Cd and Se; waist-to-hip ratio for Br; body mass index for Br, Ca, Fe, Mg and Zn; alcohol intake for B, Cs, Pb, Hg, Se and Ag; smoking status for B, Cd, Pb, Mn and Rb, and Mn; fatty-fish intake for As, Hg, and Se.

Conclusions. The blood concentrations determined in this large, total-population based cohort suggest generally a low exposure to toxic elements. Our results imply that geographical area, lifestyle, and several socio-demographic characteristics markedly influence the blood concentrations of several trace elements in humans, particularly for the elements As, Hg, Br, B, and Se, for which the marine environment may be an important source of exposure. Future studies should focus on well-characterized prospectively followed population-based cohorts, where detailed information is available on a wide range of socio-demographic and lifestyle characteristics, paying particular attention to nutritional factors and regional geochemical data.

Introduction

Several diseases have been linked to trace element imbalances and deficiencies, and to exposure to toxic trace elements [1]. The major sources of exposure to trace elements for humans are food, water and air, and the exposure routes are by ingestion, inhalation, or through the skin [2]. Establishing trace element blood levels in a general population can help elucidate potential causes of diseases related to natural and anthropogenic sources of these elements; it can also provide a baseline for potential future biomonitoring that could assess and evaluate temporal changes in the trace element status in populations [3]. As stated by Underwood and Mertz, the common property of essential elements is that they normally occur and function in living tissues in low concentrations [4]. Studying the relationships between the levels of a high number of essential trace elements and numerous factors potentially influencing these levels in a single study poses several challenges. Including non-essential elements makes it even more complicated as each of the elements has its specific mode of action, and can originate from different sources. Therefore, it is crucial in such surveys to provide detailed data on demographic characteristics and lifestyle habits, like sex, age, body mass index (BMI), socioeconomic status, and dietary habits including smoking, alcohol consumption, and food intake frequency. It is also potentially important to have access to relevant regional geochemical data, such as the elemental composition of soil, air, and water [3]. Biomonitoring of a total population-based cohort within a large health survey can provide reliable information on trace element contents and may elucidate potential health determinants within the general population, population groups and individuals [5].

Recently, studies of trace elements in different segments of the population have been conducted in several countries [5-16], including Norway [17-20].

In this study, we used whole blood samples collected in the large population-based HUNT3 Survey [21, 22]. In addition to estimating background levels for this population, a major focus was on assessing regional differences in trace element levels. Further, we investigated the relationships between the blood concentrations and various socio-demographic and lifestyle characteristics available in the HUNT database: sex, age, body mass index (BMI), waist-to-hip ratio, smoking status, fatty-fish intake and alcohol consumption.

Materials and methods

Study population

The participants for this cross-sectional study were selected from the third wave of the Nord-Trøndelag Health Study (HUNT3), one of the largest population-based health studies conducted in Norway to date. In HUNT, the entire adult population in Nord-Trøndelag County, geographically situated in the central part of Norway (Figure 1), was invited to participate in three consecutive cross-sectional surveys: HUNT1 (1984-86), HUNT2 (1995-97) and HUNT3 (2006-08) [22]. In HUNT, data has been gathered for a wide range of factors through questionnaires, interviews, clinical examinations and collection of blood and urine samples. In the HUNT3 Survey, several whole blood samples were collected and stored in a recently constructed state-of-the-art biobank. Among the 50 807 adults participating in the HUNT3 Survey (54.1% attendance rate) [21], blood samples for trace element analysis were collected from 27 962 subjects.

We selected participants from three regions of Nord-Trøndelag County (Figure 1): coastal municipalities (Nærøy, Vikna, Flatanger, Leka and Fosnes), urban (Levanger and Steinkjer) and inland-mountain municipalities (Røyrvik, Namsskogan and Grong). We applied region, sex and age stratified probability sampling design, and randomly selected equal numbers of men and women from each of the three regions, and from each of six age categories. Of the 16 808 individuals who met the eligibility criteria (age $\geq$ 20, living in the selected municipalities, and non-pregnant), 1016 participants (6.0%) were selected. We oversampled those living in the inland-mountain and coastal regions, and those aged 20-39 and $\geq$ 70, based on their smaller share of the population.

Covariate data were collected from a questionnaire which the participants had filled out at home and delivered when they attended the basic health examination (residential area, age, sex, smoking status, alcohol and fatty-fish consumption), from the interview performed at the health examination sites (current pregnancy), and from the clinical measurements at the health examination sites (weight, height, and waist and hip circumferences). Information on education level and income was obtained from Statistics Norway.

Blood sample collection and storage

Whole blood samples were collected at the health examination stations and transported daily by courier to the biobank. Blood sampling followed a strict quality protocol [21]. Five blood

samples were collected from each participant using needles for routine blood collection (Vacuette, Greiner Bio-One North America, Inc., Monroe, North Carolina). In order to minimize possible contamination of trace elements originating from the needles, the samples for trace element analysis were collected as the last of the five vacutainer tubes, a "trace element free" tube containing sodium heparin (Vacutainer; Becton, Dickinson & Co, Cat. no. 367735, Franklin Lakes, NJ, USA). 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. The selected samples were shipped on dry ice to our laboratory, where they were stored at -20 °C until analysis.

Trace element analysis

The sample preparation was performed in a clean laboratory (ISO 6) to minimize contamination from the surroundings, paying strict attention to contamination control in all steps. Approximately 0.7 mL of blood was pipetted out (Rainin E-Man Hybride, Mettler Toledo, Oakland, CA, USA) into 20 mL teflon vessels (TFM PTFE UC). The pipette tips (Bioclean) were washed with ultrapure water (PURELAB Option-Q, ELGA, UK) before use. The precise weight of each blood sample was measured (Sartorius balance, with Sartorius SartoCollect Software, Krugersdorp, South Africa) and converted back to volume by multiplying with 1.06 g/mL (the average density of whole blood). Then 1.0 mL ultrapure concentrated nitric acid was added using a 5 mL bottle-top dispenser (Seastar Chemicals, Sidney, BC, Canada). The ultrapure nitric acid was produced at NTNU from nitric acid (proanalysis grade, Merck, Darmstadt, Germany) using a quartz sub-boiling distillation system (SubPur, Milestone, Shelton, Connecticut, USA). The samples were then digested using a high performance microwave reactor (UltraClave, Milestone). The digested samples were transferred into pre-cleaned 15 mL polypropylene vials (VWR, European Catalogue no. 525-0461, batch no. 142CB) and diluted with 13.5 mL ultrapure water to achieve a final acid concentration of 0.6 M. Trace element concentrations were measured using high resolution inductively coupled plasma – mass spectrometry (HR-ICP-MS, Thermo Finnigan Element 2, Thermo Finnigan, Bremen, Germany). The sample introduction system consisted of an SC2-DX auto-sampler with ULPA filter, a prepFAST system, concentric PFA-ST nebulizer combined with a quartz micro-cyclonic Scott spray chamber with auxiliary gas port, aluminium sample and skimmer cones, and O-ring-free quartz torch and 2.5 mm injector (Elemental Scientific, Omaha, NE, USA). The radio frequency power was set to 1350 W; nebulizer and T-connection sample gas flow were 0.75 and 0.55 L/min, respectively. Cooling

gas flow was 15.5 L/min; auxiliary gas flow 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) 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 the element concentration ranges. The solutions were matrix matched for 0.6 M nitric acid and main element concentrations (160 mg/L Na and 115 mg/L K). Na- and K-solutions were prepared from single element standard solutions (10 000 ppm, Spectrapure Standards AS, Oslo, Norway). An internal standard containing 1 µg/L of rhenium was automatically mixed with the sample in the prepFAST system. The elements were determined at three different resolutions, low (LR 400; Be, Cd, Cs, Au, In, Pb, Hg, Tl, Sn, and W), medium (MR 5 000; B, Ca, Cr, Cu, Ga, Fe, Mg, Mn, Mo, Ni, Rb, Sc, Ag, Sr, and Zn), and high (HR 10 000; As, Br, and Se). Elements found to be present as contaminants in the blood collection tubes were excluded from the analysis, leaving a total of 28 elements in the study. In addition, the elements with blood levels below the limit of detection in 33% or more of the study participants were excluded from further statistical analysis.

Analytical quality control

To test for possible element leaching and contamination, blood collection tubes, pipet tips, polypropylene vials, flasks and ultrapure acid used in the samples manipulation were checked prior to the analysis. In each sample batch, three blanks were prepared by adding 0.9% NaCl solution directly to the polypropylene vials. In order to check for instrumental drift, one of the multi-element standards was analysed for every 20 samples. In each analysis batch, one sample of the certified reference material Seronorm Level 1 (Sero, Norway, Table 1) and two samples of one healthy volunteer blood specimen were analysed to verify the accuracy of the instrument. Blanks and control samples had an alternating position in each batch. The stability of the instrument was controlled by checking the internal standard concentrations and argon signals.

Statistical analyses

The data were analysed using Stata 13 (StataCorp, TX,) and SPSS 24 (SPSS, Inc., Chicago, IL). All statistical tests were two-sided. Sampling weights based on sex, age and geographic areas were calculated and used in all analyses to provide accurate estimates reflecting the population in the three regions of Nord-Trøndelag.

In the statistical analyses, natural logarithm transformation was used for the elements that were not normally distributed. We applied three linear regression models to study associations between trace element blood concentrations and three geographical areas (coastal, urban and inland-mountain). In the first model, we adjusted for sex and age (10-year categories). Then, multivariable analysis was performed adjusting for potential socio-demographic factors previously reported to be associated with trace element blood levels: body mass index (BMI, categorized according to WHO recommendations as < 25.0, 25.0-29.9, and ≥ 30 kg/m^2), education (< 10, 10-12 and ≥ 13 years), and income level (given as after-tax equivalent income – EU-equivalent scale, divided into quartiles). In the third model, we further adjusted for intercorrelated trace elements levels (Spearman's rank correlation coefficient $|r_s|> 0.5$). The levels of significance were corrected using the Bonferroni multiple-comparisons procedure. Because the total number of tests were 50, we set the level of significance at 0.05/50 = 0.001.

Additionally, we applied a univariate general linear model to estimate and compare trace element blood levels for different demographic and lifestyle categories. In the first model, we calculated crude estimates, in the second, we adjusted for sex and age, while in the third model we further adjusted for the following variables: geographical region, waist-to-hip ratio (divided into tertiles, ≤ 0.88, 0.89-0.93, and ≥ 0.94), BMI, education, income, smoking status (never smokers, former smokers and current smokers), fatty-fish consumption (< 4 meals monthly, 1-3 meals weekly and ≥ 4 meals weekly), and alcohol intake (divided into quartiles of daily amount of grams of alcohol consumed: 0 (abstainers), 0.2-2.6, 2.7-6.0 and > 6.0 g/day), and the intercorrelated trace elements (Spearman's correlation coefficient, $|r_s| > 0.5$). Daily amount of alcohol consumption in grams was calculated based on the participants' answers to the following question: "How many drinks of beer, wine or spirits do you usually drink in the course of 2 weeks?". To calculate the daily amount of alcohol for each type of beverage, the reported amount was multiplied by the alcohol content of the specified beverage (16 g for one can/bottle/glass of beer, 12 g for one glass of wine and 12 g for one standard drink of spirits) and the numbers were summed up to give the total average alcohol intake per day [23]. *P*-values were corrected for multiple testing using the Dunn-Šidák correction procedure, and $P < 0.05$ was considered statistically significant. The analyses were also performed after excluding outliers. For the essential elements we determined the minimum and maximum outliers (1st quartile – 1.5 * interquartile range and 3rd quartile +

1.5 * interquartile range, respectively), and for the non-essentials maximum outliers (3rd quartile + 1.5 * interquartile range).

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 five participants (samples were missing or contained low blood volume), a total of 1011 subjects, 505 women and 506 men, were included in the study. Characteristics of the participants are shown in Table 2. Descriptive statistics for the concentrations of the 28 trace elements are summarized in Table 3. Ten of the trace element pairs showed correlation coefficient $|r_s| > 0.5$ when we tested for bivariate correlations by Spearman's rank correlation test: As – Hg: $r_s = 0.611$, Ca – Fe: $r_s = -0.588$, Cs – Rb: $r_s = 0.539$, Cr – Ni: $r_s = 0.769$, Fe – Zn: $r_s = 0.559$, and Hg – Se: $r_s = 0.535$.

Differences in trace element blood concentrations between geographical areas

The relationships between blood concentrations in the three geographical areas are listed in Table 4 (Be, Sc and W were excluded from the analysis due to concentrations < LOD in over 33% of the samples). The multiple linear regression model adjusted for sex and age showed that people living in the urban and inland-mountain areas had significantly ($P < 0.0001$) lower levels of As (-47.2% and -55.5%, respectively), Hg (-29.5% and -32.5%), and Br (-11.8% and -11.7%), and higher levels of Ga (10.5% and 21.8%), than people living in the coastal area. These associations were not substantially changed after further adjustment for BMI, education and income in the second model. Adjusting As for correlated levels of Hg, the associations were slightly attenuated, but remained significant in both the urban (-33.1%) and inland-mountain (-41.0%) populations. Adjusting Hg for correlated levels of As and Se, the association was attenuated, but still significant in the inland-mountain population (-14.7%, $P < 0.001$) and borderline significant in the urban population (-13.1%, $P = 0.0012$).

Comparing to the coast, Fe and Zn blood concentrations were significantly higher in the urban population (Table 4). Levels of Ca, Ag and Sn were lower, while Rb and Tl levels were higher in the population living in the inland-mountain region. Lead concentrations were 15% higher in the inland-mountain population in both models. Se concentrations in the urban population, in the first two models were 4.6% and 5.8% lower, respectively, but after Se concentrations were further adjusted for Hg, no significant association was found. This may possibly be explained by Se being an antagonist of Hg, affecting absorption, distribution and elimination of Hg [24]. For the remaining elements, Cd, Cr, Cu, Au, In, Mg, Mn, Ni, and Sr, we found no statistical evidence for associations with geographical area. Unadjusted blood levels of As, Hg, Br and Se as a function of the region and fatty-fish intake are presented in Figures 2-5, respectively.

Relationships between trace element concentrations and lifestyle and socio-demographic characteristics

The means, geometric or arithmetic (GMs, AMs), with 95% confidence intervals for different lifestyle and socio-demographic indices are summarized in supplementary Tables S1 to S25. In the age-adjusted model, means of B, Br, Cd, Ca, Cu, Mn, Ni, Ag, Sr, and Sn were higher in women, and the concentrations of Cs, Ga, Fe, Pb, Mg, Hg, Rb, and Zn were higher in men.

There was a trend for increasing concentrations with increasing age for As (although the differences were not significant in the fully adjusted model), B, Cd, Cs, Au, Pb, Hg, and Ag. For Br, Ca, Cr, Cu, Ga, In, Fe, Mg, Mn, Mo, Ni, Rb, Se, Sr, Tl, Sn, and Zn, there were only small variations with age, variations which were significant in some age groups for Ca, Fe, Rb, Se, Sr and Zn.

In current smokers, the concentration of Cd was more than fourfold higher than in never-smokers in the sex and age adjusted model, and in former smokers, it was about 50% higher. Unadjusted blood levels of Cd in never smokers, former smokers and current smokers, by sex and age categories, and by sex and education are shown in Figures 6 and 7, respectively. The concentration of Au was slightly higher in both current and former smokers than in never-smokers. The concentrations of Cu, Pb, Mg, Rb, and Ag were slightly higher only in current smokers. B, Br, Mn, and Se levels were slightly lower in current smokers than in never-smokers. Unadjusted blood levels of Pb in never smokers, former smokers and current smokers, by sex and alcohol intake categories, are shown in Figure 8. We found the same associations in all statistical models, but for Se in the crude model the association with

current smoking was not significant. Cs levels were significantly lower in current smokers than in never-smokers only in the fully adjusted model.

Clear positive associations with alcohol intake were found for B, Cs, Pb (Figure 8), Hg, and Ag. In addition, the concentrations of Cr, Se and Sr were slightly higher for the highest quartile of alcohol intake, comparing to the abstainers.

Regarding fatty-fish consumption, the blood concentrations of especially As (Figure 2) and Hg (Figure 3), but also of Br (Figure 4), Cs, Se (Figure 5) and Sn, increased with increasing intake. For As and Hg, the differences in concentrations with increasing fatty-fish intake were smaller in the fully adjusted model (when As was adjusted for blood Hg, and Hg for As and Se) than in the sex and age adjusted model.

For BMI and waist-to-hip ratio, we found a negative association for Br and Ca, and a positive association for Fe. For Mg, Sr and Zn, there was a positive relationship with BMI, but not with waist-to-hip ratio. Differences between BMI groups, either in the crude or in the adjusted models, were also found for B, Cs, Cu, Ga, Pb, Hg, Mo, and Sn.

We found decreasing Cd (Figure 7) and increasing Se and B concentrations across the increasing education and economic status categories. Differences between some of the education and/or economic status strata were also found for Cs, Cr, Cu, Pb, Mg and Rb.

Discussion

The element levels determined in this study were within generally accepted reference ranges [25-27], not surprisingly since Nord-Trøndelag County is little influenced by industrial pollution [28]. The concentrations ranges of the analysed elements were generally similar to those observed in other surveys (Table 5), although Cr, Fe and Zn (mean values 0.58 µg/L, 541 mg/L, and 7.5 mg/L, respectively) were slightly higher in our study population [6, 8, 12, 27, 29]. For Zn, this difference could possibly be related to differences in diet between populations [30]. For Cr, it is quite possible that we have some contamination from the needles used in the sample collection [31].

Differences in trace element blood concentrations between geographical areas

Natural geological processes and atmospheric transport of elements derived from both natural and anthropogenic sources have a large impact on terrestrial and aquatic ecosystems, thus

potentially on humans too [32]. Monitoring of atmospheric deposition of metals through moss and soil analysis across Norway has showed large geographical variations for many elements [32, 33], which could have relevance for the clear differences in trace element concentrations we found between people living in the coastal and inland-mountain areas. In people living near the coast, higher concentrations were found particularly for As and Hg, and also for Br and B (Table 4). A major source for these elements in terrestrial ecosystems is transport through the atmosphere from ocean to land [33, 34]. Much higher Br concentrations have been reported in the coastal than in the inland-mountain area in natural surface soils [33] and in drinking water [35]. As and Hg are found in high concentrations in seafood [17, 36], and blood Br concentration has also been reported to correlate positively with high seafood intake [37, 38]. Lower levels of Br in the population of Beijing compared to Shanghai residents and to Japanese subjects were attributed to lower seafood consumption in Beijing and its inland location [39].

Se is another element related to atmospheric transport from the marine environment and to seafood consumption [17, 34, 36, 40, 41], and Se concentrations were slightly higher in the coastal than in the urban population (Table 4).

We found Ga concentrations to be significantly higher in the population from the urban and mountain area than in the coastal area, but the concentrations are much lower than those reported in studies on occupational exposures [42], or indeed in healthy volunteers [43]. Ga is used in the semiconductor industry and in “smart phone” production, and also has medical applications [44].

Sn and Ag concentrations were significantly lower in the inland-mountain than in the coastal population. Inorganic Sn is emitted into the environment from tin mining, but the largest ecological problems related to Sn are associated with organotin compounds [45]. Naturally occurring organotin compounds, mostly produced by methylation of inorganic Sn, have been observed in estuaries, sewage waters, and sediments [46]. However, the most important source of organotin compounds in the environment is man-made products, notably tributyltin (TBT). TBT has been heavily used in antifouling paints for protecting ship hulls, and although TBT use is now phased out, it is still found around marinas, harbours, and major shipping routes [45], and marine biota tend to accumulate organotin compounds [46]. Ag is released into the atmosphere by the combustion of fossil fuels, and into the aquatic environment from photographic industries and mining activities [45]. Drinking water usually

contains very low Ag concentrations, while higher levels have been reported in seawater and hence, marine organisms [47]. The general population may be exposed to Ag from dental fillings and consumption of seafood [47, 48].

The blood concentrations of Cs, Tl, Mo, Pb and Rb were found to be significantly higher in the inland-mountain than in the coastal population. Similar geographical differences were found in drinking water for Pb, Rb and Cs, but not for Mo and Tl [35].

Relationships between trace element concentrations and lifestyle and socio-demographic characteristics

We found sex to be a significant determinant ($P < 0.05$) for the concentrations of as many as 18 elements, and age for 12 elements. In agreement with previously reported findings, Cd, Cu, Mn, and Ni were higher in women, while Fe, Pb, Hg, and Zn were higher in men [6, 8, 12, 16, 25, 49-52]. Because of a lower concentration of erythrocytes in women, levels of elements bound to erythrocytes, like Fe and Pb, will be lower [27]. Conflicting with our results of higher Sn concentration in women, Sn was found to be significantly higher in men in serum, although not in whole blood in the Italian population [6]. For Cd and Mn, the concentrations were higher in women, in line with earlier reported findings [6, 51, 53]. This might be explained by competitive gastrointestinal absorption of Cd, Mn, and Fe, together with the lower Fe stores in women [50, 54].

Several elements were found to increase with increasing age. Especially for elements with a slow elimination from the body, notably Cd and Pb, advancing age is associated with increased body burden [3, 55]. Compared to the youngest age group (20-29 years), the concentration in the age group where the highest level was found was 285% higher for Hg, 184% for As, 163% for Ag, 103% for Au, 81% for Cd, 48% for Pb, and 48% for Cs. For some of these elements, peak concentrations were not found in the oldest age group (≥ 70), but at age 40-49 for Ag, 50-59 for Rb, and 60-69 for Pb, Hg, and Zn.

We found increasing values of the obesity-related parameters BMI and waist-to-hip ratio to be related to decreasing values of Ca and Br, and we found indications of relationships with B, Cs, Cu, Ga, Pb, Hg, Mo, and Sn. An Italian study found Pb, Mo, and Tl levels positively correlated with BMI [25], while positive correlations with BMI for Cu in men and Zn in women were found in a Czech population [56].

We found increasing Cd and decreasing Se and B blood levels to be associated with decreasing levels of education and economic status. For Cd, similar findings have been reported for income in Korean adolescents [57], and education level in Brazilian adults [58]. Lower levels of Se have been reported to be associated with lower socioeconomic status in the US population, possibly explained by low Se dietary intake and occupational exposure [52]. In the NHANES study in the USA, Tyrrell et al. reported that persons with higher socioeconomic status had higher serum and urine concentrations of Hg, As, Cs, and Tl, and lower concentrations of Cd, Pb, and Sb [59]. Probable mediators for these associations included fish and shellfish consumption for Hg, As, and Tl, and smoking, occupation, and diet, for Cd and Pb.

Alcohol intake was positively associated with higher blood levels of B, Cs, Cr, Pb, Hg, Se, Ag, and Sr, in line with previous studies for for Pb and Cr [25, 60] and Se [61]. We found lower blood levels of Mo for the highest quartile of alcohol consumption, a similar finding was reported in the Italian PROBE [6] study. However, in that study both abstainers and alcohol consumers had higher Mo GMs (1.24 and 1.19 µg/L) than those found in our study (0.91 and 0.76 µg/L).

Smoking is a well-known source of Cd, but also for other elements [62]. Tobacco accumulates Cd from soil and its high content is reflected in manifold higher Cd blood concentrations in smokers [54, 62], in line with our finding that current smokers had more than fourfold higher Cd levels than non-smokers. We also found Cu, Pb, Mg, Rb, and Ag to be positively, and B, Br, and Mn negatively associated with smoking. Similar findings have been reported for Pb, Cu, Mn, and Se [6, 11, 55, 63].

As and Hg were the elements most distinctively associated with fatty-fish intake, and the blood levels of Br, Cs, Se and Sn also increased with increasing intake. Similar associations have been reported previously [17, 51]. The Hg levels that we found in people consuming 0-3 meals of fish monthly (GM 1.98 µg/L) were identical to those found in a German population (2.0 µg/L) with similar fish intake frequency [64].

A major strength of our work is that it is based on a well-characterized population from the HUNT3 Survey, with large sample size and high attendance. The sex, age, and region stratified probability sampling design created a sample that is truly representative of this population. The access to a wide range of variables allowed us to compare trace elements concentrations by a wide range of demographic and lifestyle factors. As the most

comprehensive health study in Norway, the HUNT Study represents an excellent base for human biomonitoring, available for decades ahead, that can also provide data on indicators of environmental exposures.

We acknowledge some limitations to our study. The cross-sectional design does not give a strong basis for establishing causality. Furthermore, in spite of a range of biochemical laboratory measurements, the HUNT3 Survey did not include urine, serum or plasma samples collection for trace elements analysis, that would be appropriate complementary biological media for optimal assessment of the body burden of several trace elements [2]. On the other hand, whole blood is a better source for simultaneous evaluation of a broad spectrum of trace element concentration than serum, plasma, hair or urine separately [2]. The lack of detailed data on dietary habits is also one of the limitations [17, 48, 65, 66]. For example, in our study the only variable related to fish and seafood intake was "fatty-fish" intake.It is well known that seafood products can be an important source of trace elements, but this is highly dependent on product type [36]. Furthermore, the inaccuracy inherent in self-reported exposures, such as food frequency [67], alcohol intake [68], and smoking status [69] is a source of residual confounding not accounted for in our multivariable analyses. Finally, when setting the significance level for the differences in trace elements distribution, we chose Bonferroni adjustment for multiple comparisons. This approach is usually considered too conservative, and has been widely discussed [70]. Because we performed so many comparisons, we wanted to avoid falsely significant results (Type I error). This adjustment, however, may increase the number of cases where the null hypothesis is not rejected, when in fact it should have been, leading to Type 2 error [70]. Therefore, in Table 4 we highlighted all *P*-values < 0.05, but in our interpretation we emphasized results for significance levels corrected using the Bonferroni procedure, *P*-value < 0.001.

Conclusions

Our study provides information on 28 trace element whole blood levels in the general population of Nord-Trøndelag County, and assesses regional differences between populations living on the coast, in urban and in inland-mountain areas. For the first time, whole blood concentrations of a large number of trace elements were examined in such a large, total-population based Norwegian cohort. Our results demonstrate a considerable influence of regional geochemical characteristics on the blood concentrations of several elements,

particularly of elements attributed to the marine environment, namely As, Hg, Br, B and Se. For many elements, we also found differences in blood concentrations according to the participants lifestyle and socio-demographic indices, which shows the importance of considering these characteristics in evaluation of exposure to trace elements. Our results suggest low exposure to toxic trace elements in the residents of Nord-Trøndelag County, Norway. Future studies should link trace element levels in the general population to relevant regional geochemical data and detailed nutritional factors, using state-of-the-art analytical techniques and methods.

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Table 1. Results of the analysis of the certified reference material Seronorm WB1 batch 201505.

Selected isotopes	Certified concentration Mean (95 % confidence interval)	Measured value Mean (± SD)	Recovery (%)
Arsenic (^{75}As) µg/L	1.8 (1.4-2.2)	2.02 (± 0.16)	112
Beryllium (^{9}Be) µg/L	< 0.01	< 0.01	-
Boron (^{11}B) µg/L	26.3 (10.7–41.9)	44 (±3.4)	167
Bromine (^{81}Br) mg/L	1.1 (0.9–1.3)	0.38 (±0.10)	60
Cadmium (^{114}Cd) µg/L	0.74 (0.68–0.80)	0.657 (±0.036)	89
Calcium (^{43}Ca) mg/L	14.2 (13.4–15.0)	12.5 (±0.3)	88
Cesium (^{133}Cs) µg/L	2.3 (2.2–2.4)	2.09 (±0.05)	91
Chromium (^{52}Cr) µg/L	0.60 (0.42–0.78)	0.42 (±0.14)	70
Copper (^{63}Cu) µg/L	564 (531–597)	640 (±19)	114
Gallium (^{69}Ga) ng/L	46 (28–64)	52 (±12)	113
Gold (^{197}Au) µg/L	< 0.01	0.009 (±0.001)	-
Indium (^{115}In) µg/L	Not certified	0.009 (±0.002)	-
Iron (^{57}Fe) mg/L	432 (404 -460)	442 (±11)	102
Lead (^{208}Pb) µg/L	27.6 (26.2–29.0)	27.2 (±0.40)	99
Magnesium (^{25}Mg) mg/L	19.6 (18.5–20.7)	19.6 (±0.40)	100
Manganese (^{55}Mn) µg/L	10.6 (10.0–11.2)	10.7 (±0.20)	101
Mercury (^{202}Hg) µg/L	2.2 (2.0–2.4)	2.25 (±0.11)	102
Molybdenum (^{98}Mo) µg/L	0.50 (0.45–0.55)	0.42 (±0.10)	84
Nickel (^{60}Ni)) µg/L	1.6 (1.0–2.2)	1.00 (±0.14)	63
Rubidium (^{85}Rb) mg/L	1278 (1210–1346)	1217 (±22)	96
Scandium (^{45}Sc) ng/L	15 (9–21)	9.0 (±3.0)	60
Selenium (^{78}Se) µg/L	79.8 (74.4–85.2)	78.5 (±2.2)	98
Silver (^{109}Ag) µg/L	0.13 (0.11–0.15)	0.126 (±0.020)	97
Strontium (^{88}Sr) µg/L	27.8 (26.1–29.5)	27.6 (±0.5)	99
Thallium (^{205}Tl) µg/L	< 0.01	0.009 (±0.002)	-
Tin (^{118}Sn) µg/L	0.34 (0.29–0.39)	0.32 (±0.04)	94
Tungsten (^{182}W) µg/L	0.06 (0.03–0.09)	0.091 (±0.030)	152
Zinc (^{66}Zn) mg/L	5.5 (5.2–5.8)	5.61 (±0.14)	102

Table 2. General characteristics of the study population.

Characteristic	Total (n = 1011)	Men (n = 506)	Women (n = 505)
Mean age (years, SD)	50.0 (17.6)	50.2 (17.6)	49.9 (17.7)
Age group (years, n, %)			
20-29	167 (16.5)	82 (16.2)	85 (16.8)
30-39	169 (16.7)	86 (17.0)	83 (16.5)
40-49	170 (16.8)	85 (16.8)	85 (16.8)
50-59	168 (16.6)	85 (16.8)	83 (16.5)
60-69	169 (16.7)	84 (16.6)	85 (16.8)
≥ 70	168 (16.6)	84 (16.6)	84 (16.6)
Region (n, %)			
Mountain inland	335 (33.2)	166 (32.8)	169 (33.5)
Urban	336 (33.2)	169 (33.4)	167 (33.0)
Coastal	340 (33.6)	171 (33.8)	169 (33.5)
Mean waist-to-hip ratio (SD) [a]	0.90 (0.08)	0.94 (0.07)	0.87 (0.07)
Waist-to-hip ratio group (n, %)			
≤ 0.88	336 (33.4)	85 (16.9)	251 (49.8)
0.89-0.93	336 (33.4)	172 (34.2)	164 (32.5)
≥ 0.94	334 (33.3)	246 (48.9)	89 (17.7)
Mean body mass index (kg/m^2, SD) [b]	27.3 (4.5)	24.6 (4.1)	27.0 (4.9)
Body mass index group (kg/m^2, n, %)			
< 25.0	316 (31.4)	125 (24.8)	191 (38.0)
25.0-29.9	438 (43.5)	255 (50.6)	183 (36.5)
≥ 30	252 (25.1)	124 (25.5)	128 (25.5)
Education (years, n, %)			
< 10	249 (24.7)	133 (26.4)	116 (23.0)
10-12	524 (52.0)	276 (54.8)	248 (49.2)
≥ 13	235 (23.3)	95 (18.8)	140 (27.8)
Economic status level (n, %) [c]			
Quartile 1 (lowest)	252 (24.9)	116 (22.9)	136 (26.9)
Quartile 2	239 (23.6)	115 (22.7)	124 (24.6)
Quartile 3	288 (28.5)	154 (30.5)	134 (26.5)
Quartile 4	232 (23.0)	121 (23.9)	111 (22.0)
Mean alcohol intake (g/day, SD)	4.4 (5.4)	5.8 (6.2)	2.9 (4.0)
Alcohol intake in g/day in group (n, %)			
Abstainers	280 (27.7)	101 (20.0)	179 (35.4)
< 2.7	232 (23.0)	94 (18.6)	138 (27.3)
2.7-6.0	248 (24.5)	135 (26.7)	113 (22.4)
≥ 6.0	251 (24.8)	176 (34.7)	75 (14.9)
Smoking status (n, %)			
Never smokers	433 (42.8)	208 (41.1)	225 (44.5)
Former smokers	373 (36.9)	200 (39.5)	173 (34.3)
Current smokers	205 (20.3)	98 (19.4)	107 (21.2)
Fatty-fish consumption (n, %) [d]			
< 4 meals monthly	399 (9.5)	206 (40.7)	193 (38.2)
1-3 meals weekly	513 (50.7)	252 (49.8)	261 (51.7)
≥ 4 meals weekly	99 (9.8)	48 (9.5)	51 (10.1)

[a] Data available for 503 (99.4 %) men and 504 (99.8 %) women
[b] Data available for 504 (99.6 %) men and 502 (99.4 %) women
[c] Data available for 504 (99.6 %) men and 504 (99.8 %) women
[d] Fatty-fish includes salmon, trout, herring, mackerel and redfish

Table 3. Statistical data for element blood concentrations of 1011 participants (20-91 years) in the HUNT3 Survey.

Element	LOD	< LOD (%)	Mean	Range	Geometric mean	Median	Percentiles			
							5%	25%	75%	95%
Arsenic (µg/L)	0.46	4.0	4.29	< 0.46–70.5	2.64	2.55	0.61	1.36	4.95	14.5
Beryllium (µg/L)	0.0096	81.8	< 0.0096	< 0.0096–0.0260	< 0.0096	< 0.0096	< 0.0096	< 0.0096	< 0.0096	0.0122
Boron (µg/L)	2.2		30.0	6.6–144	27.2	27.7	13.1	20.6	36.9	55.2
Bromine (mg/L)	0.11		1.60	0.26–7.10	1.51	1.53	0.86	1.27	1.84	2.37
Cadmium (µg/L)	0.016	0.1	0.54	< 0.016–4.65	0.36	0.31	0.11	0.20	0.56	1.87
Calcium (mg/L)	0.14		59.1	47.8–71.3	59.0	59.0	52.3	56.6	61.6	65.6
Cesium (µg/L)	0.0039		4.68	1.45–36.0	4.44	4.38	2.66	3.62	5.39	7.43
Chromium (µg/L)	0.40	31.5	0.93	< 0.40–46.2	0.58	0.59	< 0.40	< 0.40	1.08	2.26
Copper (mg/L)	0.0013		1.03	0.44–2.18	1.01	1.01	0.82	0.92	1.10	1.27
Gallium (µg/L)	0.010		0.078	0.020–1.81	0.073	0.073	0.046	0.061	0.085	0.112
Gold (µg/L)	0.0057	21.6	0.0147	< 0.0057–0.287	0.0097	0.0093	< 0.0057	0.0068	0.0142	0.0392
Indium (µg/L)	0.002	0.1	0.032	< 0.002–0.079	0.029	0.029	0.016	0.024	0.037	0.060
Iron (mg/L)	0.133		543	81–693	541	541	468	508	575	631
Lead (µg/L)	0.41		21.5	3.46–219	18.8	18.6	8.9	13.6	25.4	45.5
Magnesium (mg/L)	0.015		39.7	27.9–61.3	39.6	39.6	33.9	37.3	42.0	45.9
Manganese (µg/L)	0.40		9.52	3.75–66.4	9.11	8.92	5.81	7.51	10.8	14.9
Mercury (µg/L)	0.036		3.63	0.24–21.6	2.74	2.73	0.84	1.58	4.66	9.69
Molybdenum (µg/L)	0.43	3.9	0.95	< 0.43–8.14	0.83	0.81	0.44	0.62	1.07	1.78
Nickel (µg/L)	0.22	11.9	0.71	< 0.22–9.71	0.50	0.49	< 0.22	0.31	0.85	1.72
Rubidium (µg/L)	0.16		2250	1266–3789	2220	2228	1715	1977	2478	2877
Scandium (µg/L)	0.0089	51.1	< 0.0089	< 0.0089–0.615	< 0.0089	< 0.0089	< 0.0089	< 0.0089	0.0112	0.0155
Selenium (µg/L)	6.5		102.0	51.4–255.7	100.2	99.3	75.4	89.5	112.8	136.9
Silver (µg/L)	0.039	16.7	0.160	< 0.039–1.019	0.112	0.122	< 0.039	0.070	0.202	0.424
Strontium (µg/L)	0.13		18.6	8.6–62.5	18.0	17.4	12.2	14.9	21.2	29.4
Thallium (µg/L)	0.0011		0.028	0.009–0.191	0.026	0.026	0.016	0.022	0.031	0.047
Tin (µg/L)	0.10	19.8	0.55	< 0.10–5.62	0.24	0.20	< 0.10	0.12	0.37	3.13
Tungsten (µg/L)	0.022	60.2	< 0.022	< 0.022–0.541	< 0.022	< 0.022	< 0.022	< 0.022	0.027	0.055
Zinc (mg/L)	0.004		7.5	3.8–11.4	7.5	7.5	5.9	6.8	8.2	9.1

Table 4. Results of linear regression between trace element blood levels and residence area given as the regression coefficient β (µg/L or mg/L) for the normally distributed elements, and as the effect (percentage of difference (%)[a] in the non-normally distributed elements (ln-transformed)) with 95% confidence intervals (CI), comparing the coastal region as the reference with the urban and inland-mountain regions.

Element	Model 1 (n = 1011) [b]		Model 2 (n = 1003) [c]		Model 3 (n = 1003) [d]	
	Urban	Inland-Mountain	Urban	Inland-Mountain	Urban	Inland-Mountain
Arsenic (%)	-47.2 (-54.5; -38.7)***	-55.5 (-61.7; -48.4)***	-48.6 (-55.9; -40.1)***	-55.5 (-61.8; -48.3)***	-33.1 (-42.0; -22.8)***	-41.0 (-49.0; -31.7)***
Boron (%)	11.2 (4.0; 18.8)*	-13.5 (-19.3; -7.3)***	8.7 (1.6; 16.4)*	-13.4 (-19.2; -7.2)***	-	-
Bromine (%)	-11.8 (-16.2; -7.2)***	-11.7 (-15.4; -7.7)***	-13.2 (-17.6; -8.6)***	-11.6 (-15.4; -7.6)***	-	-
Cadmium (%)	-13.2 (-17.6; -8.6)	-11.6 (-15.4; -7.6)	4.3 (-15.8; 9.2)	6.6 (-6.7; 21.7)	-	-
Calcium (mg/L)	-0.13 (-0.67; 0.41)	-1.58 (-2.10; -1.06)***	-0.23 (-0.78; 0.33)	-1.51 (-2.03; -0.99)***	0.19 (-0.33; 0.71)	-1.33 (-1.83; -0.83)***
Cesium (%)	0.3 (-4.3; 5.2)	11.8 (6.2; 17.6)***	-0.7 (-5.5; 4.3)	11.7 (6.0; 17.7)***	-2.2 (-6.5; 2.3)	2.6 (-2.1; 7.6)
Chromium (%)	12.6 (-2.4; 30.1)	10.8 (-4.1; 27.9)	15.5 (-0.4; 34.0)	10.5 (-4.5; 27.9)	5.3 (-4.0; 15.6)	-1.3 (-10.3; 8.5)
Copper (µg/L)	18.6 (-2.9; 40.1)	16.0 (-5.1; 37.0)	26.2 (4.0; 48.4)*	16.9 (-3.7; 37.6)	-	-
Gallium (%)	10.5 (5.2; 16.0)***	21.8 (16.5; 27.3)***	11.3 (6.0; 16.8)***	21.6 (16.2; 27.2)***	-	-
Gold (%)	18.0 (4.6; 33.1)*	-13.9 (-23.6; -3.0)*	16.2 (2.7; 31.4)*	-14.7 (-24.4; -3.7)*	-	-
Indium (%)	4.3 (-3.0; 12.2)	6.1 (-11.4; 0.2)	4.5 (-3.2; 12.8)	5.9 (-11.2; 0.5)	-	-
Iron (mg/L)	11.8 (5.1; 18.4) **	6.6 (-0.6; 13.8)	13.7 (7.0; 20.4)***	5.9 (-1.3; 13.2)	7.1 (1.6; 12.5)*	-3.5 (-9.9; 2.9)
Lead (%)	-1.1 (-8.4; 6.9)	15.6 (6.9; 25.0)**	0.0 (-7.4; 8.0)	15.7 (7.0; 25.1)**	-	-
Magnesium (mg/L)	0.62 (0.04; 1.21)*	0.13 (-0.44; 0.70)	0.76 (0.16; 1.37)*	0.14 (0.43; 0.72)	-	-
Manganese (%)	4.6 (0.0; 9.5)	5.7 (1.0; 10.6)*	4.1 (-0.8; 9.2)	5.5 (0.7; 10.4)*	-	-
Mercury (%)	-29.5 (-36.0; -22.2)***	-32.5 (-38.5; -26.0)***	-31.4 (-38.1; -24.1)***	-33.3 (-39.2; -36.8)***	-13.1 (-20.2; -5.4)*	-14.7 (-21.3; -7.5)***
Molybdenum (%)	11.9 (2.8; 21.9)*	16.4 (7.8; 25.7)**	12.8 (3.4; 22.9)*	18.1 (9.4; 27.6)***	-	-
Nickel (%)	11.9 (-2.2; 28.1)	15.0 (0.0; 32.3)	12.0 (-3.1; 29.4)	14.9 (-0.3; 32.4)	1.4 (-7.5; 11.2)	7.3 (-2.2; 17.7)
Rubidium (µg/L)	36 (-22; 94)	227 (167; 288)***	41 (-17; 100)	235 (174; 296)***	45 (-8; 98)	178 (123; 232)***
Selenium (%)	-4.6 (-7.3; -1.8)*	-4.0 (-6.6; -1.0)*	-5.8 (-8.2; -2.8)***	-3.9 (-6.8; -1.0)*	0.1 (-2.5; 2.6)	2.2 (-0.5; 4.9)
Silver (%)	4.3 (-8.8; 19.3)	-32.6 (-41.4; -22.6)***	3.4 (-9.8; 18.5)	-33.1 (-41.8; -23.0)***	-	-
Strontium (%)	1.2 (-2.8; 5.4)	-0.7 (-4.6; 3.4)	2.3 (-2.0; 6.7)	0.5 (-4.5; 3.7)	-	-
Thallium (%)	8.1 (3.0; 13.4)*	28.1 (21.0; 35.6)***	7.2 (2.1; 12.6)*	27.5 (20.4; 35.0)***	-	-
Tin (%)	14.6 (-4.0; 36.7)	-26.2 (-36.5; -14.1)***	12.9 (-5.8; 35.5)	-25.9 (-36.5; -13.6)**	-	-
Zinc (mg/L)	0.28 (0.15; 0.42)***	0.21 (0.07; 0.36)*	0.31 (0.17; 0.45)***	0.20 (0.05; 0.35)*	0.18 (0.05; 0.30)*	0.15 (0.01; 0.29)*

[a]Percentage of difference calculated as 1 subtracted from the anti-ln of the β regression coefficient for ln-transformed trace element blood levels;
[b]Adjusted for sex and age category;
[c]Adjusted for sex, age, body mass index, education and income;
[d]Adjusted for sex, age, body mass index, education, income and for moderately/highly inter-correlated trace elements level (Spearman's correlation coefficient $|r_S|>0.5$)
* $P < 0.05$; ** $P < 0.001$, significance level after Bonferroni adjustment for multiple comparisons; *** $P < 0.0001$;

Table 5. Comparison of trace element whole blood concentrations found in the present study with data from selected recent surveys.

Element	Present study (HUNT3) (n = 1011)	Germany [26] (n = 130)	Benin [29] (n = 70)	Canada CHMS [12]	Norway [17] (n = 184)	China [16] (n = 648)	Italy PROBE [6] (n = 1423)	Sweden [71] (n = 243–343, adolescents)	France [43] n = 100	France IMEPOGE [11] (n = 1992)
	GM P(5th – 95th)	GM P(5th – 95th)	GM P(5th – 95th)	RV95 (n)	Median P(5th – 95th)	GM P(25th – 75th)	GM P(5th – 95th)	Median (Range)	Median P(5th – 95th)	GM P(10th-95th)
Arsenic (µg/L)	2.64 (0.61 - 14.50)	0.71 (0.16 – 2.3)	5.810 (3.520 – 10.550)	2.0 (n = 996)	5.9 (0.8 – 41.0)		1.14 (0.28 – 5.32)		5.0 (2.6 – 1.8)	1.67 (0.50 – 6.72)
Beryllium (µg/L)	< 0.0096 (< 0.0096 - 0.0122)	< 0.008 (< 0.008 – 0.015)	0.020 (<0.010 – 0.196)				0.085 (< 0.045 – 0.156)		0.02 (0.02 – 0.09)	0.003 (<LOD – 0.09)
Boron (µg/L)	27.2 (13.1 - 55.2)	36 (14 – 41)	-	-		-	-	-	26 (14 – 44)	
Cadmium (µg/L)	0.36 (0.11 - 1.87)	0.38 (0.12 – 1.9)	0.319 (0.150 – 0.630)	0.83 (n = 2507)	0.45 (0.11 – 1.8)	0.68 (0.42 – 1.14)	0.53 (0.23 – 1.42)	< 0.2 (<0.2 – 2.6)	0.31 (0.15 – 2.04)	0.39 (0.17 – 1.67)
Cesium (µg/L)	4.44 (2.66 - 7.43)	3.4 (2 – 5.5)	-	-		-	-	-		
Chromium (µg/L)	0.58 (< 0.40 - 2.26)		<0.240 (<0.240 – 0.990)				0.24 (0.06 – 1.09)			0.42 (0.10 – 1.26)
Copper (mg/L)	1.01 (0.82 - 1.27)	1.02 (0.80 – 1.62)	0.870 (0.720 – 1.027)	1.30 (f = 3124) 1.00 (m = 2940)		0.802 (0.685 – 0.885)		0.92 (0.61 – 1.9)		
Gallium (µg/L)	0.073 (0.046 - 0.112)	< 0.2 (< 0.2 - < 0.2)	-	-		-	-	-	3.5 (2.65 – 4.71)	
Gold (µg/L)	0.0097 (< 0.0057 - 0.0392)	0.02 (< 0.012 – 0.45)	-	-		-	-	-		
Indium (µg/L)	0.029 (0.016 - 0.060)	< 0.009 (< 0.009 – 0.014)	-	-		-	-	-		
Iron (mg/L)	541 (468 - 631)		469 (387 – 554)	-		-	-	-		
Lead (µg/L)	18.8 (8.9 - 45.5)	19 (8 – 47)	47.39 (29.37 – 74.78)	33 (n = 3142)	24.5 (8.6 – 65.1)	42.55 (31.31 – 59.89)	19.9 (7.38 – 51.7)	16 (3.5 – 170)	26 (11.4 – 62.8)	18.8 (8.86 – 49.3)
Magnesium (mg/L)	39.6 (33.9 - 45.9)		27.7 (23.4 – 34.1)							
Manganese (µg/L)	9.11 (5.81 - 14.92)	8.6 (5.7 – 14.6)	19.71 (15.70 – 25.14)	16 (f = 1937) 14 (m = 1676)		11.42 (8.81 – 14.72)	8.19 (4.41 – 12.8)		7.6 (5.0 – 12.8)	7.71 (5.26 – 12.9)
Mercury (µg/L)	2.74 (0.84 - 9.69)	0.9 (0.2 – 3.3)	3.12 (1.11 – 7.64)	2.3 (n = 1229)	4.0 (1.2 – 12.6)		1.19 (0.35 – 5.16)	1.1 (<0.7 – 6.1)	3.0 (0.94 – 8.3)	1.38 (0.49 – 5.06)
Molybdenum (µg/L)	0.83 (0.44 - 1.78)	0.33 (0.14 – 1.1)	0.912 (0.370 – 3.160)	1.6 (n = 1759)			1.21 (0.69 – 2.05)		2.9 (0.77 – 7.86)	
Nickel (µg/L)	0.50 (< 0.22 - 1.72)	0.08 (0.03 – 0.22)	-	1.1 (n = 5924)			0.89 (<0.35 – 2.62)		2.1 (0.09 – 4.18)	1.31 (0.74 – 2.67)
Rubidium (µg/L)	2220 (1715 – 2877)	2369 (1768 – 3131)	-					2800 (1500 – 4400)	1680 (1289 – 2358)	
Selenium (µg/L)	100.2 (75.4 - 136.9)	132 (105 – 164)	163.0 (123.0 – 205.0)	240 (n = 3598)	95 (63 – 153)			110 (55 – 180)	119 (89 – 154)	
Silver (µg/L)	< 0.039 (< 0.039 - 0.424)	0.04 (0.009 – 0.236)	-						1.4 (0.69 – 4.51)	
Strontium (µg/L)	18.0 (12.2 - 29.4)	19 (11 – 39)	30.53 (21.17 – 48.42)						16 (9 – 41)	
Thallium (µg/L)	0.026 (0.016 - 0.047)	0.016 (< 0.01 – 0.035)	0.123 (0.050 – 0.270)				0.037 (0.018 – 0.098)	< 0.06 (< 0.06 – 0.15)	0.02 (0.011 – 0.035)	0.02 (0.01 – 0.14)
Tin (µg/L)	0.24 (< 0.10 - 3.13)	0.12 (0.03 – 0.55)	0.211 (<0.100 – 0.480)				0.539 (0.124 – 2.250)		1.1 (0.11 – 1.75)	
Tungsten (µg/L)	< 0.022 (< 0.022 - 0.055)	< 0.011 (< 0.011 – 0.017)	<0.002 (<0.002 – 0.143)				0.028 (0.011 – 0.075)	< 0.1 (< 0.1 – 0.94)	0.006 (0.004 – 0.0822)	
Zinc (mg/L)	7.5 (5.9 - 9.1)		4.85 (3.68 – 6.67)	6.7 (f = 947) 7.9 (m = 821)		4.67 (3.78 – 5.56)		6.1 (3.1 – 9.8)		5.81 (4.77 – 7.27)

GM – Geometric mean; P (5th, 10th, 25th, 75th and 95th): 5th, 10th, 25th, 75th and 95th percentiles; RV95: Reference values at the 95th percentile; f: females; m: males

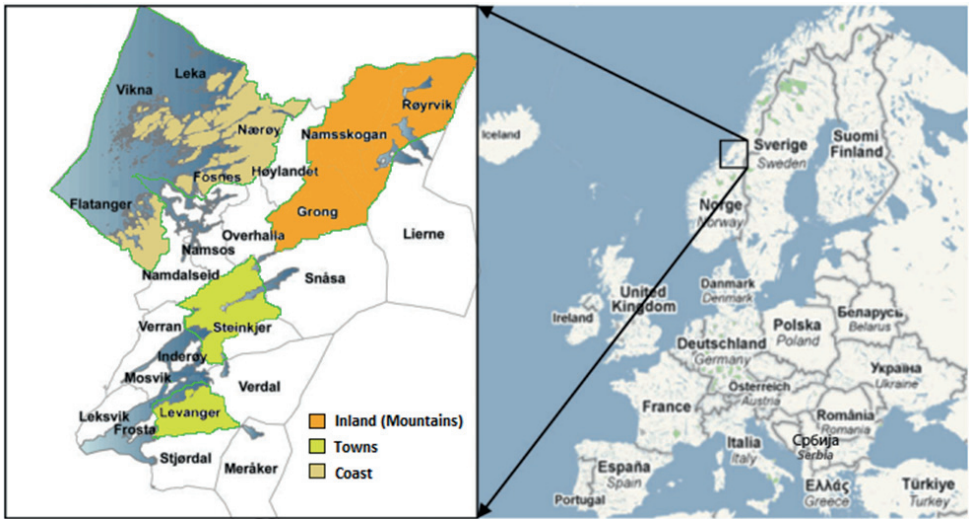

Figure 1. Map of Nord-Trøndelag County and the selected geographical regions in the study. (Modified and reproduced with permission of the author Dr. Steinar Krokstad from [21])

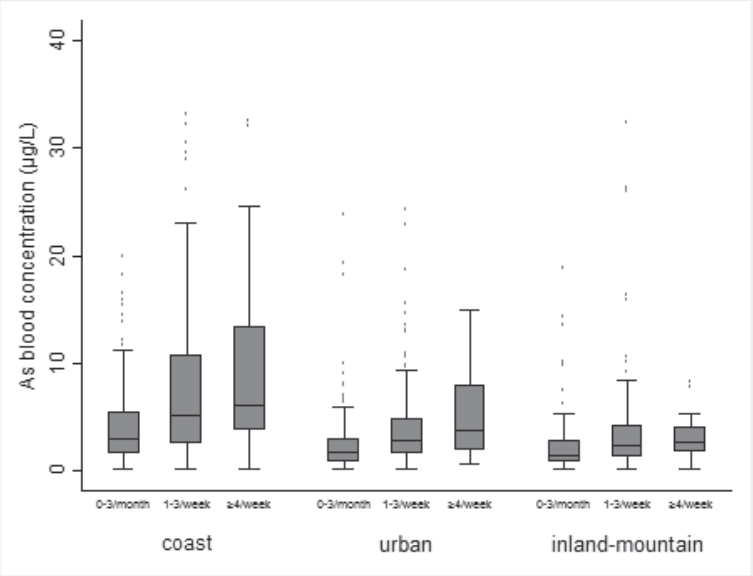

Figure 2. Unadjusted arsenic blood levels (with bars indicating median, 25th and 75th percentiles, maximum and minimum excluding outliers, and outliers) as a function of fatty-fish intake and the region.

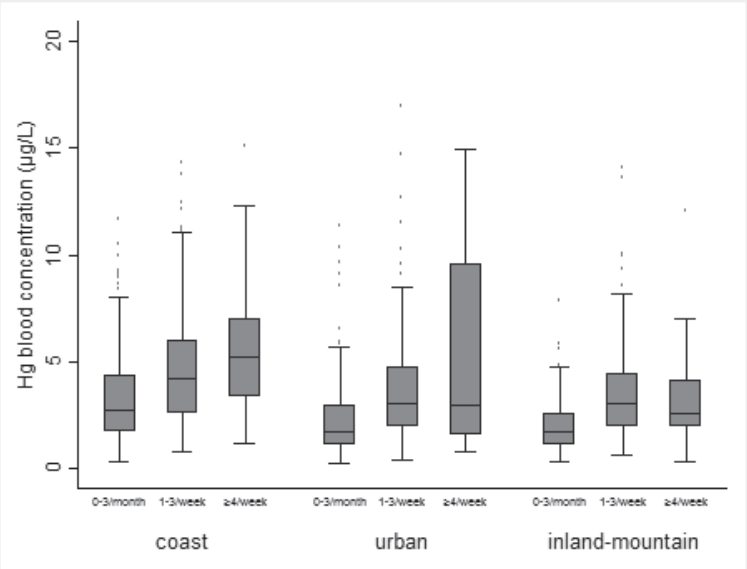

Figure 3. Unadjusted mercury blood levels (with bars indicating median, 25th and 75th percentiles, maximum and minimum excluding outliers, and outliers) as a function of fatty-fish intake and the region.

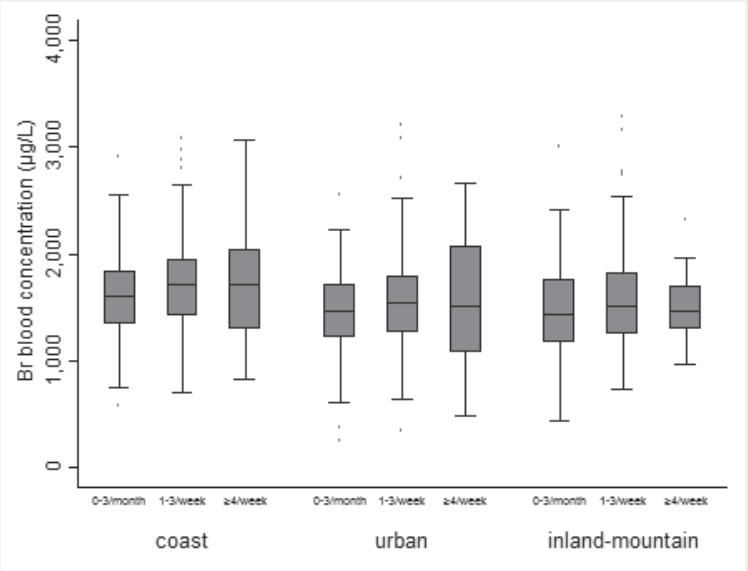

Figure 4. Unadjusted bromine blood levels (with bars indicating median, 25th and 75th percentiles, maximum and minimum excluding outliers, and outliers) as a function of fatty-fish intake and the region.

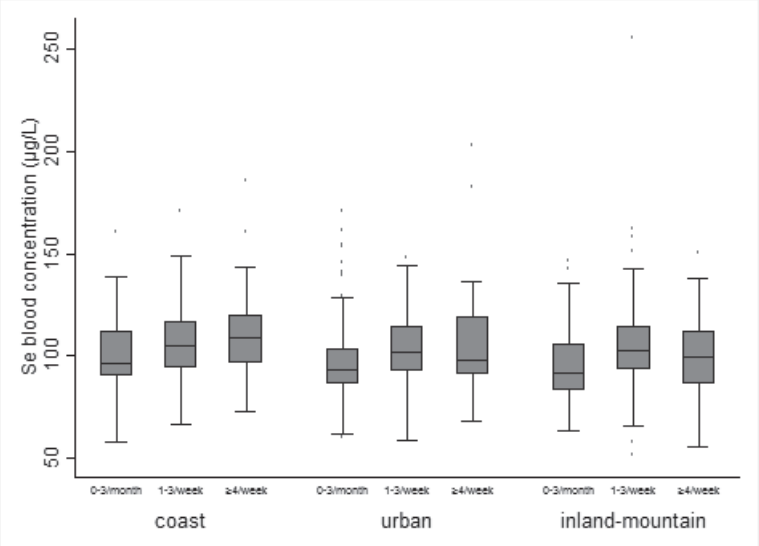

Figure 5. Unadjusted selenium blood levels (with bars indicating median, 25th and 75th percentiles, maximum and minimum excluding outliers, and outliers) as a function of fatty-fish intake and the region.

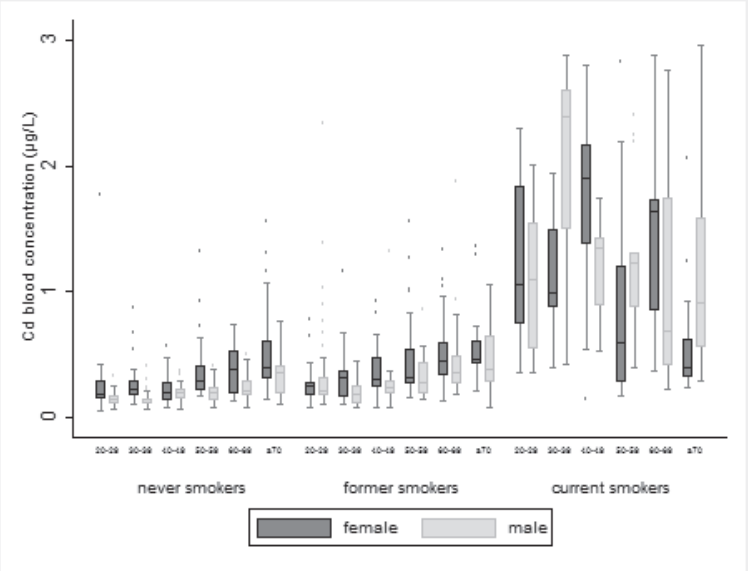

Figure 6. Unadjusted cadmium blood levels (with bars indicating median, 25th and 75th percentiles, maximum and minimum excluding outliers, and outliers) by sex and age categories (years) in never smokers, former smokers and current smokers.

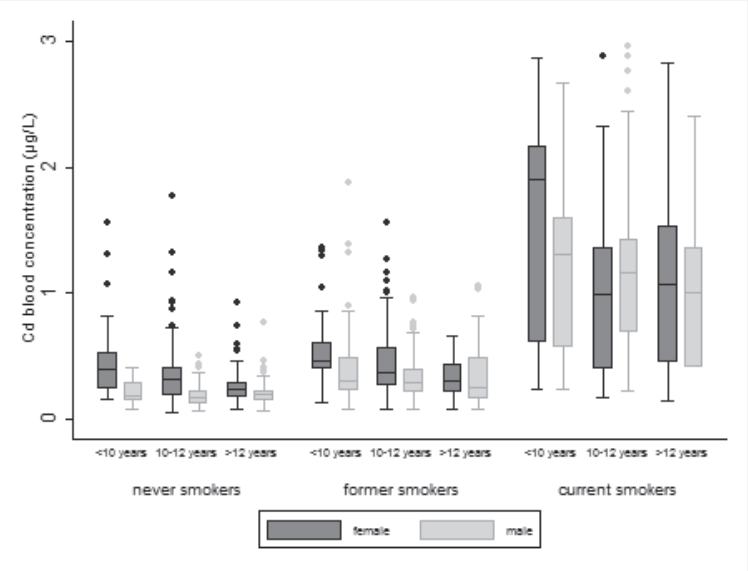

Figure 7. Unadjusted cadmium blood levels (with bars indicating median, 25th and 75th percentiles, maximum and minimum excluding outliers, and outliers) by sex and years of education in never smokers, former smokers and current smokers.

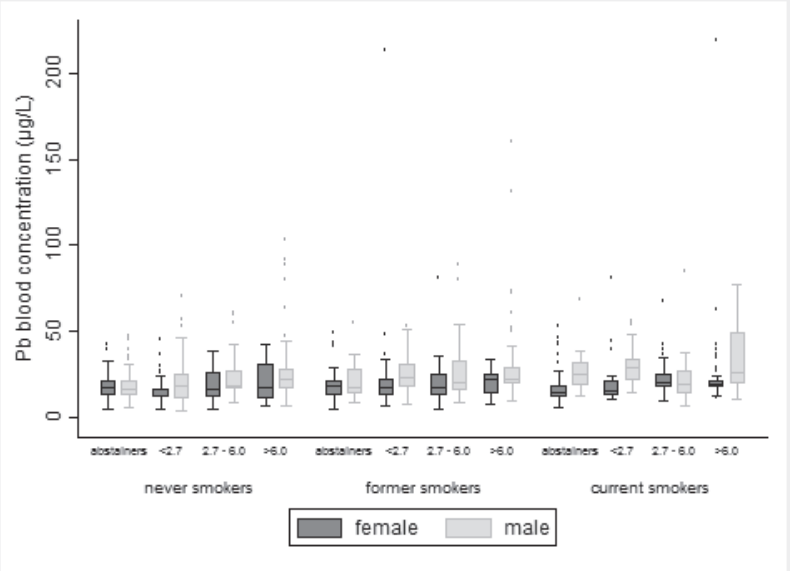

Figure 8. Unadjusted lead blood levels (with bars indicating median, 25th and 75th percentiles, maximum and minimum excluding outliers, and outliers) by sex and alcohol intake (g/day) in never smokers, former smokers and current smokers.

Table S1. Arsenic whole blood levels (µg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	2.56 (2.35 – 2.79)	2.33 (2.14 – 2.53)	2.89 (2.56 – 3.26)
Men	2.75 (2.52 – 2.99)	2.46 (2.25 – 2.69)	2.82 (2.53 – 3.14)
Age (year) [e]			
20-29 [d]	1.35 (1.16 – 1.56)	1.35 (1.06 – 1.72)	2.77 (2.19 - 3.50)
30-39	1.71 (1.51 – 1.93)	1.71 (1.45 – 2.02)	2.74 (2.30 – 3.27)
40-49	2.16 (1.84 – 2.54)**	2.17 (1.91 – 2.46)**	2.71 (2.36 – 3.12)
50-59	2.74 (2.37 – 3.16)***	2.74 (2.43 – 3.09)***	2.40 (2.09 – 2.75)
60-69	3.55 (3.10 – 4.05)***	3.55 (3.10 – 4.03)***	2.96 (2.56 – 3.43)
≥70	3.83 (3.35 – 4.37)***	3.83 (3.34 – 4.40)***	3.69 (3.13 – 4.35)
Region			
Inland-Mountains	2.03 (1.83 – 2.24)***	1.80 (1.48 – 2.19)***	2.23 (1.84 – 2.70)***
Urban	2.34 (2.12 – 2.58)***	2.14 (1.99 – 2.29)***	2.57 (2.42 – 2.89)***
Coastal [d]	4.53 (4.06 – 5.04)	4.04 (3.56 – 4.59)	3.83 (3.47 – 4.49)
Waist-to-hip ratio			
≤ 0.865 [d]	2.48 (2.23 – 2.76)	2.53 (2.26 – 2.84)	3.15 (2.75 – 3.60)
0.866-0.932	2.58 (2.32 – 2.87)	2.38 (2.15 – 2.63)	2.75 (2.44 – 3.10)
≥ 0.933	2.88 (2.60 – 3.20)	2.27 (2.02 – 2.54)	2.68 (2.35 – 3.05)
Body mass index (kg/m^2)			
≤ 24 [d]	2.55 (2.27 – 2.86)	2.46 (2.21 – 2.74)	2.60 (2.29 – 2.95)
25-29	2.74 (2.50 – 3.01)	2.44 (2.22 – 2.68)	3.04 (2.72 – 3.41)
≥ 30	2.60 (2.33 – 2.91)	2.24 (1.98 – 2.54)	2.93 (2.55 – 3.37)
Education (years)			
< 10 [d]	2.92 (2.57 – 3.32)	2.34 (2.04 – 2.67)	2.75 (2.39 – 3.16)
10-12	2.53 (2.32 – 2.75)	2.27 (2.08 – 2.47)	2.73 (2.45 – 3.05)
≥ 13	2.66 (2.35 – 3.00)	2.72 (2.42 – 3.06)	3.09 (2.70 – 3.54)
Economic status level			
Quartile 1 [d]	2.73 (2.41 – 3.10)	2.29 (2.01 – 2.62)	2.70 (2.34 – 3.12)
Quartile 2	2.84 (2.53 – 3.18)	2.56 (2.26 – 2.90)	2.94 (2.57 – 3.36)
Quartile 3	2.20 (1.96 – 2.46)	2.13 (1.90 – 2.38)	2.82 (2.49 – 3.20)
Quartile 4 (highest)	2.99 (2.62 – 3.41)	2.71 (2.39 – 3.06)	2.95 (2.57 – 3.40)
Alcohol consumption (g/day)			
Abstainers [d]	2.72 (2.43 – 3.03)	2.22 (1.96 – 2.51)	2.94 (2.57 – 3.38)
< 2.7	2.50 (2.43 – 3.03)	2.24 (1.98 – 2.54)	2.65 (2.32 – 3.02)
2.7-6.0	2.57 (2.27 – 2.92)	2.33 (2.07 – 2.63)	2.77 (2.43 – 3.16)
≥ 6.1	2.76 (2.43 – 2.92)	2.76 (2.45 – 3.11)	3.07 (2.65 – 3.51)
Smoking status			
Never-smokers [d]	2.64 (2.43 – 2.88)	2.54 (2.31 – 2.78)	2.98 (2.66 – 3.33)
Former smokers	2.64 (2.38 – 2.93)	2.27 (2.06 – 2.51)	2.72 (2.42 – 3.05)
Current smokers	2.63 (2.27 – 3.06)	2.31 (2.02 – 2.64)	2.87 (2.49 – 3.31)
Fatty-fish consumption [f]			
0-3/month [c]	1.82 (1.65 – 2.01)	1.80 (1.64 – 1.98)	2.37 (2.21 – 2-78)
1-3/week	3.22 (2.98 – 3.47)***	2.86 (2.62 – 3.11)***	2.91 (2.66 – 3.24)**
≥ 4/week	4.19 (3.46 – 5.06)***	3.65 (3.02 – 4.40)***	3.17 (2.66 – 3.85)**

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using the Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table and mercury blood levels (Spearman's correlation coefficient, $r_S = 0.611$).

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S2. Boron whole blood levels (µg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	28.48 (27.45 - 29.54)	27.6 (26.6 - 28.7)	25.18 (23.84 - 26.59)
Men	25.88 (24.88 - 26.92) ***	24.9 (24.0 - 26.0)***	22.40 (21.07 - 23.82)***
Age (year) [e]			
20-29 [d]	20.14 (23.27 - 23.27)	21.5 (19.3 - 24.0)	18.89 (16.84 - 21.19)
30-39	24.12 (22.47 - 25.90)	23.9 (22.2 - 25.8)	21.13 (19.36 - 23.07)
40-49	24.95 (23.60 - 26.38)	24.9 (23.5 - 26.3)	22.30 (20.75 - 23.97)
50-59	28.39 (26.55 - 30.37)***	28.3 (26.8 - 29.9)***	24.32 (22.70 - 26.06)**
60-69	29.12 (27.19 - 31.19)***	29.1 (27.4 - 30.8)***	26.57 (24.72 - 28.55)***
≥70	31.03 (29.26 - 32.90)***	30.9 (29.1 - 32.9)***	31.18 (28.73 - 33.84)***
Region			
Inland-Mountains	22.25 (21.13 - 23.42)	21.3 (19.4 - 23.3)*	20.89 (18.94 - 23.05)
Urban	28.33 (27.06 - 29.65)***	27.3 (26.4 - 28.2)**	26.46 (25.31 - 27.67)***
Coastal [d]	25.69 (24.55 - 26.88)*	24.6 (23.2 - 26.1)	24.23 (22.69 -)25.87)**
Waist-to-hip ratio			
≤ 0.865 [d]	28.35 (27.02 - 29.75)	27.7 (26.3 - 29.1)	24.98 (23.32 - 26.77)
0.866-0.932	27.31 (26.10 - 28.57)	26.5 (25.4 - 27.8)	23.43 (22.04 - 24.90)*
≥ 0.933	26.18 (24.97 - 27.45)	24.5 (23.2 - 25.8)**	22.88 (21.41 - 24.46)*
Body mass index (kg/m^2)			
≤ 24 [d]	27.15 (25.84 - 28.52)	26.3 (25.1 - 27.6)	22.79 (21.35 - 24.31)
25-29	28.21 (27.09 - 29.38)	27.4 (26.3 - 28.6)	25.15 (23.72 - 26.65)*
≥ 30	25.61 (24.28 - 27.02)	24.1 (22.8 - 25.5)	23.38 (21.78 - 25.10)
Education (years)			
< 10 [d]	24.75 (23.39 - 26.19)	22.6 (21.3 - 24.0)	21.44 (19.97 - 23.01)
10-12	27.27 (26.27 - 28.31)	26.2 (25.2 - 27.2)***	24.08 (22.78 - 25.46)**
≥ 13	29.45 (27.93 - 31.06)	29.5 (28.0 - 31.1)***	25.95 (24.21 - 27.81)***
Economic status level			
Quartile 1 [d]	26.56 (25.17 - 28.03)	24.8 (23.3 - 26.3)	23.78 (22.10 - 25.59)
Quartile 2	26.30 (24.84 - 27.85)	25.4 (24.0 - 26.8)	23.32 (21.77 - 24.97)
Quartile 3	26.21 (24.95 - 27.52)	25.9 (24.6 - 27.2)	23.11 (21.67 - 24.64)
Quartile 4 (highest)	30.03 (28.40 - 31.76)*	29.3 (27.7 - 31.0)***	24.83 (23.12 - 26.66)
Alcohol consumption (g/day)			
Abstainers [d]	26.44 (25.15 - 27.80)	23.6 (22.3 - 24.9)	22.38 (20.87 - 23.99)
< 2.7	25.25 (23.88 - 26.71)	23.8 (22.5 - 25.2)	21.42 (20.02 - 22.90)
2.7-6.0	27.97 (26.66 - 29.35)	27.4 (26.0 - 28.9)**	25.03 (23.40 - 26.78)*
≥ 6.1	29.18 (27.48 - 30.99)*	30.1 (28.5 - 31.7)***	26.52 (24.76 - 28.40)***
Smoking status			
Never-smokers [d]	27.67 (26.61 - 28.77)	27.1 (26.0 - 28.3)	24.80 (23.42 - 26.26)
Former smokers	28.52 (27.22 - 29.87)	27.1 (25.9 - 28.3)	24.85 (23.41 - 26.38)
Current smokers	24.08 (22.73 - 25.51)**	22.8 (21.4 - 24.2)***	21.74 (20.21 - 23.37)**
Fatty-fish consumption [f]			
0-3/month [c]	25.23 (24.22 - 26.28)	25.3 (24.2 - 26.4)	23.05 (21.77 - 24.40)
1-3/week	28.62 (27.53 - 29.75)***	27.1 (26.0 - 28.2)	24.06 (22.87 - 25.30)
≥ 4/week	28.50 (26.26 - 30.92)*	26.4 (24.1 - 28.8)	24.15 (21.98 - 26.54)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using the Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S3. Bromine whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	1.61 (1.56 - 1.65)	1.60 (1.55 - 1.65)	1.58 (1.51 - 1.64)
Men	1.40 (1.36 - 1.45)***	1.40 (1.35 - 1.44)***	1.42 (1.36 - 1.49)***
Age (year) [e]			
20-29 [d]	1.47 (1.42 - 1.53)	1.46 (1.34 - 1.59)	1.45 (1.32 - 1.59)
30-39	1.50 (1.42 - 1.58)	1.48 (1.40 - 1.57)	1.46 (1.37 - 1.57)
40-49	1.52 (1.45 - 1.59)	1.51 (1.45 - 1.58)	1.51 (1.43 - 1.60)
50-59	1.60 (1.52 - 1.69)	1.60 (1.53 - 1.67)	1.59 (1.51 - 1.68)
60-69	1.42 (1.35 - 1.50)	1.42 (1.36 - 1.48)	1.44 (1.37 - 1.52)
≥70	1.50 (1.42 - 1.59)	1.49 (1.42 - 1.57)	1.52 (1.43 - 1.61)
Region			
Inland-Mountains	1.47 (1.43 - 1.52)**	1.46 (1.36 - 1.57)**	1.44 (1.34 - 1.55)**
Urban	1.47 (1.41 - 1.53)***	1.46 (1.42 - 1.50)***	1.42 (1.37 - 1.47)***
Coastal [d]	1.67 (1.62 - 1.72)	1.65 (1.58 - 1.73)	1.63 (1.55 - 1.71)
Waist-to-hip ratio			
≤ 0.865 [d]	1.68 (1.62 - 1.73)	1.61 (1.55 - 1.68)	1.59 (1.51 - 1.68)
0.866-0.932	1.45 (1.40 - 1.51)***	1.44 (1.39 - 1.50)***	1.45 (1.39 - 1.52)**
≥ 0.933	1.42 (1.37 - 1.47)***	1.43 (1.37 - 1.49)***	1.44 (1.37 - 1.52)**
Body mass index (kg/m^2)			
≤ 24 [d]	1.60 (1.54 - 1.65)	1.55 (1.49 - 1.61)	1.53 (1.46 - 1.61)
25-29	1.52 (1.47 - 1.57)	1.52 (1.47 - 1.57)	1.55 (1.48 - 1.62)
≥ 30	1.39 (1.33 - 1.45)***	1.36 (1.30 - 1.42)***	1.41 (1.33 - 1.49)*
Education (years)			
< 10 [d]	1.48 (1.42 - 1.54)	1.47 (1.40 - 1.54)	1.48 (1.41 - 1.56)
10-12	1.49 (1.44 - 1.53)	1.47 (1.43 - 1.52)	1.48 (1.42 - 1.54)
≥ 13	1.58 (1.51 - 1.65)	1.55 (1.49 - 1.62)	1.53 (1.45 - 1.61)
Economic status level			
Quartile 1 [d]	1.47 (1.41 - 1.53)	1.46 (1.39 - 1.53)	1.45 (1.37 - 1.53)
Quartile 2	1.50 (1.42 - 1.58)	1.49 (1.43 - 1.56)	1.51 (1.43 - 1.59)
Quartile 3	1.55 (1.49 - 1.60)	1.52 (1.46 - 1.59)	1.55 (1.48 - 1.63)
Quartile 4 (highest)	1.51 (1.45 - 1.58)	1.49 (1.43 - 1.56)	1.47 (1.39 - 1.55)
Alcohol consumption (g/day)			
Abstainers [d]	1.58 (1.52 - 1.63)	1.53 (1.46 - 1.60)	1.54 (1.46 - 1.62)
< 2.7	1.45 (1.38 - 1.52)*	1.42 (1.35 - 1.48)	1.43 (1.35 - 1.50)
2.7-6.0	1.49 (1.44 - 1.54)	1.49 (1.43 - 1.56)	1.48 (1.40 - 1.56)
≥ 6.1	1.52 (1.45 - 1.60)	1.53 (1.46 - 1.60)	1.54 (1.46 - 1.62)
Smoking status			
Never-smokers [d]	1.56 (1.51 - 1.60)	1.54 (1.49 - 1.59)	1.56 (1.49 - 1.63)
Former smokers	1.51 (1.46 - 1.56)	1.50 (1.44 - 1.55)	1.52 (1.45 - 1.59)
Current smokers	1.42 (1.34 - 1.50)**	1.38 (1.32 - 1.45)**	1.42 (1.34 - 1.49)**
Fatty-fish consumption [f]			
0-3/month [c]	1.42 (1.38 - 1.47)	1.42 (1.37 - 1.47)	1.43 (1.37 - 1.49)
1-3/week	1.56 (1.52 - 1.61)***	1.55 (1.50 - 1.60)**	1.53 (1.47 - 1.59)*
≥ 4/week	1.58 (1.45 - 1.71)*	1.55 (1.44 - 1.66)	1.53 (1.42 - 1.64)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using the Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S4. Cadmium whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	0.42 (0.39 - 0.44)	0.39 (0.37 - 0.42)	0.47 (0.44 - 0.51)
Men	0.31 (0.28 - 0.33)***	0.29 (0.27 - 0.31)***	0.33 (0.31 - 0.36)***
Age (year) [e]			
20-29[d]	0.26 (0.23 - 0.30)	0.26 (0.21 - 0.32)	0.32 (0.27 - 0.38)
30-39	0.24 (0.21 - 0.27)	0.23 (0.20 - 0.27)	0.34 (0.30 - 0.38)
40-49	0.36 (0.31 - 0.42)	0.36 (0.32 - 0.40)	0.39 (0.36 - 0.43)
50-59	0.35 (0.31 - 0.40)	0.35 (0.32 - 0.39)	0.42 (0.38 - 0.46)
60-69	0.40 (0.36 - 0.45)**	0.40 (0.36 - 0.45)**	0.49 (0.44 - 0.54)***
≥70	0.47 (0.43 - 0.52)***	0.47 (0.41 - 0.52)***	0.46 (0.41 - 0.51)**
Region			
Inland-Mountains	0.41 (0.37 - 0.45)	0.38 (0.31 - 0.45)	0.41 (0.36 - 0.47)
Urban	0.35 (0.32 - 0.38)	0.33 (0.31 - 0.35)	0.37 (0.35 - 0.40)
Coastal [d]	0.39 (0.35 - 0.43)	0.36 (0.32 - 0.40)	0.41 (0.37 - 0.44)
Waist-to-hip ratio			
≤ 0.865 [d]	0.36 (0.33 - 0.40)	0.32 (0.29 - 0.36)	0.39 (0.36 - 0.43)
0.866-0.932	0.37 (0.34 - 0.41)	0.36 (0.33 - 0.39)	0.39 (0.36 - 0.43)
≥ 0.933	0.35 (0.31 - 0.38)	0.32 (0.29 - 0.36)	0.41 (0.37 - 0.44)
Body mass index (kg/m^2)			
≤ 24[d]	0.36 (0.33 - 0.39)	0.33 (0.30 - 0.36)	0.40 (0.36 - 0.44)
25-29	0.36 (0.32 - 0.40)	0.34 (0.32 - 0.37)	0.42 (0.39 - 0.46)*
≥ 30	0.36 (0.33 - 0.39)	0.32 (0.29 - 0.36)	0.37 (0.34 - 0.41)
Education (years)			
< 10 [d]	0.51 (0.45 - 0.57)	0.45 (0.40 - 0.50)	0.45 (0.41 - 0.49)
10-12	0.36 (0.34 - 0.39)***	0.34 (0.32 - 0.36)***	0.40 (0.37 - 0.43)*
≥ 13	0.27 (0.25 - 0.30)***	0.26 (0.24 - 0.29)***	0.35 (0.32 - 0.39)***
Economic status level			
Quartile 1 [d]	0.46 (0.41 - 0.50)	0.41 (0.36 - 0.45)	0.41 (0.37 - 0.45)
Quartile 2	0.37 (0.33 - 0.41)*	0.34 (0.31 - 0.38)	0.40 (0.36 - 0.44)
Quartile 3	0.34 (0.30 - 0.37)***	0.32 (0.29 - 0.35)**	0.38 (0.35 - 0.41)
Quartile 4 (highest)	0.31 (0.29 - 0.34)***	0.29 (0.26 - 0.32)***	0.40 (0.36 - 0.44)
Alcohol consumption (g/day)			
Abstainers [d]	0.39 (0.35 - 0.42)	0.32 (0.29 - 0.36)	0.40 (0.36 - 0.43)
< 2.7	0.36 (0.33 - 0.40)	0.33 (0.30 - 0.37)	0.39 (0.35 - 0.43)
2.7-6.0	0.35 (0.31 - 0.38)	0.33 (0.30 - 0.37)	0.39 (0.35 - 0.43)
≥ 6.1	0.35 (0.31 - 0.39)	0.36 (0.32 - 0.40)	0.42 (0.38 - 0.46)
Smoking status			
Never-smokers [d]	0.23 (0.22 - 0.24)	0.22 (0.21 - 0.24)	0.22 (0.20 - 0.24)
Former smokers	0.34 (0.32 - 0.36)***	0.32 (0.30 - 0.34)***	0.31 (0.28 - 0.33)***
Current smokers	1.05 (0.94 - 1.17)***	0.97 (0.89 - 1.05)***	0.93 (0.84 - 1.02)***
Fatty-fish consumption [f]			
0-3/month [c]	0.34 (0.31 - 0.37)	0.34 (0.31 - 0.37)	0.43 (0.40 - 0.47)
1-3/week	0.38 (0.35 - 0.40)	0.34 (0.31 - 0.36)	0.42 (0.40 - 0.46)
≥ 4/week	0.36 (0.30 - 0.43)	0.31 (0.26 - 0.36)	0.34 (0.30 - 0.39)**

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S5. Calcium whole blood levels (mg/L) by subjects' characteristics.

Factors	Arithmetic means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	60.83 (60.54 - 61.12)	60.93 (60.63 - 61.24)	59.68 (59.26 - 60.10)
Men	57.09 (56.78 - 57.40)***	57.21 (56.89 - 57.54)***	57.76 (57.31 - 58.20)***
Age (year) [e]			
20-29 [d]	59.98 (59.00 - 60.97)	59.77 (58.91 - 60.64)	59.58 (58.71 - 60.45)
30-39	59.57 (58.89 - 60.25)	59.28 (58.68 - 59.88)	58.98 (58.33 - 59.62)
40-49	59.44 (58.89 - 59.97)	59.32 (58.86 - 59.78)	59.02 (58.50 - 59.53)
50-59	59.30 (58.91 - 59.79)	59.22 (58.79 - 59.66)	58.78 (58.27 - 59.29)
60-69	57.94 (57.42 - 58.47)**	57.86 (57.40 - 58.32)**	57.62 (57.10 - 58.13)**
≥70	58.11 (58.54 - 59.68)	58.98 (58.48 - 59.48)	58.35 (57.76 - 58.93)
Region			
Inland Mountains	57.68 (57.83 - 58.53)**	57.71 (56.97 - 58.45)**	57.74 (57.03 - 58.45)**
Urban	59.18 (58.90 - 59.47)	59.16 (58.90 - 59.43)	59.33 (59.01 - 59.66)
Coastal [d]	59.31 (58.77 - 59.84)	59.29 (58.81 - 59.76)	59.08 (58.61 - 59.56)
Waist-to-hip ratio			
≤ 0.865 [d]	60.75 (60.35 - 61.16)	59.60 (59.20 - 60.01)	59.09 (58.59 - 59.59)
0.866-0.932	58.79 (58.42 - 59.16)***	59.01 (58.64 - 59.38)	58.68 (58.23 - 59.13)
≥ 0.933	57.58 (57.15 - 58.02)***	58.56 (58.15 - 58.97)**	58.39 (57.91 - 58.87)
Body mass index (kg/m^2)			
≤ 24 [d]	60.14 (59.71 - 60.44)	59.52 (59.13 - 59.91)	58.62 (58.14 - 59.10)
25-29	58.75 (58.40 - 59.10)***	59.14 (58.80 - 59.47)	58.96 (58.55 - 59.38)
≥ 30	58.38 (57.89 - 58.87)***	58.28 (57.83 - 58.73)***	58.58 (58.06 - 59.09)
Education (years)			
< 10 [d]	58.76 (58.25 - 59.28)	58.82 (58.34 - 59.30)	58.71 (58.21 - 59.22)
10-12	58.84 (58.50 - 59.17)	58.92 (58.60 - 59.23)	58.60 (58.20 - 58.99)
≥ 13	59.82 (59.36 - 60.29)**	59.53 (59.10 - 59.95)	58.85 (58.34 - 59.36)
Economic status level			
Quartile 1 [d]	59.07 (58.54 - 59.59)	59.00 (58.52 - 59.48)	58.59 (58.07 - 59.12)
Quartile 2	59.19 (58.69 - 59.69)	59.22 (58.77 - 59.67)	59.02 (58.52 - 59.52)
Quartile 3	59.18 (58.75 - 59.62)	59.00 (58.59 - 59.41)	58.69 (58.22 - 59.16)
Quartile 4 (highest)	58.91 (58.44 - 59.39)	59.10 (58.65 - 59.55)	58.57 (58.05 - 59.09)
Alcohol consumption (g/day)			
Abstainers [d]	59.68 (59.21 - 60.15)	59.08 (58.63 - 59.53)	58.85 (58.35 - 59.35)
< 2.7	59.27 (58.77 - 59.77)	58.94 (58.48 - 59.39)	58.61 (58.11 - 59.10)
2.7-6.0	58.80 (58.32 - 59.27)	59.14 (58.71 - 59.58)	58.69 (58.19 - 59.18)
≥ 6.1	58.63 (58.16 - 59.10)*	59.11 (58.67 - 59.55)	58.73 (58.23 - 59.23)
Smoking status			
Never-smokers [d]	59.15 (58.78 - 59.53)	59.03 (58.69 - 59.37)	58.79 (58.37 - 59.20)
Former smokers	59.10 (58.72 - 59.49)	59.30 (58.94 - 59.66)	58.91 (58.47 - 59.34)
Current smokers	58.93 (58.39 - 59.47)	58.72 (58.23 - 59.21)	58.46 (57.95 - 58.98)
Fatty-fish consumption [f]			
0-3/month [d]	58.70 (58.32 - 58.09)	58.78 (58.43 - 59.13)	58.27 (57.86 - 58.68)
1-3/week	59.26 (58.92 - 59.59)	59.26 (58.94 - 59.58)	58.88 (58.52 - 59.25)*
≥ 4/week	59.78 (58.99 - 60.56)*	59.49 (58.79 - 60.20)	59.00 (58.32 - 59.68)

Arithmetic means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table and iron blood levels (Spearman's correlation coefficient, r_S = -0.588).

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S6. Cesium whole blood levels (µg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	4.34 (4.23 - 4.46)	4.16 (4.05 - 4.27)	4.37 (4.23 - 4.50)
Men	4.56 (4.43 - 4.69)*	4.34 (4.21 - 4.46)*	4.24 (4.10 - 4.39)
Age (year) [e]			
20-29[d]	3.31 (3.16 - 3.47)	3.32 (3.08 - 3.58)	3.51 (3.27 - 3.76)
30-39	3.93 (3.79 - 4.08)**	3.95 (3.75 - 4.16)**	3.92 (3.72 - 4.12)
40-49	4.48 (4.28 - 4.68)***	4.48 (4.31 - 4.67)***	4.29 (4.12 - 4.47)***
50-59	4.84 (4.62 - 5.08)***	4.85 (4.67 - 5.03)***	4.67 (4.48 - 4.86)***
60-69	4.90 (4.69 - 5.12)***	4.91 (4.71 - 5.11)***	4.88 (4.68 - 5.08)***
≥70	4.19 (4.01 - 4.39)***	4.20 (4.02 - 4.39)***	4.73 (4.52 - 4.95)***
Region			
Inland-Mountains	4.91 (4.72 - 5.10)*	4.69 (4.40 - 5.01)*	4.43 (4.18 - 4.69)
Urban	4.40 (4.26 - 4.55)	4.21 (4.12 - 4.31)	4.16 (4.07 - 4.26)
Coastal [d]	4.41 (4.26 - 4.56)	4.20 (4.03 - 4.38)	4.33 (4.17 - 4.49)
Waist-to-hip ratio			
≤ 0.865 [d]	4.40 (4.24 - 4.55)	4.39 (4.24 - 4.55)	4.39 (4.22 - 4.57)
0.866-0.932	4.48 (4.35 - 4.62)	4.27 (4.14 - 4.41)	4.34 (4.19 - 4.49)
≥ 0.933	4.44 (4.29 - 4.60)	4.07 (3.92 - 4.21)*	4.18 (4.03 - 4.34)
Body mass index (kg/m^2)			
≤ 24[d]	4.27 (4.12 - 4.42)	4.21 (4.07 - 4.35)	4.20 (4.05 - 4.36)
25-29	4.60 (4.47 - 4.73)**	4.34 (4.21 - 4.47)	4.37 (4.23 - 4.51)
≥ 30	4.39 (4.22 - 4.58)	4.14 (3.98 - 4.30)	4.35 (4.18 - 4.53)
Education (years)			
< 10 [d]	4.31 (4.12 - 4.51)	4.13 (3.96 - 4.31)	4.20 (4.03 - 4.37)
10-12	4.44 (4.33 - 4.55)*	4.20 (4.09 - 4.32)	4.26 (4.13 - 4.39)
≥ 13	4.54 (4.37 - 4.72)**	4.41 (4.25 - 4.57)	4.46 (4.29 - 4.64)
Economic status level			
Quartile 1 [d]	4.03 (3.87 - 4.18)	3.99 (3.83 - 4.16)	4.17 (4.00 - 4.35)
Quartile 2	4.53 (4.35 - 4.71)***	4.36 (4.19 - 4.53)*	4.39 (4.22 - 4.57)
Quartile 3	4.48 (4.32 - 4.64)***	4.30 (4.15 - 4.45)*	4.34 (4.19 - 4.51)
Quartile 4 (highest)	4.69 (4.51 - 4.88)***	4.35 (4.18 - 4.52)*	4.31 (4.14 - 4.49)
Alcohol consumption (g/day)			
Abstainers [d]	3.99 (3.85 - 4.13)	3.82 (3.68 - 3.97)	3.94 (3.79 - 4.10)
< 2.7	4.31 (4.13 - 4.49)*	4.10 (3.94 - 4.26)*	4.21 (4.05 - 4.38)*
2.7-6.0	4.67 (4.51 - 4.84)***	4.41 (4.25 - 4.58)***	4.41 (4.25 - 4.58)***
≥ 6.1	4.83 (4.65 - 5.01)***	4.65 (4.49 - 4.83)***	4.69 (4.51 - 4.88)***
Smoking status			
Never-smokers [d]	4.35 (4.23 - 4.47)	4.23 (4.11 - 4.36)	4.43 (4.49 - 4.33)
Former smokers	4.63 (4.49 - 4.78)*	4.34 (4.21 - 4.48)	4.43 (4.50 - 4.34)
Current smokers	4.27 (4.09 - 4.47)	4.10 (3.93 - 4.28)	4.06 (4.11 - 3.93)***
Fatty-fish consumption [f]			
0-3/month [c]	4.17 (4.05 - 4.29)	4.06 (3.94 - 4.19)	4.14 (4.00 - 4.28)
1-3/week	4.62 (4.49 - 4.75)***	4.39 (4.27 - 4.51)**	4.38 (4.24 - 4.51)**
≥ 4/week	4.63 (4.37 - 4.91)**	4.41 (4.15 - 4.69)*	4.57 (4.32 - 4.83)**

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table and rubidium blood levels (Spearman's correlation coefficient $r_S = 0.539$)

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S7. Chromium whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	0.61 (0.57 - 0.66)	0.62 (0.57 - 0.67)	0.59 (0.55 - 0.64)
Men	0.55 (0.51 - 0.60)	0.56 (0.51 - 0.61)	0.60 (0.55 - 0.65)
Age (year) [e]			
20-29[d]	0.65 (0.56 - 0.75)	0.64 (0.51 - 0.81)	0.61 (0.51 - 0.72)
30-39	0.60(0.52 - 0.69)	0.59 (0.51 - 0.70)	0.63 (0.56 - 0.71)
40-49	0.64 (0.56 - 0.73)	0.64 (0.57 - 0.72)	0.61 (0.55 - 0.67)
50-59	0.56 (0.49 - 0.65)	0.56 (0.50 - 0.63)	0.57 (0.52 - 0.63)
60-69	0.58 (0.51 - 0.67)	0.58 (0.51 - 0.65)	0.60 (0.54 - 0.67)
≥70	0.52 (0.45 - 0.60)	0.52 (0.45 - 0.59)	0.56 (0.50 - 0.63)
Region			
Inland-Mountains	0.58 (0.53 - 0.64)	0.59 (0.49 - 0.72)	0.58 (0.51 - 0.66)
Urban	0.60 (0.54 - 0.66)	0.60 (0.56 - 0.65)	0.62 (0.58 - 0.66)
Coastal [d]	0.53 (0.48 - 0.58)	0.54 (0.47 - 0.61)	0.59 (0.54 - 0.65)
Waist-to-hip ratio			
≤ 0.865 [d]	0.60 (0.55 - 0.66)	0.58 (0.52 - 0.64)	0.60 (0.55 - 0.66)
0.866-0.932	0.61 (0.55 - 0.68)	0.62 (0.56 - 0.68)	0.62 (0.57 - 0.68)
≥ 0.933	0.54 (0.49 - 0.60)	0.57 (0.51 - 0.63)	0.56 (0.51 - 0.62)
Body mass index (kg/m^2)			
≤ 24[d]	0.57 (0.52 - 0.63)	0.56 (0.51 - 0.62)	0.55 (0.50 - 0.60)
25-29	0.58 (0.53 - 0.63)	0.59 (0.54 - 0.64)	0.59 (0.54 - 0.64)
≥ 30	0.63 (0.56 - 0.70)	0.64 (0.57 - 0.72)	0.65 (0.59 - 0.72)*
Education (years)			
< 10 [d]	0.63 (0.56 - 0.71)	0.66 (0.58 - 0.75)	0.66 (0.60 - 0.73)
10-12	0.54 (0.51 - 0.59)	0.55 (0.51 - 0.60)*	0.56 (0.52 - 0.61)**
≥ 13	0.62 (0.55 - 0.70)	0.61 (0.54 - 0.68)	0.57 (0.51 - 0.63)*
Economic status level			
Quartile 1 [d]	0.56 (0.50 - 0.62)	0.57 (0.50 - 0.65)	0.57 (0.52 - 0.63)
Quartile 2	0.57 (0.51 - 0.64)	0.58 (0.51 - 0.65)	0.56 (0.51 - 0.61)
Quartile 3	0.59 (0.53 - 0.65)	0.59 (0.53 - 0.65)	0.60 (0.54 - 0.65)
Quartile 4 (highest)	0.61 (0.54 - 0.69)	0.62 (0.55 - 0.70)	0.66 (0.60 - 0.73)
Alcohol consumption (g/day)			
Abstainers [d]	0.51 (0.46 - 0.56)	0.51 (0.45 - 0.57)	0.57 (0.52 - 0.63)
< 2.7	0.63 (0.56 - 0.71)	0.62 (0.55 - 0.70)	0.62 (0.56 - 0.68)
2.7 – 6.0	0.57 (0.51 - 0.63)	0.57 (0.51 - 0.64)	0.58 (0.53 - 0.64)
≥ 6.1	0.64 (0.57 - 0.72)*	0.65 (0.58 - 0.73)*	0.61 (0.56 - 0.68)
Smoking status			
Never-smokers [d]	0.56 (0.51 - 0.61)	0.56 (0.51 - 0.61)	0.59 (0.54 - 0.64)
Former smokers	0.62 (0.57 - 0.68)	0.64 (0.58 - 0.70)	0.61 (0.56 - 0.66)
Current smokers	0.56 (0.50 - 0.64)	0.57 (0.50 - 0.65)	0.59 (0.53 - 0.65)
Fatty-fish consumption [f]			
0-3/month [c]	0.58 (0.53 - 0.63)	0.57 (0.52 - 0.63)	0.59 (0.54 - 0.64)
1-3/week	0.58 (0.54 - 0.63)	0.59 (0.54 - 0.65)	0.59 (0.55 - 0.63)
≥ 4/week	0.64 (0.54 - 0.75)	0.62 (0.57 - 0.67)	0.59 (0.55 - 0.64)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table and nickel blood levels (Spearman's correlation coefficient $r_S = 0.769$)

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S8. Copper whole blood levels (mg/L) by subjects' characteristics.

Factors	Arithmetic means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	1.08 (1.06 - 1.09)	1.08 (1.07 - 1.10)	1.08 (1.06 - 1.10)
Men	0.95 (0.94 - 0.95)***	0.95 (0.94 - 0.96)***	0.95 (0.93 - 0.97)***
Age (year) [e]			
20-29 [d]	1.06 (1.02 - 1.09)	1.05 (1.01 - 1.08)	1.04 (1.00 - 1.08)
30-39	1.01 (0.98 - 1.04)	1.00 (0.98 - 1.02)	1.01 (0.98 - 1.04)
40-49	1.00 (0.98 - 1.02)	0.99 (0.98 - 1.01)	1.00 (0.97 - 1.02)
50-59	1.02 (0.99 - 1.04)	1.01 (1.00 - 1.03)	1.02 (1.00 - 1.04)
60-69	1.01 (0.99 - 1.03)	1.01 (0.99 - 1.03)	1.01 (0.99 - 1.04)
≥70	1.02 (1.00 - 1.04)	1.02 (1.00 - 1.04)	1.01 (0.98 - 1.03)
Region			
Inland-Mountains	1.01 (1.00 - 1.03)	1.02 (0.99 - 1.05)	1.02 (0.99 - 1.05)
Urban	1.02 (1.00 - 1.03)	1.02 (1.01 - 1.03)	1.02 (1.01 - 1.04)
Coastal [d]	1.00 (0.99 - 1.02)	1.00 (0.98 - 1.02)	1.00 (0.98 - 1.02)
Waist-to-hip ratio			
≤ 0.865 [d]	1.05 (1.03 - 1.07)	1.01 (0.99 - 1.02)	1.01 (0.99 - 1.04)
0.866-0.932	1.02 (1.00 - 1.03)**	1.02 (1.01 - 1.04)	1.02 (1.00 - 1.04)
≥ 0.933	0.98 (0.97 - 0.99)***	1.01 (0.99 - 1.02)	1.00 (0.98 - 1.02)
Body mass index (kg/m^2)			
≤ 24[d]	1.03 (1.01 - 1.05)	1.01 (1.00 - 1.03)	1.01 (0.99 - 1.03)
25-29	0.99 (0.98 - 1.01)**	1.01 (1.00 - 1.02)	1.01 (0.99 - 1.03)
≥ 30	1.03 (1.02 - 1.05)	1.02 (1.01 - 1.04)	1.03 (1.00 - 1.05)
Education (years)			
< 10 [d]	1.02 (1.01 - 1.04)	1.03 (1.01 - 1.05)	1.02 (1.00 - 1.04)
10-12	1.02 (1.00 - 1.03)	1.02 (1.01 - 1.03)	1.02 (1.00 - 1.04)
≥ 13	1.00 (0.98 - 1.02)	0.99 (0.98 - 1.01)*	1.00 (0.98 - 1.02)
Economic status level			
Quartile 1 [d]	1.04 (1.02 - 1.06)	1.04 (1.02 - 1.06)	1.03 (1.00 - 1.05)
Quartile 2	1.02 (1.00 - 1.04)	1.02 (1.00 - 1.04)	1.01 (0.99 - 1.04)
Quartile 3	1.00 (0.99 - 1.02)*	1.00 (0.98 - 1.01)*	1.00 (0.98 - 1.02)
Quartile 4 (highest)	1.00 (0.99 - 1.02)*	1.01 (0.99 - 1.02)	1.01 (0.99 - 1.04)
Alcohol consumption (g/day)			
Abstainers [d]	1.05 (1.03 - 1.07)	1.02 (1.01 - 1.04)	1.02 (1.00 - 1.04)
< 2.7	1.02 (1.00 - 1.04)	1.00 (0.99 - 1.02)	1.01 (0.99 - 1.03)
2.7-6.0	0.99 (0.97 - 1.01)***	1.00 (0.98 - 1.02)	1.00 (0.98 -.1.02)
≥ 6.1	1.00 (0.98 - 1.02)**	1.02 (1.01 - 1.04)	1.03 (1.00 - 1.05)
Smoking status			
Never-smokers[d]	1.01 (1.00 - 1.02)	1.01 (0.99 - 1.02)	1.00 (0.99 - 1.02)
Former smokers	1.00 (0.99 - 1.01)	1.00 (0.99 - 1.02)	1.00 (0.98 - 1.02)
Current smokers	1.05 (1.03 - 1.08)**	1.05 (1.03 - 1.07)**	1.04 (1.02 - 1.06)*
Fatty-fish consumption[f]			
0-3/month[c]	1.01 (0.99 - 1.02)	1.02 (1.00 - 1.03)	1.02 (1.00 - 1.03)
1-3/week	1.02 (1.00 - 1.03)	1.01 (1.00 - 1.02)	1.01 (1.00 - 1.03)
≥ 4/week	1.03 (1.00 - 1.06)	1.01 (0.99 - 1.04)	1.01 (0.98 - 1.04)

Arithmetic means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S9. Gallium whole blood levels (µg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	0.069 (0.068 - 0.071)	0.069 (0.067 - 0.071)	0.068 (0.065 - 0.071)
Men	0.077 (0.075 - 0.080)***	0.077 (0.075 - 0.080)***	0.078 (0.074 - 0.081)***
Age (year)[e]			
20-29 [d]	0.075 (0.070 - 0.080)	0.075 (0.069 - 0.082)	0.075 (0.069 - 0.082)
30-39	0.070 (0.067 - 0.073)	0.071 (0.067 - 0.075)	0.068 (0.064 - 0.073)
40-49	0.074 (0.071 - 0.077)	0.074 (0.071 - 0.078)	0.075 (0.071 - 0.079)
50-59	0.070 (0.067 - 0.072)	0.070 (0.067 - 0.073)	0.070 (0.066 - 0.073)
60-69	0.079 (0.074 - 0.084)	0.079 (0.076 - 0.083)	0.079 (0.075 - 0.083)
≥70	0.070 (0.067 - 0.074)	0.071 (0.067 - 0.074)	0.069 (0.066 - 0.074)
Region			
Inland-Mountains	0.081 (0.079 - 0.084)***	0.082 (0.076 - 0.088)***	0.080 (0.074 - 0.085)***
Urban	0.074 (0.071 - 0.076)***	0.074 (0.072 - 0.076)***	0.073 (0.071 - 0.076)***
Coastal [d]	0.067 (0.065 - 0.069)	0.067 (0.064 - 0.070)	0.066 (0.063 - 0.069)
Waist-to-hip ratio			
≤ 0.865 [d]	0.070 (0.068 - 0.072)	0.073 (0.070 - 0.076)	0.069 (0.065 - 0.072)
0.866-0.932	0.074 (0.071 - 0.077)	0.074 (0.071 - 0.077)	0.073 (0.070 - 0.076)
≥ 0.933	0.075 (0.072 - 0.077)*	0.073 (0.070 - 0.076)	0.077 (0.073 - 0.081)
Body mass index (kg/m^2)			
≤ 24[d]	0.069 (0.067 - 0.071)	0.070 (0.068 - 0.073)	0.069 (0.065 - 0.072)
25-29	0.074 (0.072 - 0.076)*	0.074 (0.071 - 0.076)	0.073 (0.070 - 0.076)*
≥ 30	0.077 (0.073 - 0.080)***	0.077 (0.074 - 0.081)**	0.077 (0.073 - 0.081)**
Education (years)			
< 10 [d]	0.072 (0.069 - 0.074)	0.072 (0.068 - 0.075)	0.071 (0.068 - 0.075)
10-12	0.074 (0.072 - 0.076)	0.074 (0.072 - 0.076)	0.073 (0.070 - 0.076)
≥ 13	0.073 (0.070 - 0.076)	0.074 (0.071 - 0.077)	0.074 (0.070 - 0.077)
Economic status level			
Quartile 1 [d]	0.073 (0.070 - 0.075)	0.073 (0.070 - 0.077)	0.074 (0.070 - 0.078)
Quartile 2	0.075 (0.072 - 0.077)	0.075 (0.072 - 0.078)	0.074 (0.070 - 0.077)
Quartile 3	0.072 (0.070 - 0.075)	0.073 (0.070 - 0.076)	0.072 (0.069 - 0.076)
Quartile 4 (highest)	0.072 (0.068 - 0.076)	0.072 (0.069 - 0.075)	0.071 (0.067 - 0.075)
Alcohol consumption (g/day)			
Abstainers [d]	0.073 (0.071 - 0.075)	0.073 (0.071 - 0.075)	0.075 (0.072 - 0.079)
< 2.7	0.075 (0.071 - 0.078)	0.075 (0.071 - 0.078)	0.075 (0.071 - 0.078)
2.7-6.0	0.072 (0.069 - 0.075)	0.072 (0.069 - 0.075)	0.071 (0.067 - 0.074)
≥ 6.1	0.072 (0.070 - 0.075)	0.072 (0.070 - 0.075)	0.070 (0.067 - 0.074)
Smoking status			
Never-smokers [d]	0.074 (0.072 - 0.076)	0.075 (0.072 - 0.077)	0.074 (0.071 - 0.077)
Former smokers	0.073 (0.071 - 0.075)	0.073 (0.070 - 0.075)	0.073 (0.070 - 0.076)
Current smokers	0.070 (0.067 - 0.074)	0.071 (0.068 - 0.074)	0.071 (0.067 - 0.075)
Fatty-fish consumption [f]			
0-3/month [c]	0.074 (0.072 - 0.077)	0.074 (0.071 - 0.076)	0.074 (0.071 - 0.077)
1-3/week	0.073 (0.071 - 0.075)	0.074 (0.072 - 0.076)	0.075 (0.072 - 0.077)
≥ 4/week	0.067 (0.063 - 0.071)*	0.068 (0.064 - 0.073)	0.070 (0.065 - 0.075)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S10. Gold whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	0.0095 (0.0088 - 0.0102)	0.0091 (0.0085 - 0.0098)	0.0083 (0.0075 - 0.0091)
Men	0.0100 (0.0093 - 0.0107)	0.0095 (0.0088 - 0.0102)	0.0093 (0.0083 - 0.0103)*
Age (year) [e]			
20-29[d]	0.0065 (0.0059 - 0.0072)	0.0065 (0.0053 - 0.0080)	0.0060 (0.0048 - 0.0075)
30-39	0.0082 (0.0074 - 0.0091)	0.0082 (0.0071 - 0.0094)	0.0074 (0.0063 - 0.0086)
40-49	0.0086 (0.0079 - 0.0094)*	0.0086 (0.0077 - 0.0096)	0.0080 (0.0070 - 0.0091)
50-59	0.0087 (0.0077 - 0.0098)*	0.0087 (0.0079 - 0.0097)	0.0082 (0.0072 - 0.0093)
60-69	0.0120 (0.0105 - 0.0137)***	0.0120 (0.0108 - 0.0134)***	0.0112 (0.0098 - 0.0127)***
≥70	0.0132 (0.0114 - 0.0153)***	0.0132 (0.0118 - 0.0149)***	0.0138 (0.0119 - 0.0159)***
Region			
Inland-Mountains	0.0077 (0.0070 - 0.0084)	0.0072 (0.0060 - 0.0085)	0.0075 (0.0063 - 0.0090)
Urban	0.0103 (0.0094 - 0.0113)*	0.0098 (0.0092 - 0.0105)*	0.0101 (0.0093 - 0.0110)
Coastal [d]	0.0088 (0.0083 - 0.0094)	0.0083 (0.0075 - 0.0093)	0.0088 (0.0078 - 0.0099)
Waist-to-hip ratio			
≤ 0.865 [d]	0.0098 (0.0089 - 0.0108)	0.0102 (0.0093 - 0.0113)	0.0098 (0.0087 - 0.0111)
0.866-0.932	0.0096 (0.0088 - 0.0103)	0.0091 (0.0084 - 0.0100)	0.0087 (0.0078 - 0.0097)
≥ 0.933	0.0099 (0.0090 - 0.0108)	0.0085 (0.0077 - 0.0093)*	0.0078 (0.0070 - 0.0088)*
Body mass index (kg/m^2)			
≤ 24[d]	0.0097 (0.0088 - 0.0107)	0.0096 (0.0087 - 0.0105)	0.0085 (0.0075 - 0.0096)
25-29	0.0096 (0.0089 - 0.0102)	0.0090 (0.0083 - 0.0097)	0.0084 (0.0075 - 0.0093)
≥ 30	0.0102 (0.0091 - 0.0113)	0.0095 (0.0085 - 0.0105)	0.0094 (0.0083 - 0.0107)
Education (years)			
< 10 [d]	0.0098 (0.0089 - 0.0107)	0.0084 (0.0075 - 0.0094)	0.0080 (0.0071 - 0.0091)
10-12	0.0100 (0.0093 - 0.0108)	0.0096 (0.0089 - 0.0103)	0.0092 (0.0084 - 0.0102)
≥ 13	0.0090 (0.0082 - 0.0100)	0.0093 (0.0084 - 0.0102)	0.0090 (0.0079 - 0.0102)
Economic status level			
Quartile 1 [d]	0.0097 (0.0087 - 0.0107)	0.0084 (0.0075 - 0.0094)	0.0079 (0.0069 - 0.0090)
Quartile 2	0.0104 (0.0093 - 0.0117)	0.0098 (0.0088 - 0.0109)	0.0095 (0.0084 - 0.0108)
Quartile 3	0.0099 (0.0091 - 0.0108)	0.0100 (0.0091 - 0.0110)	0.0092 (0.0082 - 0.0103)
Quartile 4 (highest)	0.0090 (0.0081 - 0.0100)	0.0089 (0.0080 - 0.0099)	0.0085 (0.0075 - 0.0097)
Alcohol consumption (g/day)			
Abstainers [d]	0.0104 (0.0094 - 0.0115)	0.0092 (0.0083 - 0.0102)	0.0092 (0.0081 - 0.0104)
< 2.7	0.0089 (0.0080 - 0.0100)	0.0085 (0.0076 - 0.0094)	0.0078 (0.0069 - 0.0089)
2.7-6.0	0.0110 (0.0100 - 0.0120)	0.0105 (0.0095 - 0.0116)	0.0098 (0.0086 - 0.0110)
≥ 6.1	0.0088 (0.0080 - 0.0096)	0.0090 (0.0081 - 0.0100)	0.0083 (0.0074 - 0.0094)
Smoking status			
Never-smokers [d]	0.0086 (0.0080 - 0.0093)	0.0084 (0.0078 - 0.0091)	0.0078 (0.0070 - 0.0087)
Former smokers	0.0105 (0.0097 - 0.0115)**	0.0099 (0.0091 - 0.0108)*	0.0090 (0.0081 - 0.0101)*
Current smokers	0.0108 (0.0097 - 0.0120)**	0.0101 (0.0090 - 0.0114)*	0.0095 (0.0084 - 0.0108)*
Fatty-fish consumption[f]			
0-3/month [c]	0.0101 (0.0093 - 0.0108)	0.0101 (0.0093 - 0.0109)	0.0091 (0.0082 - 0.0101)
1-3/week	0.0094 (0.0087 - 0.0101)	0.0086 (0.0080 - 0.0092)**	0.0079 (0.0072 - 0.0086)*
≥ 4/week	0.0107 (0.0090 - 0.0129)	0.0096 (0.0081 - 0.0113)	0.0093 (0.0079 - 0.0110)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S11. Indium whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	0.029 (0.028 - 0.030)	0.029 (0.028 - 0.030)	0.029 (0.027 - 0.030)
Men	0.030 (0.029 - 0.031)	0.030 (0.029 - 0.031)	0.030 (0.028 - 0.032)
Age (year) [e]			
20-29 [d]	0.029 (0.027 - 0.031)	0.029 (0.026 - 0.032)	0.029 (0.026 - 0.033)
30-39	0.030 (0.028 - 0.032)	0.030 (0.028 - 0.032)	0.029 (0.026 - 0.032)
40-49	0.030 (0.028 - 0.032)	0.030 (0.028 - 0.032)	0.029 (0.027 - 0.032)
50-59	0.030 (0.028 - 0.032)	0.030 (0.028 - 0.032)	0.029 (0.027 - 0.031)
60-69	0.027 (0.025 - 0.030)	0.027 (0.026 - 0.029)	0.028 (0.026 - 0.030)
≥70	0.031 (0.029 - 0.033)	0.031 (0.029 - 0.033)	0.032 (0.030 - 0.035)
Region			
Inland-Mountains	0.027 (0.026 - 0.028)	0.027 (0.024 - 0.030)	0.030 (0.028 - 0.032)
Urban	0.030 (0.028 - 0.031)	0.030 (0.029 - 0.031)	0.030 (0.029 - 0.032)
Coastal [d]	0.029 (0.027 - 0.030)	0.029 (0.027 - 0.031)	0.028 (0.026 - 0.030)
Waist-to-hip ratio			
≤ 0.865 [d]	0.029 (0.028 - 0.031)	0.030 (0.028 - 0.031)	0.030 (0.028 - 0.032)
0.866-0.932	0.031 (0.029 - 0.032)	0.030 (0.029 - 0.032)	0.030 (0.029 - 0.032)
≥ 0.933	0.028 (0.027 - 0.030)	0.028 (0.026 - 0.029)	0.028 (0.026 - 0.030)
Body mass index (kg/m^2)			
≤ 24[d]	0.028 (0.027 - 0.030)	0.028 (0.027 - 0.030)	0.028 (0.026 - 0.030)
25-29	0.031 (0.029 - 0.032)*	0.031 (0.029 - 0.032)	0.031 (0.029 - 0.033)*
≥ 30	0.028 (0.026 - 0.030)	0.028 (0.027 - 0.030)	0.029 (0.027 - 0.032)
Education (years)			
< 10 [d]	0.029 (0.027 - 0.031)	0.029 (0.027 - 0.030)	0.029 (0.027 - 0.031)
10-12	0.029 (0.028 - 0.030)	0.029 (0.028 - 0.030)	0.029 (0.028 - 0.031)
≥ 13	0.031 (0.029 - 0.032)	0.031 (0.029 - 0.033)	0.031 (0.029 - 0.033)
Economic status level			
Quartile 1 [d]	0.028 (0.027 - 0.030)	0.028 (0.026 - 0.030)	0.028 (0.026 - 0.030)
Quartile 2	0.029 (0.028 - 0.031)	0.029 (0.028 - 0.031)	0.030 (0.028 - 0.032)
Quartile 3	0.030 (0.028 - 0.031)	0.030 (0.028 - 0.032)	0.030 (0.028 - 0.032)
Quartile 4 (highest)	0.030 (0.028 - 0.032)	0.030 (0.028 - 0.032)	0.030 (0.028 - 0.032)
Alcohol consumption (g/day)			
Abstainers [d]	0.030 (0.028 - 0.031)	0.029 (0.028 - 0.031)	0.030 (0.028 - 0.032)
< 2.7	0.028 (0.026 - 0.029)	0.028 (0.026 - 0.030)	0.028 (0.026 - 0.030)
2.7-6.0	0.030 (0.029 - 0.032)	0.031 (0.029 - 0.032)	0.030 (0.028 - 0.033)
≥ 6.1	0.030 (0.028 - 0.031)	0.030 (0.028 - 0.031)	0.030 (0.028 - 0.032)
Smoking status			
Never-smokers [d]	0.029 (0.028 - 0.030)	0.029 (0.028 - 0.031)	0.029 (0.028 - 0.031)
Former smokers	0.030 (0.028 - 0.031)	0.030 (0.028 - 0.031)	0.030 (0.028 - 0.032)
Current smokers	0.029 (0.027 - 0.031)	0.029 (0.027 - 0.031)	0.029 (0.027 - 0.031)
Fatty-fish consumption[f]			
0-3/month [c]	0.030 (0.028 - 0.031)	0.030 (0.028 - 0.031)	0.029 (0.028 - 0.031)
1-3/week	0.029 (0.028 - 0.030)	0.029 (0.028 - 0.030)	0.029 (0.027 - 0.030)
≥ 4/week	0.030 (0.028 - 0.032)	0.030 (0.027 - 0.033)	0.030 (0.027 - 0.033)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S12. Iron whole blood levels (mg/L) by subjects' characteristics.

Factors	Arithmetic means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	519 (516 - 523)	520 (516 – 524)	530 (525 – 535)
Men	571 (567 - 575)***	572 (568 – 576)***	553 (548 – 558)***
Age (year)[e]			
20-29[d]	547 (535 - 560)	550 (539 – 561)	555 (545 – 565)
30-39	545 (536 - 554)	549 (541 – 556)	550 (542 – 557)
40-49	543 (537 - 550)	545 (539 – 551)	544 (538 – 549)
50-59	543 (537 - 550)	545 (539 – 550)	540 (534 – 545)
60-69	549 (543 - 556)	551 (545 – 556)	534 (528 – 540)**
≥70	533 (526 - 541)	535 (529 – 542)	526 (519 – 532)***
Region			
Inland-Mountains	541 (530 - 552)	543 (534 – 553)	536 (528 – 544)
Urban	546 (543 - 550)**	548 (545 – 552)**	548 (544 – 551)*
Coastal [d]	534 (527 - 541)	537 (531 – 543)	540 (535 – 546)
Waist-to-hip ratio			
≤ 0.865 [d]	524 (519 - 529)	540 (535 – 546)	542 (537 – 548)
0.866-0.932	546 (541 - 551)***	546 (542 – 551)	542 (537 – 547)
≥ 0.933	558 (553 - 563)***	551 (546 – 556)*	539 (534 – 545)
Body mass index (kg/m^2)			
≤ 24[d]	522 (518 - 527)	533 (529 – 538)	533 (527 – 538)
25-29	549 (543 - 554)***	549 (545 – 553)***	543 (539 – 548)**
≥ 30	550 (544 - 557)***	558 (552 – 564)***	548 (542 – 554)***
Education (years)			
< 10 [d]	546 (539 - 552)	550 (544 – 556)	547 (541 – 553)
10-12	546 (542 - 550)	547 (543 – 551)	540 (536 – 545)
≥ 13	536 (530 - 542)	539 (534 – 545)*	537 (531 – 543)**
Economic status level			
Quartile 1 [d]	540 (534 - 547)	546 (540 – 552)	538 (532 – 544)
Quartile 2	547 (540 - 553)	549 (543 – 555)	543 (537 – 548)
Quartile 3	543 (537 - 548)	546 (540 – 551)	542 (537 – 548)
Quartile 4 (highest)	544 (538 - 550)	543 (537 – 548)	542 (536 – 548)
Alcohol consumption (g/day)			
Abstainers [d]	533 (527 - 539)	545 (540 – 551)	540 (534 – 546)
< 2.7	538 (531 - 544)	545 (539 – 550)	541 (536 – 547)
2.7-6.0	547 (541 - 553)**	545 (539 – 550)	542 (536 – 547)
≥ 6.1	555 (549 - 561)***	548 (543 – 554)	543 (537 – 548)
Smoking status			
Never-smokers [d]	546 (541 - 551)	548 (544 – 553)	545 (540 – 549)
Former smokers	542 (537 - 547)	542 (538 – 547)	540 (535 – 545)
Current smokers	541 (534 - 548)	546 (540 – 553)	539 (533 – 545)
Fatty-fish consumption[f]			
0-3/month [c]	545 (540 - 550)	544 (539 – 548)	537 (533 – 542)
1-3/week	544 (540 - 548)	548 (544 – 552)	546 (542 – 551)**
≥ 4/week	533 (522 - 543)	542 (533 – 551)	540 (533 – 548)

Arithmetic means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table and calcium and zinc blood levels (Spearman's correlation coefficient, r_S = -0.588, r_S = 0.559, respectively).

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S13. Lead whole blood levels (µg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	16.7 (16.0 - 17.4)	16.1 (15.4 - 16.8)	17.7 (1.03 - 16.64)
Men	21.6 (20.7 - 22.6)***	20.7 (19.8 - 21.7)***	21.32 (1.03 - 19.98)***
Age (year)[e]			
20-29[d]	14.5 (13.5 - 15.5)	14.7 (13.0 - 16.6)	15.6 (13.7 - 17.8)
30-39	16.5 (15.2 - 18.0)	16.9 (15.5 - 18.3)	19.0 (17.2 - 20.9)
40-49	18.0 (16.9 - 19.3)*	18.2 (17.0 - 19.4)*	18.7 (17.3 - 20.2)
50-59	19.8 (18.3 - 21.3)***	19.9 (18.7 - 21.1)***	20.0 (18.5 - 21.5)*
60-69	21.6 (20.0 - 23.3)***	21.7 (20.3 - 23.1)***	22.9 (21.2 - 24.7)***
≥70	19.0 (17.7 - 20.4)**	19.1 (17.9 - 20.5)**	21.1 (19.3 - 23.0)**
Region			
Inland-Mountains	21.8 (20.6 - 23.0)	21.0 (18.9 - 23.3)	21.4 (19.3 - 23.8)
Urban	18.5 (17.6 - 19.5)*	18.0 (17.3 - 18.7)	18.3 (17.4 - 19.2)*
Coastal [d]	18.8 (17.8 - 19.9)	18.2 (17.0 - 19.4)	18.6 (17.4 - 20.0)
Waist-to-hip ratio			
≤ 0.865 [d]	17.0 (16.1 - 17.9)	18.3 (17.2 - 19.3)	19.8 (18.3 - 21.3)
0.866-0.932	18.8 (17.8 - 19.8)*	18.1 (17.2 - 19.1)	18.8 (17.6 - 20.1)
≥ 0.933	20.8 (19.7 - 21.9)***	18.5 (17.4 - 19.6)	19.7 (18.3 - 21.1)
Body mass index (kg/m^2)			
≤ 24[d]	17.1 (16.1 - 18.2	17.6 (16.7 - 18.6)	18.7 (17.4 - 20.0)
25-29	20.1 (19.2 - 21.0)***	18.9 (18.0 - 19.8)	20.2 (19.0 - 21.5)
≥ 30	18.9 (17.8 - 20.0)	18.1 (17.0 - 19.3)	19.4 (17.9 - 20.9)
Education (years)			
< 10 [d]	19.7 (18.6 - 20.8)	19.0 (17.7 - 20.3)	20.3 (18.9 - 21.9)
10-12	19.7 (18.9 - 20.6)	18.9 (18.1 - 19.7)	20.1 (19.0 - 21.3)
≥ 13	16.7 (15.6 - 17.9)**	16.7 (15.7 - 17.7)*	17.9 (16.5 - 19.3)*
Economic status level			
Quartile 1 [d]	17.8 (16.7 - 19.0)	17.5 (16.4 - 18.7)	18.5 (17.1 - 20.0)
Quartile 2	19.0 (17.8 - 20.2)	18.4 (17.3 - 19.6)	19.3 (17.9 - 20.8)
Quartile 3	18.7 (17.6 - 19.9)	18.5 (17.5 - 19.6)	19.6 (18.2 - 21.0)
Quartile 4 (highest)	19.7 (18.6 - 20.9)	18.6 (17.4 - 19.8)	20.3 (18.8 - 21.9)
Alcohol consumption (g/day)			
Abstainers [d]	16.6 (15.7 - 17.6)	16.2 (15.3 - 17.3)	17.5 (16.2 - 18.8)
< 2.7	17.3 (16.3 - 18.5)	17.0 (15.9 - 18.1)	18.0 (16.7 - 19.4)
2.7-6.0	19.7 (18.5 - 20.9)***	18.6 (17.5 - 19.8)*	19.9 (18.5 - 21.4)*
≥ 6.1	22.0 (20.7 - 23.3)***	21.2 (19.9 - 22.5)***	22.7 (21.0 - 24.4)***
Smoking status			
Never-smokers [d]	17.3 (16.5 - 18.1)	17.2 (16.4 - 18.0)	18.1 (17.0 - 19.3)
Former smokers	19.6 (18.7 - 20.6)**	18.6 (17.7 - 19.5)	19.2 (18.0 - 20.5)
Current smokers	20.6 (19.2 - 22.1)***	20.3 (19.0 - 21.8)***	21.0 (19.4 - 22.7)**
Fatty-fish consumption[f]			
0-3/month [c]	18.3 (17.4 – 19.2)	18.0 (17.2 - 18.9)	19.0 (17.8 - 20.1)
1-3/week	19.1 (18.2 - 19.9)	18.3 (17.5 - 19.2)	19.2 (18.2 - 20.3)
≥ 4/week	19.7 (17.8 - 21.8)	19.2 (17.4 - 21.2)	20.1 (18.1 - 22.2)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S14. Magnesium whole blood levels (mg/L) by subjects' characteristics.

Factors	Arithmetic means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	39.0 (38.7 - 39.3)	39.0 (38.6 - 39.3)	39.2 (38.7 - 39.6)
Men	40.6 (40.3 - 40.9)***	40.6 (40.2 - 40.9)***	40.8 (40.3 - 41.3)***
Age (year) [e]			
20-29 [d]	39.2 (38.3 - 40.2)	39.3 (38.4 - 40.2)	39.8 (38.8 - 40.8)
30-39	40.0 (39.3 - 40.6)	40.1 (39.4 - 40.7)	40.4 (39.6 - 41.1)
40-49	39.5 (39.0 - 40.0)	39.6 (39.1 - 40.1)	39.7 (39.1 - 40.3)
50-59	39.9 (39.4 - 40.4)	39.9 (39.5 - 40.4)	40.1 (39.5 - 40.7)
60-69	39.9 (39.4 - 40.4)	40.0 (39.5 - 40.4)	40.2 (39.6 - 40.8)
≥70	39.6 (39.1 - 40.2)	39.7 (39.1 - 40.2)	39.8 (39.1 - 40.5)
Region			
Inland Mountains	39.4 (38.6 - 40.2)	39.4 (38.6 - 40.2)	39.8 (39.0 - 40.7)
Urban	39.9 (39.6 - 40.2)	39.9 (39.6 - 40.2)	40.4 (40.1 - 40.8)
Coastal [d]	39.3 (38.8 - 39.8)	39.3 (38.8 - 39.8)	39.7 (39.2 - 40.2)
Waist-to-hip ratio			
≤ 0.865 [d]	38.9 (38.5 - 39.3)	39.4 (39.0 - 39.8)	40.1 (39.5 - 40.6)
0.866-0.932	40.1 (39.7 - 40.5)***	40.0 (39.6 - 40.4)	40.2 (39.7 - 40.7)
≥ 0.933	40.1 (39.8 - 40.5)***	39.8 (39.4 - 40.3)	39.7 (39.2 - 40.3)
Body mass index (kg/m^2)			
≤ 24 [d]	38.8 (38.4 - 39.3)	39.1 (38.7 - 39.5)	39.2 (38.6 - 39.7)
25-29	40.0 (39.7 - 40.4)***	39.9 (39.5 - 40.2)*	40.1 (39.6 - 40.6)**
≥ 30	40.4 (39.9 - 40.9)***	40.5 (40.0 - 41.0)***	40.7 (40.2 - 41.3)***
Education (years)			
< 10 [d]	40.3 (39.8 - 40.8)	40.4 (39.9 - 40.9)	40.4 (39.9 - 41.0)
10-12	39.5 (39.2 - 39.9)*	39.5 (39.2 - 39.8)**	39.6 (39.1 - 40.0) **
≥ 13	39.7 (39.3 - 40.2)	39.8 (39.3 - 40.2)	40.0 (39.4 - 40.6)
Economic status level			
Quartile 1 [d]	39.6 (39.2 - 40.1)	39.8 (39.2 - 40.3)	39.8 (39.2 - 40.5)
Quartile 2	39.6 (39.1 - 40.1)	39.6 (39.1 - 40.1)	39.8 (39.2 - 40.4)
Quartile 3	39.8 (39.4 - 40.2)	39.9 (39.4 - 40.3)	40.2 (39.6 - 40.7)
Quartile 4 (highest)	39.9 (39.4 - 40.3)	39.8 (39.3 - 40.3)	40.2 (39.6 - 40.8)
Alcohol consumption (g/day)			
Abstainers [d]	39.6 (39.2 - 40.1)	39.9 (39.4 - 40.4)	40.1 (39.6 - 40.7)
< 2.7	39.7 (39.2 - 40.1)	39.8 (39.3 - 40.3)	40.1 (39.5 - 40.6)
2.7-6.0	39.4 (39.0 - 39.9)	39.3 (38.8 - 39.7)	39.5 (38.9 - 40.0)
≥ 6.1	40.3 (39.8 - 40.7)	40.1 (39.6 - 40.5)	40.3 (39.7 - 40.9)
Smoking status			
Never-smokers [d]	39.6 (39.2 - 39.9)	39.6 (39.3 - 40.0)	39.7 (39.2 - 40.2)
Former smokers	39.6 (39.2 - 40.0)	39.5 (39.2 - 39.9)	39.7 (39.2 - 40.2)
Current smokers	40.4 (39.9 - 40.9)*	40.5 (40.0 - 41.1)**	40.6 (40.0 - 41.2)*
Fatty-fish consumption [f]			
0-3/month [d]	39.9 (39.5 - 40.3)	39.8 (39.5 - 40.2)	39.8 (39.3 - 40.3)
1-3/week	39.6 (39.2 - 39.9)	39.6 (39.2 - 39.9)	39.7 (39.3 - 40.1)
≥ 4/week	40.2 (39.5 - 41.0)	40.4 (39.7 - 41.2)	40.5 (39.7 - 41.3)

Arithmetic means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S15. Manganese whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	9.66 (9.43 - 9.89)	9.74 (9.50 - 9.99)	9.46 (9.12 - 9.81)
Men	8.51 (8.29 - 8.73)***	8.58 (8.35 - 8.82)***	8.29 (7.97 - 8.63)***
Age (year) [e]			
20-29 [d]	9.71 (9.01 - 10.45)	9.64 (8.97 - 10.36)	9.46 (8.74 - 10.24)
30-39	9.18 (8.72 - 9.66)	9.09 (8.64 - 9.55)	8.63 (8.13 - 9.15)
40-49	8.84 (8.50 - 9.20)	8.81 (8.48 - 9.16)	8.61 (8.21 - 9.02)
50-59	8.70 (8.38 - 9.03)	8.68 (8.37 - 9.00)	8.37 (7.99 - 8.77)
60-69	9.57 (9.20 - 9.95)	9.54 (9.18 - 9.92)	9.21 (8.79 - 9.66)
≥70	9.19 (8.81 - 9.59)	9.15 (8.78 - 9.54)	8.91 (8.45 - 9.39)
Region			
Inland Mountains	9.26 (8.69 - 9.87)	9.32 (8.75 - 9.92)	9.04 (8.47 - 9.64)
Urban	9.18 (8.99 - 9.38)	9.22 (9.02 - 9.43)	8.96 (8.70 - 9.23)
Coastal [d]	8.78 (8.43 - 9.14)	8.81 (8.47 - 9.17)	8.58 (8.22 - 8.96)
Waist-to-hip ratio			
≤ 0.865 [d]	9.30 (9.01 - 9.60)	9.32 (8.75 - 9.91)	8.73 (8.34 - 9.14)
0.866-0.932	9.10 (8.82 - 9.38)	9.22 (9.02 - 9.43)	8.95 (8.60 - 9.33)
≥ 0.933	8.94 (8.67 - 9.22)	8.81 (8.47 - 9.17)	8.88 (8.50 - 9.28)
Body mass index (kg/m^2)			
≤ 24 [d]	9.03 (8.74 - 9.33)	8.90 (8.62 - 9.20)	8.66 (8.29 - 9.04)
25-29	9.06 (8.82 - 9.30)	9.27 (9.01 - 9.54)	8.98 (8.64 - 9.32)
≥ 30	9.26 (8.92 - 9.61)	9.23 (8.89 - 9.59)	8.93 (8.52 - 9.36)
Education (years)			
< 10 [d]	8.87 (8.53 - 9.22)	8.83 (8.48 - 9.20)	8.70 (8.31 - 9.11)
10-12	9.17 (8.94 - 9.40)	9.23 (9.00 - 9.48)	8.98 (8.66 - 9.31)
≥ 13	9.18 (8.87 - 9.51)	9.23 (8.90 - 9.56)	8.89 (8.49 - 9.32)
Economic status level			
Quartile 1 [d]	9.15 (8.80 - 9.51)	9.03 (8.67 - 9.40)	8.84 (8.43 - 9.28)
Quartile 2	9.10 (8.76 - 9.45)	9.10 (8.77 - 9.45)	8.76 (8.37 - 9.17)
Quartile 3	9.18 (8.88 - 9.48)	9.26 (8.95 - 9.58)	9.00 (8.62 - 9.40)
Quartile 4 (highest)	9.00 (8.68 - 9.32)	9.17 (8.83 - 9.52)	8.82 (8.41 - 9.24)
Alcohol consumption (g/day)			
Abstainers [d]	9.29 (8.97 - 9.62)	9.12 (8.78 - 9.47)	8.80 (8.41 - 9.21)
< 2.7	9.59 (9.23 - 9.95)	9.48 (9.13 - 9.85)	9.21 (8.80 - 9.63)
2.7-6.0	8.85 (8.54 - 9.17)	8.96 (8.64 - 9.29)	8.66 (8.27 - 9.05)
≥ 6.1	8.78 (8.48 - 9.10)	9.08 (8.75 - 9.41)	8.77 (8.38 - 9.18)
Smoking status			
Never-smokers [d]	9.48 (9.22 - 9.75)	9.49 (9.23 - 9.76)	9.31 (8.96 - 9.66)
Former smokers	8.99 (8.74 - 9.25)*	9.03 (8.77 - 9.31)*	8.81 (8.47 - 9.17)*
Current smokers	8.59 (8.25 - 8.95)***	8.60 (8.25 - 8.95)***	8.47 (8.08 - 8.88)**
Fatty-fish consumption [f]			
0-3/month [d]	8.98 (8.73 - 9.25)	9.07 (8.81 - 9.34)	8.84 (8.52 - 9.18)
1-3/week	9.26 (9.03 - 9.49)	9.29 (9.04 - 9.54)	9.13 (8.83 - 9.44)
≥ 4/week	8.78 (8.28 - 9.31)	8.69 (8.19 - 9.22)	8.60 (8.09 - 9.15)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S16. Mercury whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	2.51 (2.36 - 2.68)	2.18 (2.06 - 2.31)	2.39 (2.25 - 2.55)
Men	3.04 (2.84 - 3.25)***	2.58 (2.42 - 2.74)***	2.78 (2.60 - 2.97)***
Age (year) [e]			
20-29 [d]	1.06 (0.96 - 1.18)	1.07 (0.91 - 1.26)	1.43 (1.25 - 1.64)
30-39	1.45 (1.34 - 1.57)*	1.47 (1.31 - 1.64)*	1.88 (1.70 - 2.08)**
40-49	2.25 (2.05 - 2.47)***	2.26 (2.07 - 2.46)***	2.49 (2.30 - 2.69)***
50-59	3.29 (3.00 - 3.62)***	3.31 (3.05 - 3.58)***	3.16 (2.93 - 3.42)***
60-69	4.11 (3.71 - 4.55)***	4.12 (3.78 - 4.49)***	3.65 (3.37 - 3.96)***
≥70	3.63 (3.27 - 4.02)***	3.65 (3.32 - 4.00)***	3.80 (3.47 - 4.16)***
Region			
Inland Mountains	2.50 (2.34 - 2.69)***	2.12 (1.85 - 2.43)***	2.40 (2.15 - 2.68)*
Urban	2.55 (2.34 - 2.77)***	2.22 (2.11 - 2.33)***	2.49 (2.37 - 2.62)**
Coastal [d]	3.71 (3.46 - 3.98)	3.15 (2.88 - 3.43)	2.86 (2.65 - 3.08)
Waist-to-hip ratio			
≤ 0.865 [d]	2.30 (2.13 - 2.49)	2.35 (2.17 - 2.53)	2.43 (2.25 - 2.62)
0.866-0.932	2.71 (2.49 - 2.94)*	2.39 (2.23 - 2.56)	2.56 (2.39 - 2.75)
≥ 0.933	3.28 (3.04 - 3.55)***	2.38 (2.20 - 2.57)	2.75 (2.56 - 2.96)*
Body mass index (kg/m^2)			
≤ 24 [d]	2.67 (2.44 - 2.93)	2.58 (2.40 - 2.78)	2.79 (2.59 - 3.00)
25-29	2.74 (2.56 - 2.94)	2.26 (2.12 - 2.41)*	2.41 (2.26 - 2.57)**
≥ 30	2.87 (2.63 - 3.14)	2.32 (2.13 - 2.52)	2.55 (2.36 - 2.76)
Education (years)			
< 10 [d]	2.90 (2.62 - 3.21)	2.24 (2.04 - 2.45)	2.51 (2.32 - 2.71)
10-12	2.82 (2.64 - 3.01)	2.38 (2.24 - 2.52)	2.72 (2.56 - 2.89)
≥ 13	2.49 (2.28 - 2.73)	2.47 (2.28 - 2.68)	2.51 (2.32 - 2.72)
Economic status level			
Quartile 1 [d]	2.66 (2.39 - 2.97)	2.26 (2.06 - 2.47)	2.61 (2.41 - 2.83)
Quartile 2	2.90 (2.63 - 3.19)	2.53 (2.32 - 2.75)	2.69 (2.49 - 2.90)
Quartile 3	2.42 (2.24 - 2.62)	2.24 (2.08 - 2.41)	2.54 (2.36 - 2.73)
Quartile 4 (highest)	3.11 (2.83 - 3.40)	2.51 (2.31 - 2.74)	2.48 (2.29 - 2.69)
Alcohol consumption (g/day)			
Abstainers [d]	2.51 (2.30 - 2.73)	2.03 (1.86 - 2.20)	2.33 (2.16 - 2.52)
< 2.7	2.71 (2.46 - 2.98)	2.34 (2.15 - 2.55)	2.73 (2.53 - 2.94)**
2.7-6.0	2.89 (2.63 - 3.18)	2.45 (2.26 - 2.65)**	2.71 (2.51 - 2.93)**
≥ 6.1	2.89 (2.61 - 3.18)	2.68 (2.47 - 2.90)***	2.56 (2.37 - 2.77)
Smoking status			
Never-smokers [d]	2.50 (2.33 - 2.68)	2.35 (2.20 - 2.50)	2.49 (2.34 - 2.66)
Former smokers	2.97 (2.74 - 3.21)**	2.37 (2.22 - 2.54)	2.53 (2.37 - 2.71)
Current smokers	2.86 (2.58 - 3.17)	2.42 (2.21 - 2.65)	2.71 (2.50 - 2.94)
Fatty-fish consumption [f]			
0-3/month [d]	1.98 (1.84 - 2.13)	1.92 (1.80 - 2.04)	2.32 (2.18 - 2.47)
1-3/week	3.27 (3.09 - 3.46)***	2.70 (2.55 - 2.86)***	2.66 (2.52 - 2.82)***
≥ 4/week	4.01 (3.37 - 4.76)***	3.26 (2.87 - 3.70)***	2.78 (2.50 - 3.08)**

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table and blood levels of arsenic and selenium (Spearman's correlation coefficient $r_S = 0.611$ and $r_S = 0.535$, respectively).

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model .

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S17. Molybdenum whole blood levels (µg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	0.84 (0.80 - 0.87)	0.85 (0.81 - 0.89)	0.82 (0.77 - 0.87)
Men	0.81 (0.78 - 0.85)	0.83 (0.79 - 0.87)	0.84 (0.78 - 0.90)
Age (year) [e]			
20-29 [d]	0.86 (0.80 - 0.92)	0.86 (0.75 - 0.97)	0.83 (0.72 - 0.95)
30-39	0.92 (0.86 - 0.97)	0.91 (0.84 - 1.00)	0.87 (0.78 - 0.96)
40-49	0.80 (0.73 - 0.87)	0.80 (0.74 - 0.85)	0.79 (0.73 - 0.86)
50-59	0.75 (0.69 - 0.80)	0.75 (0.70 - 0.80)	0.77 (0.71 - 0.84)
60-69	0.79 (0.73 - 0.86)	0.79 (0.74 - 0.85)	0.80 (0.73 - 0.87)
≥70	0.95 (0.88 - 1.02)	0.95 (0.88 - 1.02)	0.92 (0.84 - 1.01)
Region			
Inland Mountains	0.88 (0.85 - 0.92)*	0.89 (0.80 - 0.99)	0.89 (0.79 - 1.00)*
Urban	0.84 (0.80 - 0.89)*	0.86 (0.82 - 0.89)*	0.85 (0.81 - 0.89)*
Coastal [d]	0.75 (0.71 - 0.80)	0.77 (0.71 - 0.82)	0.75 (0.70 - 0.81)
Waist-to-hip ratio			
≤ 0.865 [d]	0.85 (0.81 - 0.89)	0.87 (0.82 - 0.92)	0.85 (0.78 - 0.92)
0.866-0.932	0.87 (0.82 - 0.92)	0.88 (0.83 - 0.93)	0.87 (0.81 - 0.93)
≥ 0.933	0.76 (0.72 - 0.80)*	0.77 (0.72 - 0.82)*	0.77 (0.72 - 0.83)
Body mass index (kg/m^2)			
≤ 24 [d]	0.86 (0.81 - 0.91)	0.86 (0.81 - 0.91)	0.84 (0.78 - 0.91)
25-29	0.84 (0.80 - 0.88)	0.86 (0.82 - 0.90)	0.85 (0.80 - 0.91)
≥ 30	0.76 (0.72 - 0.80)*	0.78 (0.73 - 0.83)	0.79 (0.73 - 0.86)
Education (years)			
< 10 [d]	0.83 (0.79 - 0.88)	0.82 (0.76 - 0.88)	0.80 (0.74 - 0.87)
10-12	0.81 (0.78 - 0.85)	0.83 (0.80 - 0.87)	0.82 (0.77 - 0.87)
≥ 13	0.85 (0.79 - 0.90)	0.86 (0.81 - 0.92)	0.86 (0.80 - 0.94)
Economic status level			
Quartile 1 [d]	0.91 (0.85 - 0.97)	0.88 (0.82 - 0.95)	0.86 (0.79 - 0.93)
Quartile 2	0.87 (0.83 - 0.92)	0.88 (0.82 - 0.94)	0.88 (0.81 - 0.95)
Quartile 3	0.78 (0.74 - 0.83)**	0.80 (0.75 - 0.84)	0.78 (0.73 - 0.84)
Quartile 4 (highest)	0.78 (0.73 - 0.83)**	0.82 (0.76 - 0.87)	0.80 (0.73 - 0.87)
Alcohol consumption (g/day)			
Abstainers [d]	0.91 (0.86 - 0.95)	0.89 (0.84 - 0.96)	0.89 (0.82 - 0.96)
< 2.7	0.84 (0.79 - 0.89)	0.85 (0.80 - 0.91)	0.84 (0.78 - 0.91)
2.7-6.0	0.81 (0.76 - 0.87)	0.83 (0.78 - 0.89)	0.82 (0.76 - 0.89)
≥ 6.1	0.76 (0.71 - 0.82)**	0.79 (0.74 - 0.84)	0.77 (0.71 - 0.83)*
Smoking status			
Never-smokers [d]	0.82 (0.79 - 0.86)	0.83 (0.79 - 0.87)	0.81 (0.76 - 0.86)
Former smokers	0.82 (0.77 - 0.86)	0.84 (0.80 - 0.89)	0.83 (0.77 - 0.89)
Current smokers	0.86 (0.80 - 0.93)	0.87 (0.81 - 0.93)	0.85 (0.78 - 0.92)
Fatty-fish consumption [f]			
0-3/month [d]	0.86 (0.82 - 0.90)	0.87 (0.83 - 0.92)	0.87 (0.81 - 0.93)
1-3/week	0.81 (0.78 - 0.85)	0.82 (0.78 - 0.86)	0.81 (0.77 - 0.86)
≥ 4/week	0.80 (0.71 - 0.89)	0.80 (0.72 - 0.89)	0.80 (0.72 - 0.90)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S18. Nickel whole blood levels (µg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	0.53 (0.50 - 0.57)	0.54 (0.50 - 0.58)	0.53 (0.49 - 0.56)
Men	0.47 (0.43 - 0.50)**	0.47 (0.44 - 0.51)**	0.48 (0.45 - 0.52)*
Age (year) [e]			
20-29 [d]	0.57 (0.51 - 0.65)	0.57 (0.46 - 0.70)	0.53 (0.45 - 0.62)
30-39	0.50 (0.44 - 0.56)	0.49 (0.43 - 0.57)	0.48 (0.43 - 0.54)
40-49	0.54 (0.48 - 0.61)	0.54 (0.48 - 0.60)	0.51 (0.47 - 0.56)
50-59	0.49 (0.43 - 0.55)	0.49 (0.44 - 0.54)	0.51 (0.46 - 0.55)
60-69	0.48 (0.42 - 0.55)	0.48 (0.43 - 0.53)	0.48 (0.44 - 0.53)
≥70	0.48 (0.43 - 0.54)	0.48 (0.42 - 0.54)	0.52 (0.47 - 0.57)
Region			
Inland Mountains	0.52 (0.48 - 0.57)	0.53 (0.44 - 0.64)	0.53 (0.46 - 0.60)
Urban	0.51 (0.47 - 0.56)	0.52 (0.48 - 0.55)	0.50 (0.47 - 0.53)
Coastal [d]	0.46 (0.42 - 0.50)	0.46 (0.41 - 0.52)	0.49 (0.45 - 0.53)
Waist-to-hip ratio			
≤ 0.865 [d]	0.53 (0.49 - 0.58)	0.51 (0.46 - 0.56)	0.50 (0.46 - 0.54)
0.866-0.932	0.50 (0.46 - 0.55)	0.51 (0.47 - 0.56)	0.49 (0.45 - 0.53)
≥ 0.933	0.47 (0.43 - 0.52)	0.50 (0.45 - 0.55)	0.52 (0.48 - 0.57)
Body mass index (kg/m^2)			
≤ 24 [d]	0.52 (0.47 - 0.57)	0.51 (0.46 - 0.56)	0.53 (0.49 - 0.57)
25-29	0.50 (0.46 - 0.53)	0.51 (0.47 - 0.55)	0.51 (0.48 - 0.55)
≥ 30	0.50 (0.45 - 0.55)	0.50 (0.45 - 0.56)	0.47 (0.43 - 0.52)
Education (years)			
< 10 [d]	0.51 (0.45 - 0.57)	0.51 (0.46 - 0.58)	0.47 (0.43 - 0.52)
10-12	0.48 (0.45 - 0.52)	0.49 (0.45 - 0.53)	0.51 (0.48 - 0.55)
≥ 13	0.53 (0.49 - 0.58)	0.53 (0.48 - 0.58)	0.53 (0.48 - 0.58)
Economic status level			
Quartile 1 [d]	0.50 (0.45 - 0.55)	0.50 (0.45 - 0.56)	0.52 (0.47 - 0.57)
Quartile 2	0.53 (0.48 - 0.58)	0.53 (0.48 - 0.60)	0.54 (0.50 - 0.59)
Quartile 3	0.50 (0.46 - 0.55)	0.50 (0.46 - 0.56)	0.50 (0.46 - 0.54)
Quartile 4 (highest)	0.48 (0.43 - 0.54)	0.49 (0.44 - 0.54)	0.46 (0.42 - 0.50)
Alcohol consumption (g/day)			
Abstainers [d]	0.45 (0.41 - 0.50)	0.45 (0.40 - 0.50)	0.49 (0.45 - 0.54)
< 2.7	0.53 (0.48 - 0.59)	0.53 (0.47 - 0.59)	0.50 (0.46 - 0.55)
2.7-6.0	0.49 (0.44 - 0.54)	0.50 (0.45 - 0.55)	0.51 (0.47 - 0.56)
≥ 6.1	0.54 (0.48 - 0.61)	0.56 (0.50 - 0.62)*	0.51 (0.47 - 0.56)
Smoking status			
Never-smokers [d]	0.48 (0.44 - 0.52)	0.48 (0.44 - 0.52)	0.50 (0.46 - 0.54)
Former smokers	0.53 (0.49 - 0.58)	0.55 (0.50 - 0.60)	0.51 (0.48 - 0.56)
Current smokers	0.49 (0.44 - 0.55)	0.49 (0.44 - 0.55)	0.50 (0.46 - 0.55)
Fatty-fish consumption [f]			
0-3/month [d]	0.48 (0.44 - 0.53)	0.48 (0.44 - 0.53)	0.49 (0.46 - 0.53)
1-3/week	0.51 (0.47 - 0.54)	0.52 (0.48 - 0.56)	0.51 (0.48 - 0.54)
≥ 4/week	0.54 (0.47 - 0.64)	0.56 (0.47 - 0.66)	0.51 (0.45 - 0.58)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table and chromium blood levels (Spearman's correlation coefficient, $r_S = 0.7694$).

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S19. Rubidium whole blood levels (mg/L) by subjects' characteristics.

Factors	Arithmetic means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	2.16 (2.13 - 2.19)	2.14 (2.11 - 2.17)	2.19 (2.15 - 2.23)
Men	2.29 (2.26 - 2.32)***	2.26 (2.22 - 2.29)***	2.31 (2.26 - 2.35)***
Age (year) [e]			
20-29[d]	2.05 (2.01 - 2.10)	2.06 (1.98 - 2.14)	2.23 (2.15 - 2.32)
30-39	2.17 (2.12 - 2.22)	2.18 (2.12 - 2.24)	2.30 (2.23 - 2.36)
40-49	2.28 (2.23 - 2.33)***	2.28 (2.24 - 2.33)***	2.30 (2.25 - 2.35)
50-59	2.28 (2.23 - 2.33)***	2.28 (2.23 - 2.33)***	2.28 (2.23 - 2.34)
60-69	2.26 (2.19 - 2.32)**	2.26 (2.21 - 2.31)**	2.24 (2.19 - 2.29)
≥70	2.13 (2.08 - 2.18)	2.13 (2.08 - 2.18)	2.15 (2.09 - 2.21)
Region			
Inland-Mountains	2.40 (2.36 - 2.44)***	2.38 (2.29 - 2.46)***	2.34 (2.27 - 2.42)***
Urban	2.21 (2.18 - 2.25)	2.19 (2.16 - 2.22)	2.23 (2.19 - 2.26)
Coastal [d]	2.17 (2.13 - 2.21)	2.15 (2.10 - 2.20)	2.18 (2.14 - 2.23)
Waist-to-hip ratio			
≤ 0.865 [d]	2.21 (2.17 - 2.25)	2.24 (2.20 - 2.28)	2.28 (2.23 - 2.33)
0.866-0.932	2.22 (2.19 - 2.26)	2.19 (2.15 - 2.23)	2.24 (2.19 - 2.28)
≥ 0.933	2.22 (2.18 - 2.26)	2.16 (2.12 - 2.20)*	2.23 (2.19 - 2.28)
Body mass index (kg/m^2)			
≤ 24 [d]	2.19 (2.15 - 2.23)	2.20 (2.16 - 2.24)	2.26 (2.21 - 2.30)
25-29	2.22 (2.19 - 2.26)*	2.22 (2.18 - 2.25)	2.27 (2.23 - 2.31)
≥ 30	2.22 (2.18 - 2.26)	2.16 (2.11 - 2.20)	2.23 (2.18 - 2.28)
Education (years)			
< 10 [d]	2.25 (2.21 - 2.30)	2.26 (2.21 - 2.31)	2.30 (2.25 - 2.36)
10-12	2.21 (2.18 - 2.24)	2.18 (2.15 - 2.22)*	2.24 (2.20 - 2.28)*
≥ 13	2.20 (2.15 - 2.24)	2.17 (2.13 - 2.21)*	2.21 (2.16 - 2.26)*
Economic status level			
Quartile 1 [d]	2.17 (2.12 - 2.22)	2.20 (2.15 - 2.25)	2.25 (2.19 - 2.30)
Quartile 2	2.25 (2.20 - 2.30)	2.23 (2.18 - 2.28)	2.27 (2.22 - 2.32)
Quartile 3	2.22 (2.18 - 2.26)	2.19 (2.15 - 2.23)	2.24 (2.19 - 2.28)
Quartile 4 (highest)	2.24 (2.19 - 2.28)	2.18 (2.13 - 2.23)	2.24 (2.19 - 2.30)
Alcohol consumption (g/day)			
Abstainers [d]	2.15 (2.11 - 2.20)	2.17 (2.12 - 2.22)	2.28 (2.23 - 2.33)
< 2.7	2.17 (2.13 - 2.22)	2.16 (2.11 - 2.21)	2.23 (2.18 - 2.28)
2.7-6.0	2.27 (2.22 - 2.32)**	2.23 (2.18 - 2.27)	2.26 (2.21 - 2.31)
≥ 6.1	2.28 (2.24 - 2.32)***	2.23 (2.18 - 2.27)	2.23 (2.18 - 2.28)
Smoking status			
Never-smokers [d]	2.17 (2.13 - 2.20)	2.15 (2.12 - 2.19)	2.19 (2.15 - 2.23)
Former smokers	2.23 (2.19 - 2.27)*	2.20 (2.16 - 2.23)	2.21 (2.17 - 2.26)
Current smokers	2.31 (2.26 - 2.36)***	2.30 (2.25 - 2.35)***	2.35 (2.30 - 2.40)***
Fatty-fish consumption [f]			
0-3/month [d]	2.22 (2.18 - 2.25)	2.19 (2.15 - 2.22)	2.28 (2.24 - 2.33)
1-3/week	2.23 (2.20 - 2.27)	2.21 (2.18 - 2.24)	2.26 (2.22 - 2.30)
≥ 4/week	2.17 (2.11 - 2.24)	2.17 (2.10 - 2.24)	2.21 (2.14 - 2.27)

Arithmetic means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table and cesium blood levels (Spearman's correlation coefficient $r_S = 0.539$)

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S20. Selenium whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	100.4 (98.7 - 102.2)	99.3 (97.7 - 100.9)	104.4 (102.4 - 106.3)
Men	100.0 (98.5 - 101.5)	98.6 (97.0 - 100.3)	100.2 (98.2 - 102.2)***
Age (year)[e]			
20-29[d]	94.6 (92.6 - 96.7)	94.6 (90.4 - 99.0)	110.1 (105.5 - 114.9)
30-39	96.0 (93.6 - 98.3)	95.9 (92.9 - 99.0)	105.7 (102.6 - 109.1)
40-49	00.6 (97.9 - 103.5)	100.6 (98.2 - 103.1)	103.9 (101.4 - 106.4)
50-59	104.2 (101.7 - 106.8)**	104.2 (101.9 - 106.6)**	100.6 (98.2 - 103.1)**
60-69	105.5 (102.4 - 108.7)**	105.5 (103.0 - 108.1)**	100.6 (98.1 - 103.2)**
≥70	93.7 (90.9 - 96.5)	93.6 (91.2 - 96.1)	93.3 (90.7 - 95.9)***
Region			
Inland-Mountains	99.7 (97.8 - 101.8)	98.8 (95.0 - 102.7)	103.8 (100.4 - 107.3)
Urban	99.2 (97.3 - 101.2)	98.0 (96.6 - 99.4)**	101.3 (99.7 - 102.8)
Coastal [d]	104.1 (102.2 - 106.0)	102.7 (100.2 - 105.3)	101.7 (99.4 - 104.0)
Waist-to-hip ratio			
≤ 0.865 [d]	100.1 (98.0 - 102.2)	99.2 (97.1 - 101.4)	102.3 (100.0 - 104.7)
0.866-0.932	101.6 (99.6 - 103.6)	100.3 (98.4 - 102.3)	103.6 (101.5 - 105.9)
≥ 0.933	99.0 (97.1 - 100.9)	97.2 (95.1 - 99.3)	100.8 (98.5 - 103.0)
Body mass index (kg/m^2)			
≤ 24 [d]	100.9 (98.7 - 103.2)	100.4 (98.4 - 102.5)	101.8 (99.6 - 104.1)
25-29	100.7 (98.9 - 102.5)	99.2 (97.4 - 100.9)	103.2 (101.2 - 105.3)
≥ 30	98.8 (97.0 - 100.7)	96.7 (94.4 - 99.0)*	101.7 (99.3 - 104.1)
Education (years)			
< 10 [d]	97.0 (94.7 - 99.3)	96.7 (94.3 - 99.2)	101.9 (99.5 - 104.3)
10-12	99.4 (97.9 - 101.0)	97.6 (96.0 - 99.2)	100.5 (98.7 - 102.4)
≥ 13	104.4 (102.1 - 106.8)***	103.4 (101.1 - 105.7)***	104.3 (101.9 - 106.8)
Economic status level			
Quartile 1 [d]	94.6 (92.5 - 96.8)	95.4 (93.0 - 97.8)	99.1 (96.7 - 101.6)
Quartile 2	99.5 (97.3 - 101.8)*	98.7 (96.4 - 101.0)	101.7 (99.3 - 104.1)
Quartile 3	99.0 (97.0 - 101.1)*	97.8 (95.8 - 99.9)	102.1 (99.9 - 104.4)
Quartile 4 (highest)	107.2 (104.8 - 109.8)***	104.7 (102.3 - 107.2)***	106.2 (103.6 - 108.8)***
Alcohol consumption (g/day)			
Abstainers [d]	96.5 (94.5 - 98.7)	95.9 (93.7 - 98.1)	102.5 (100.1 - 104.9)
< 2.7	96.6 (94.6 - 98.6)	94.7 (92.5 - 97.0)	98.6 (96.3 - 100.8)*
2.7-6.0	101.4 (99.1 - 103.7)*	99.4 (97.2 - 101.6)	101.6 (99.3 - 104.0)
≥ 6.1	106.2 (103.8 - 108.8)***	105.1 (102.7 - 107.5)***	106.5 (104.1 - 109.0)
Smoking status			
Never-smokers [d]	100.2 (98.4 - 102.0)	99.4 (97.7 - 101.2)	102.9 (101.0 - 105.0)
Former smokers	102.2 (100.3 - 104.2)	100.1 (98.2 - 102.0)	104.3 (102.1 - 106.4)
Current smokers	96.5 (94.5 - 98.6)	95.8 (93.3 - 98.3)*	99.6 (97.2 - 102.0)*
Fatty-fish consumption [f]			
0-3/month [d]	95.7 (94.1 - 97.4)	94.8 (93.1 - 96.5)	100.4 (98.4 - 102.4)
1-3/week	102.5 (101.0 - 104.0)***	101.4 (99.8 - 103.1)***	101.4 (99.7 - 103.2)
≥ 4/week	107.4 (102.6 - 112.4)***	106.7 (102.9 - 110.6)***	105.0 (101.7 -108.4)*

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table and blood levels of mercury (Spearman's correlation coefficient $r_S = 0.535$).

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S21. Silver whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	0.122 (0.114 - 0.131)	0.111 (0.102 - 0.119)	0.103 (0.092 - 0.114)
Men	0.102 (0.094 - 0.111)**	0.091 (0.084 - 0.099)***	0.079 (0.070 - 0.089)***
Age (year)[e]			
20-29[d]	0.057 (0.050 - 0.065)	0.056 (0.045 - 0.070)	0.048 (0.038 - 0.061)
30-39	0.087 (0.076 - 0.099)*	0.086 (0.074 - 0.100)*	0.077 (0.065 - 0.092)**
40-49	0.148 (0.132 - 0.166)***	0.147 (0.131 - 0.165)***	0.124 (0.108 - 0.143)***
50-59	0.142 (0.126 - 0.159)***	0.141 (0.127 - 0.157)***	0.124 (0.108 - 0.142)***
60-69	0.099 (0.085 - 0.115)***	0.099 (0.088 - 0.111)***	0.088 (0.077 - 0.101)***
≥70	0.104 (0.091 - 0.119)***	0.104 (0.091 - 0.117)***	0.105 (0.090 - 0.122)***
Region			
Inland-Mountains	0.076 (0.069 - 0.084)**	0.068 (0.056 - 0.081)**	0.068 (0.056 - 0.082)***
Urban	0.117 (0.107 - 0.129)	0.105 (0.098 - 0.112)	0.103 (0.095 - 0.112)
Coastal [d]	0.114 (0.104 - 0.124)	0.101 (0.089 - 0.113)	0.104 (0.091 - 0.118)
Waist-to-hip ratio			
≤ 0.865 [d]	0.115 (0.105 - 0.125)	0.100 (0.091 - 0.111)	0.088 (0.077 - 0.101)
0.866-0.932	0.119 (0.108 - 0.132)	0.107 (0.097 - 0.117)	0.094 (0.084 - 0.106)
≥ 0.933	0.104 (0.094 - 0.114)	0.094 (0.085 - 0.104)	0.087 (0.077 - 0.099)
Body mass index (kg/m^2)			
≤ 24 [d]	0.117 (0.106 - 0.129)	0.105 (0.095 - 0.116)	0.093 (0.082 - 0.105)
25-29	0.112 (0.103 - 0.122)	0.100 (0.092 - 0.108)	0.087 (0.078 - 0.098)
≥ 30	0.110 (0.099 - 0.122)	0.097 (0.087 - 0.109)	0.090 (0.078 - 0.103)
Education (years)			
< 10 [d]	0.102 (0.091 - 0.114)	0.091 (0.081 - 0.103)	0.085 (0.074 - 0.098)
10-12	0.116 (0.107 - 0.125)	0.104 (0.096 - 0.113)	0.095 (0.085 - 0.105)
≥ 13	0.114 (0.102 - 0.126)	0.100 (0.090 - 0.111)	0.090 (0.079 - 0.103)
Economic status level			
Quartile 1 [d]	0.100 (0.088 - 0.113)	0.098 (0.087 - 0.110)	0.089 (0.078 - 0.103)
Quartile 2	0.109 (0.097 - 0.122)	0.100 (0.089 - 0.112)	0.090 (0.078 - 0.103)
Quartile 3	0.118 (0.107 - 0.131)	0.102 (0.092 - 0.113)	0.089 (0.079 - 0.102)
Quartile 4 (highest)	0.120 (0.108 - 0.134)	0.102 (0.091 - 0.114)	0.091 (0.079 - 0.105)
Alcohol consumption (g/day)			
Abstainers [d]	0.094 (0.084 - 0.105)	0.082 (0.073 - 0.092)	0.076 (0.066 - 0.087)
< 2.7	0.110 (0.098 - 0.123)	0.097 (0.087 - 0.109)	0.088 (0.077 - 0.100)
2.7-6.0	0.120 (0.108 - 0.134)**	0.109 (0.098 - 0.121)**	0.096 (0.084 - 0.110)*
≥ 6.1	0.128 (0.115 - 0.143)***	0.116 (0.104 - 0.129)***	0.102(0.089 - 0.117)**
Smoking status			
Never-smokers [d]	0.101 (0.093 - 0.110)	0.093 (0.086 - 0.101)	0.082 (0.074 - 0.092)
Former smokers	0.114 (0.104 - 0.125)	0.102 (0.093 - 0.111)	0.087 (0.077 - 0.097)
Current smokers	0.136 (0.121 - 0.154)***	0.118 (0.104 - 0.133)**	0.102 (0.089 - 0.117)*
Fatty-fish consumption [f]			
0-3/month [d]	0.112 (0.103 - 0.122)	0.103 (0.095 - 0.113)	0.095 (0.085 - 0.106)
1-3/week	0.114 (0.105 - 0.123)	0.099 (0.092 - 0.107)	0.089 (0.081 - 0.099)
≥ 4/week	0.109 (0.091 - 0.129)	0.094 (0.079 - 0.112)	0.086 (0.071 - 0.103)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S22. Strontium whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	18.4 (18.0 - 18.8)	18.2 (17.7 - 18.6)	18.1 (17.6 - 18.8)
Men	17.5 (17.1 - 17.9)**	17.3 (16.8 - 17.7)**	17.0 (16.4 - 17.6)**
Age (year)[e]			
20-29[d]	16.4 (15.8 - 17.1)	16.4 (15.3 - 17.5)	16.1 (14.9 - 17.3)
30-39	16.8 (16.2 - 17.5)	16.8 (16.0 - 17.5)	16.7 (15.8 - 17.6)
40-49	16.9 (16.3 - 17.5)	16.9 (16.3 - 17.5)	16.6 (15.9 - 17.3)
50-59	17.9 (17.2 - 18.7)	17.9 (17.3 - 18.5)	17.6 (16.9 - 18.4)
60-69	18.4 (17.7 - 19.1)*	18.4 (17.8 - 19.0)**	18.4 (17.6 - 19.2)**
≥70	20.3 (19.4 - 21.1)***	20.2 (19.5 - 21.0)***	20.3 (19.4 - 21.3)***
Region			
Inland-Mountains	17.8 (17.3 - 18.3)	17.4 (16.5 - 18.5)	17.4 (16.4 - 18.5)
Urban	18.0 (17.5 - 18.5)	17.8 (17.4 - 18.1)	17.8 (17.3 - 18.2)
Coastal [d]	17.9 (17.4 - 18.3)	17.6 (16.9 - 18.2)	17.5 (16.8 - 18.2)
Waist-to-hip ratio			
≤ 0.865 [d]	17.8 (17.3 - 18.3)	17.8 (17.3 - 18.3)	17.7 (17.0 - 18.5)
0.866-0.932	18.0 (17.5 - 18.6)	18.0 (17.5 - 18.6)	17.7 (17.0 - 18.3)
≥ 0.933	18.0 (17.5 - 18.5)	18.0 (17.5 - 18.5)	17.3 (16.6 - 18.0)
Body mass index (kg/m^2)			
≤ 24 [d]	17.4 (16.9 - 17.9)	17.1 (16.6 - 17.6)	16.8 (16.2 - 17.5)
25-29	18.1 (17.6 - 18.5)	18.0 (17.5 - 18.4)*	17.8 (17.2 - 18.4)*
≥ 30	18.5 (17.9 - 19.0)*	18.1 (17.5 - 18.7)*	18.1 (17.3 - 18.9)*
Education (years)			
< 10 [d]	18.6 (18.0 - 19.3)	17.8 (17.2 - 18.5)	17.7 (17.0 - 18.4)
10-12	17.9 (17.5 - 18.3)	17.7 (17.3 - 18.1)	17.4 (16.9 - 18.0)
≥ 13	17.6 (17.1 - 18.1)	17.7 (17.2 - 18.3)	17.5 (16.8 - 18.3)
Economic status level			
Quartile 1 [d]	18.7 (18.0 - 19.3)	17.8 (17.2 - 18.5)	17.9 (17.1 - 18.7)
Quartile 2	17.9 (17.4 - 18.6)	17.7 (17.1 - 18.3)	17.4 (16.7 - 18.1)
Quartile 3	17.5 (16.9 - 18.0)*	17.5 (16.9 - 18.0)	17.3 (16.6 - 18.0)
Quartile 4 (highest)	18.0 (17.4 - 18.6)	17.9 (17.3 - 18.5)	17.7 (16.9 - 18.5)
Alcohol consumption (g/day)			
Abstainers [d]	18.5 (17.9 - 19.2)	17.5 (16.9 - 18.1)	17.3 (16.6 - 18.0)
< 2.7	17.6 (17.1 - 18.2)	17.2 (16.6 - 17.8)	17.1 (16.4 - 17.8)
2.7-6.0	17.4 (16.9 - 17.9)	17.3 (16.8 - 17.9)	17.3 (16.6 - 18.0)
≥ 6.1	18.2 (17.6 - 18.9)	18.7 (18.1 - 19.3)*	18.6 (17.9 - 19.4)*
Smoking status			
Never-smokers [d]	17.9 (17.5 - 18.3)	17.8 (17.4 - 18.3)	17.7 (17.1 - 18.3)
Former smokers	18.0 (17.5 - 18.5)	17.7 (17.2 - 18.2)	17.6 (17.0 - 18.3)
Current smokers	17.9 (17.3 - 18.6)	17.5 (16.9 - 18.2)	17.3 (16.6 - 18.1)
Fatty-fish consumption [f]			
0-3/month [d]	17.6 (17.2 - 18.0)	17.8 (17.3 - 18.2)	17.7 (17.1 - 18.3)
1-3/week	18.2 (17.7 - 18.6)	17.7 (17.2 - 18.1)	17.5 (17.0 - 18.1)
≥ 4/week	18.4 (17.3 - 19.5)	17.6 (16.7 - 18.6)	17.5 (16.5 - 18.5)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S23. Thallium whole blood levels (µg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.027)	0.028 (0.027 - 0.029)
Men	0.027 (0.026 - 0.027)	0.027 (0.026 - 0.028)	0.029 (0.027 - 0.030)
Age (year)[e]			
20-29[d]	0.028 (0.027 - 0.029)	0.028 (0.026 - 0.030)	0.030 (0.028 - 0.033)
30-39	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.027)	0.027 (0.026 - 0.029)
40-49	0.025 (0.024 - 0.027)	0.025 (0.024 - 0.026)	0.027 (0.025 - 0.028)
50-59	0.027 (0.025 - 0.028)	0.027 (0.025 - 0.028)	0.028 (0.026 - 0.029)
60-69	0.029 (0.027 - 0.030)	0.029 (0.027 - 0.030)	0.030 (0.028 - 0.032)
≥70	0.025 (0.023 - 0.026)	0.025 (0.024 - 0.026)	0.027 (0.026 - 0.029)
Region			
Inland-Mountains	0.031 (0.030 - 0.033)***	0.031 (0.029 - 0.034)***	0.032 (0.030 - 0.035)
Urban	0.026 (0.026 - 0.027)**	0.027 (0.026 - 0.027)**	0.027 (0.026 - 0.028)
Coastal [d]	0.024 (0.024 - 0.025)	0.025 (0.023 - 0.026)	0.025 (0.024 - 0.027)
Waist-to-hip ratio			
≤ 0.865 [d]	0.026 (0.025 - 0.027)	0.027 (0.026 - 0.028)	0.028 (0.027 - 0.030)
0.866-0.932	0.027 (0.026 - 0.028)	0.027 (0.026 - 0.028)	0.028 (0.027 - 0.030)
≥ 0.933	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.027)	0.028 (0.027 - 0.029)
Body mass index (kg/m^2)			
≤ 24 [d]	0.026 (0.025 - 0.027)	0.027 (0.026 - 0.028)	0.028 (0.027 - 0.029)
25-29	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.027)	0.028 (0.027 - 0.029)
≥ 30	0.027 (0.026 - 0.028)	0.027 (0.026 - 0.028)	0.029 (0.027 - 0.030)
Education (years)			
< 10 [d]	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.028)	0.028 (0.027 - 0.030)
10-12	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.027)	0.027 (0.026 - 0.029)
≥ 13	0.027 (0.026 - 0.028)	0.028 (0.027 - 0.029)	0.029 (0.027 - 0.030)
Economic status level			
Quartile 1 [d]	0.025 (0.024 - 0.026)	0.025 (0.024 - 0.027)	0.027 (0.026 - 0.029)
Quartile 2	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.027)	0.028 (0.027 - 0.030)
Quartile 3	0.027 (0.026 - 0.028)	0.027 (0.026 - 0.028)	0.028 (0.027 - 0.030)
Quartile 4 (highest)	0.027 (0.026 - 0.028)*	0.027 (0.026 - 0.028)	0.029 (0.027 - 0.030)
Alcohol consumption (g/day)			
Abstainers [d]	0.025 (0.024 - 0.026)	0.026 (0.024 - 0.027)	0.028 (0.026 - 0.029)
< 2.7	0.027 (0.026 - 0.028)	0.027 (0.026 - 0.028)	0.028 (0.027 - 0.030)
2.7-6.0	0.028 (0.027 - 0.029)**	0.028 (0.027 - 0.029)*	0.029 (0.028 - 0.031)
≥ 6.1	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.027)	0.027 (0.026 - 0.029)
Smoking status			
Never-smokers [d]	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.027)	0.028 (0.027 - 0.029)
Former smokers	0.027 (0.026 - 0.028)	0.027 (0.026 - 0.028)	0.029 (0.028 - 0.030)
Current smokers	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.027)	0.028 (0.026 - 0.029)
Fatty-fish consumption [f]			
0-3/month [d]	0.026 (0.025 - 0.027)	0.026 (0.025 - 0.027)	0.027 (0.026 - 0.028)
1-3/week	0.027 (0.026 - 0.027)	0.027 (0.026 - 0.027)	0.028 (0.027 - 0.029)
≥ 4/week	0.027 (0.026 - 0.029)	0.028 (0.026 - 0.030)	0.029 (0.027 - 0.031)

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S24. Tin whole blood levels (μg/L) by subjects' characteristics.

Factors	Geometric means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	0.27 (0.25 - 0.30)	0.27 (0.24 - 0.29)	0.22 (0.19 - 0.25)
Men	0.21 (0.19 - 0.23)***	0.20 (0.18 - 0.22)***	0.19 (0.16 - 0.22)*
Age (year)[e]			
20-29[d]	0.21 (0.17 - 0.25)	0.20 (0.15 - 0.27)	0.18 (0.13 - 0.25)
30-39	0.23 (0.19 - 0.28)	0.23 (0.19 - 0.28)	0.19 (0.15 - 0.24)
40-49	0.20 (0.17 - 0.24)	0.20 (0.17 - 0.23)	0.18 (0.15 - 0.22)
50-59	0.30 (0.25 - 0.37)	0.30 (0.26 - 0.35)	0.24 (0.20 - 0.29)
60-69	0.22 (0.19 - 0.26)	0.22 (0.19 - 0.26)	0.20 (0.16 - 0.24)
≥70	0.25 (0.21 - 0.30)	0.24 (0.21 - 0.29)	0.23 (0.18 - 0.28)
Region			
Inland-Mountains	0.16 (0.15 - 0.18)	0.16 (0.12 - 0.20)	0.16 (0.12 - 0.21)
Urban	0.25 (0.22 - 0.29)**	0.25 (0.22 - 0.27)	0.25 (0.22 - 0.28)
Coastal [d]	0.22 (0.20 - 0.25)	0.21 (0.18 - 0.25)	0.21 (0.18 - 0.25)
Waist-to-hip ratio			
≤ 0.865 [d]	0.29 (0.25 - 0.33)	0.27 (0.23 - 0.31)	0.24 (0.20 - 0.29)
0.866-0.932	0.22 (0.20 - 0.25)**	0.22 (0.19 - 0.25)	0.19 (0.16 - 0.22)*
≥ 0.933	0.21 (0.19 - 0.24)**	0.21 (0.18 - 0.24)*	0.18 (0.15 - 0.21)*
Body mass index (kg/m^2)			
≤ 24 [d]	0.24 (0.21 - 0.27)	0.24 (0.21 - 0.27)	0.18 (0.15 - 0.21)
25-29	0.25(0.22 - 0.28)	0.25 (0.22 - 0.28)	0.22 (0.19 - 0.26)
≥ 30	0.22 (0.20 - 0.25)	0.22 (0.20 - 0.25)	0.21 (0.17 - 0.25)
Education (years)			
< 10 [d]	0.20 (0.18 - 0.23)	0.20 (0.17 - 0.23)	0.18 (0.15 - 0.22)
10-12	0.25 (0.23 - 0.28)	0.24 (0.21 - 0.26)	0.22 (0.19 - 0.25)
≥ 13	0.25 (0.22 - 0.29)	0.25 (0.21 - 0.29)	0.21 (0.17 - 0.25)
Economic status level			
Quartile 1 [d]	0.21 (0.19 - 0.24)	0.21 (0.18 - 0.24)	0.18 (0.15 - 0.22)
Quartile 2	0.24 (0.20 - 0.28)	0.23 (0.20 - 0.27)	0.20 (0.17 - 0.25)
Quartile 3	0.24 (0.21 - 0.28)	0.23 (0.20 - 0.27)	0.21 (0.18 - 0.25)
Quartile 4 (highest)	0.26 (0.23 - 0.31)	0.25 (0.22 - 0.29)	0.21 (0.18 - 0.26)
Alcohol consumption (g/day)			
Abstainers [d]	0.27 (0.24 - 0.31)	0.25 (0.21 - 0.29)	0.23 (0.19 - 0.28)
< 2.7	0.21 (0.18 - 0.24)	0.20 (0.17 - 0.23)	0.17 (0.15 - 0.21)
2.7-6.0	0.23 (0.20 - 0.26)	0.23 (0.20 - 0.27)	0.20 (0.17 - 0.24)
≥ 6.1	0.24 (0.20 - 0.28)	0.24 (0.21 - 0.28)	0.21 (0.17 - 0.25)
Smoking status			
Never-smokers [d]	0.26 (0.23 - 0.29)	0.26 (0.23 - 0.29)	0.22 (0.19 - 0.26)
Former smokers	0.23 (0.21 - 0.26)	0.22 (0.19 - 0.25)	0.20 (0.17 - 0.23)
Current smokers	0.21 (0.18 - 0.25)	0.20 (0.17 - 0.24)	0.19 (0.15 - 0.23)
Fatty-fish consumption [f]			
0-3/month [d]	0.20 (0.18 - 0.22)	0.21 (0.18 - 0.23)	0.17 (0.14 - 0.19)
1-3/week	0.26 (0.23 - 0.29)**	0.25 (0.22 - 0.27)	0.21 (0.18 - 0.23)*
≥ 4/week	0.30 (0.24 - 0.37)*	0.28 (0.22 - 0.35)	0.24 (0.19 - 0.31)*

Geometric means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table.

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table S25. Zinc whole blood levels (mg/L) by subjects' characteristics.

Factors	Arithmetic means (95% CI)		
	Crude	Sex & age adjusted [a]	Fully adjusted [b]
Sex [c]			
Women [d]	7.18 (7.11 - 7.26)	7.15 (7.07 - 7.23)	7.36 (7.25 - 7.46)
Men	7.93 (7.84 - 8.01)***	7.89 (7.81 - 7.98)***	7.63 (7.52 - 7.75)***
Age (year)[e]			
20-29[d]	7.30 (7.06 - 7.55)	7.35 (7.12 - 7.57)	7.23 (7.01 - 7.45)
30-39	7.30 (7.13 - 7.47)	7.36 (7.20 - 7.52)	7.29 (7.13 - 7.46)
40-49	7.43 (7.30 - 7.56)	7.46 (7.34 - 7.58)	7.46 (7.33 - 7.59)
50-59	7.45 (7.33 - 7.57)	7.47 (7.35 - 7.58)	7.48 (7.35 - 7.61)
60-69	7.74 (7.61 - 7.87)*	7.76 (7.64 - 7.88)*	7.71 (7.58 - 7.84)**
≥70	7.73 (7.59 - 7.87)*	7.75 (7.62 - 7.89)*	7.80 (7.65 - 7.95)***
Region			
Inland-Mountains	7.54 (7.33 - 7.75)	7.51 (7.32 - 7.71)	7.54 (7.36 - 7.72)
Urban	7.59 (7.52 - 7.66)**	7.58 (7.51 - 7.65)***	7.56 (7.48 - 7.64)*
Coastal [d]	7.31 (7.18 - 7.45)	7.30 (7.18 - 7.43)	7.39 (7.27 - 7.51)
Waist-to-hip ratio			
≤ 0.865 [d]	7.25 (7.14 - 7.35)	7.50 (7.39 - 7.61)	7.57 (7.45 - 7.70)
0.866-0.932	7.51 (7.41 - 7.61)**	7.48 (7.38 - 7.58)	7.43 (7.32 - 7.54)
≥ 0.933	7.81 (7.71 - 7.91)***	7.60 (7.49 - 7.71)	7.49 (7.36 - 7.61)
Body mass index (kg/m^2)			
≤ 24 [d]	7.24 (7.13 - 7.35)	7.35 (7.25 - 7.45)	7.41 (7.29 - 7.53)
25-29	7.64 (7.55 - 7.73)***	7.56 (7.48 - 7.65)**	7.51 (7.41 - 7.62)
≥ 30	7.68 (7.56 - 7.81)***	7.70 (7.58 - 7.82)***	7.57 (7.44 - 7.70)
Education (years)			
< 10 [d]	7.58 (7.45 - 7.71)	7.49 (7.37 – 7.62)	7.40 (7.27 - 7.52)
10-12	7.61 (7.53 - 7.69)	7.60 (7.51 - 7.68)	7.58 (7.47 - 7.68)*
≥ 13	7.32 (7.21 - 7.44)*	7.41 (7.30 - 7.52)	7.52 (7.39 - 7.65)
Economic status level			
Quartile 1 [d]	7.66 (7.53 - 7.79)	7.62 (7.50 - 7.75)	7.61 (7.48 - 7.74)
Quartile 2	7.62 (7.50 - 7.75)	7.61 (7.49 - 7.72)	7.53 (7.40 - 7.66)
Quartile 3	7.44 (7.33 - 7.55)	7.47 (7.37 - 7.58)	7.44 (7.32 - 7.56)
Quartile 4 (highest)	7.44 (7.32 - 7.56)	7.40 (7.28 - 7.51)	7.40 (7.27 - 7.53)
Alcohol consumption (g/day)			
Abstainers [d]	7.52 (7.40 - 7.64)	7.58 (7.46 - 7.70)	7.55 (7.42 - 7.68)
< 2.7	7.43 (7.30 - 7.55)	7.49 (7.37 - 7.61)	7.46 (7.33 - 7.58)
2.7-6.0	7.50 (7.38 - 7.62)	7.43 (7.32 - 7.55)	7.44 (7.32 - 7.57)
≥ 6.1	7.66 (7.54 - 7.77)	7.58 (7.46 - 7.69)	7.53 (7.40 - 7.66)
Smoking status			
Never-smokers [d]	7.51 (7.41 - 7.60)	7.53 (7.45 - 7.62)	7.46 (7.36 - 7.57)
Former smokers	7.50 (7.41 - 7.60)	7.46 (7.36 - 7.55)	7.44 (7.33 - 7.55)
Current smokers	7.62 (7.48 - 7.76)	7.63 (7.50 - 7.76)	7.58 (7.45 - 7.71)
Fatty-fish consumption [f]			
0-3/month [d]	7.55 (7.45 - 7.64)	7.54 (7.45 - 7.64)	7.50 (7.40 - 7.61)
1-3/week	7.51 (7.43 - 7.60)	7.49 (7.41 - 7.58)	7.42 (7.32 - 7.51)
≥ 4/week	7.55 (7.35 - 7.74)	7.58 (7.39 - 7.77)	7.57 (7.40 - 7.74)

Arithmetic means and 95% confidence intervals (CI) were calculated using a univariate general linear model. *P* – values were corrected for multiple testing using Dunn-Šidâk procedure.

[a] Sex and age adjusted.

[b] Adjusted for all the variables in the table and iron blood levels (Spearman's correlation coefficient, $r_S = 0.559$).

[c] Age adjusted in model 1.

[d] Reference level for comparison of geometric means in each variable.

[e] Sex adjusted in model 1.

[f] Fatty-fish includes salmon, trout, herring, mackerel and redfish.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Paper II

A. Simić, A.F. Hansen, B.O. Åsvold, P.R. Romundstad, K. Midthjell, T. Syversen, T.P. Flaten. **Trace element status in patients with type 2 diabetes in Norway: The HUNT3 Survey.** *Journal of Trace Elements in Medicine and Biology* 2017; **41**: 91-98.

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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.

1. 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

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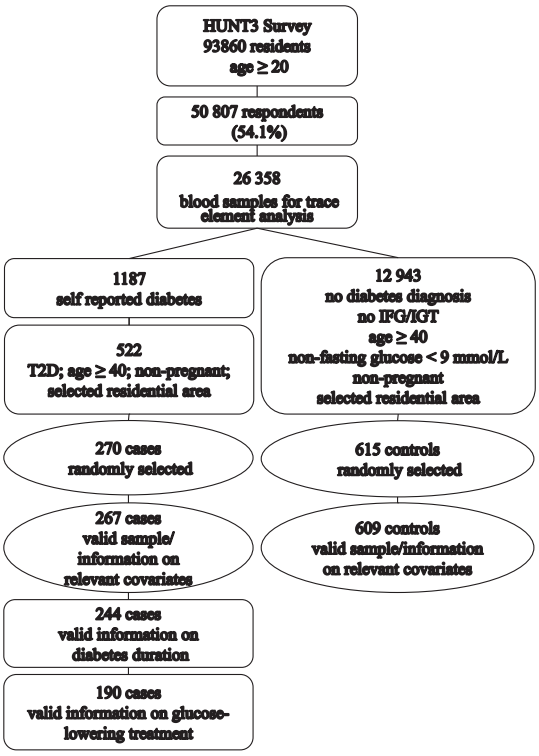

Fig. 1. Flow chart for selection of diagnosed type 2 diabetes cases and control subjects from the HUNT3 cohort.

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.

2. Methods

2.1. 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 (Fig. 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 Fig. 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 (Fig. 1).

2.2. 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.

2.3. 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.

2.4. 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.

2.5. 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.

2.6. 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.

2.7. 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.

2.8. 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.

3. 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 (Fig. 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.

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.

	Age- and sex-adjusted model			Multivariable model[a]		
Trace element	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.

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 (Fig. 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.

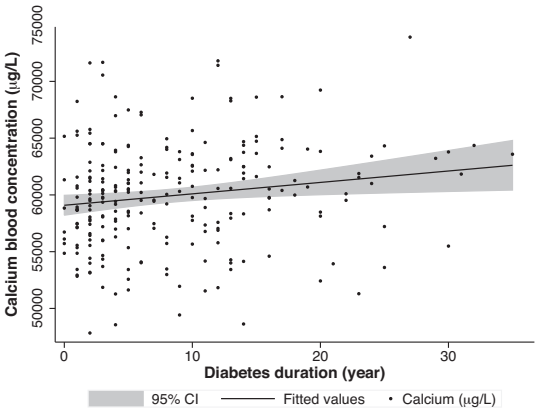

Fig. 2. Calcium whole blood levels as a function of diabetes duration.

4. 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 stud-

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.

ies 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 prediabetes. 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.

5. 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.

Competing financial interests

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

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jtemb.2017.03.001.

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Supplementary table 1. Crude and adjusted odds ratios (OR) and 95% confidence intervals (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 1[a]			Multivariable model 2[b]		
	OR (95% CI)	P_{trend}	Q_{trend}[c]	OR (95% CI)	P_{trend}	Q_{trend}[c]	OR (95% CI)	P_{trend}	Q_{trend}[c]
Arsenic	0.83 (0.53, 1.30)	0.351	0.480	0.75 (0.41, 1.34)	0.333	0.509	0.73 (0.40, 1.31)	0.312	0.312
Calcium	3.11(1.95, 4.96)	<0.001	<0.001	3.85 (2.11, 7.00)	<0.001	<0.001	3.51 (1.87, 6.60)	<0.001	<0.001
Lead	0.31 (0.20, 0.49)	<0.001	<0.001	0.28 (0.15, 0.51)	<0.001	0.007	0.24 (0.13, 0.47)	<0.001	0.002
Magnesium	0.46 (0.30, 0.71)	<0.001	0.001	0.42 (0.24, 0.74)	0.003	0.026	0.53 (0.30, 0.94)	0.033	0.055
Mercury	0.83 (0.53, 1.29)	0.609	0.720	0.64 (0.36, 1.14)	0.259	0.509	0.61 (0.34, 1.10)	0.259	0.312

[a]Adjusted for BMI, waist-to-hip ratio, first-degree family history of diabetes, smoking habits, leaving area, education and economic status.
[b]Multivariable model 1adjusted for element specific confounders: arsenic and mercury adjusted for fat fish consumption; calcium adjusted for milk and alcohol intake and magnesium blood levels; lead and magnesium adjusted for alcohol intake and calcium blood levels.
[c] P_{trend} values corrected for multiple testing.

Paper III

A.F. Hansen, A. Simić, B.O. Åsvold, P.R. Romundstad, K. Midthjell, T. Syversen, T.P. Flaten. **Trace elements in early phase type 2 diabetes mellitus—A population-based study. The HUNT study in Norway.** *Journal of Trace Elements in Medicine and Biology* 2017; **40**: 46-53.

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Epidemiology

Trace elements in early phase type 2 diabetes mellitus—A population-based study. The HUNT study in Norway

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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.

1. 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 involve-

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ment 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.

2. Methods

2.1. 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 Fig. 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 2513 participants identified as being at high risk of type 2 diabetes and also sampled for trace element analysis, 1172 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-h glucose concentration ≥11.1 mmol/L.

Glutamic acid decarboxylase antibodies (GADA), islet antigen-2 antibodies (IA-2A) and fasting C-peptide were analyzed in serum samples at the Hormone Laboratory of Aker University Hospital (Oslo, Norway) as previously described [13]. GADA measurements were used for classification of diabetes and those who had GADA <0.08 ai (antibody index relative to a standard serum) were classified as having type 2 diabetes [14]. Among the 157 individuals, we excluded one individual with GADA ≥0.08 ai and 22 individuals whose GADA levels had not been analyzed. None of the cases had C-peptide levels <150 pmol/L and for the 119 cases with measurements for IA-2A, none had IA-2A levels ≥0.11 ai (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 (Fig. 1).

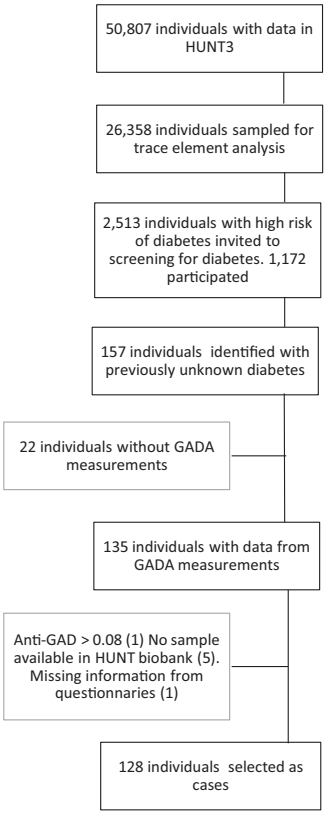

Fig. 1. Flow chart for selection of cases from the HUNT3 cohort. FINDRISC: Finnish Diabetes Risk Score. HUNT3: the third survey of the Nord-Trøndelag Health Study. GADA: Glutamic acid decarboxylase antibodies.

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.

2.2. 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 high resolution inductively coupled plasma mass spectrometry (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.

2.3. 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.

Table 1
Key characteristics of cases and controls.

	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%)

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

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

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).

3. 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,

Table 2
Trace element concentrations in whole blood in cases and controls.

			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

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).

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

	Age- and sex-adjusted model			Multivariable model[a]		
Trace element	OR (95% CI)[d]	P_{trend}[b]	Q_{trend}[c]	OR (95% CI)[d]	P_{trend}[b]	Q_{trend}[c]
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

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).

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

silver and zinc and a decreasing prevalence across quartiles for bromine after adjustment for the aforementioned confounders (Fig. 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 $P_{trend} < 0.05$ (Fig. 2). The

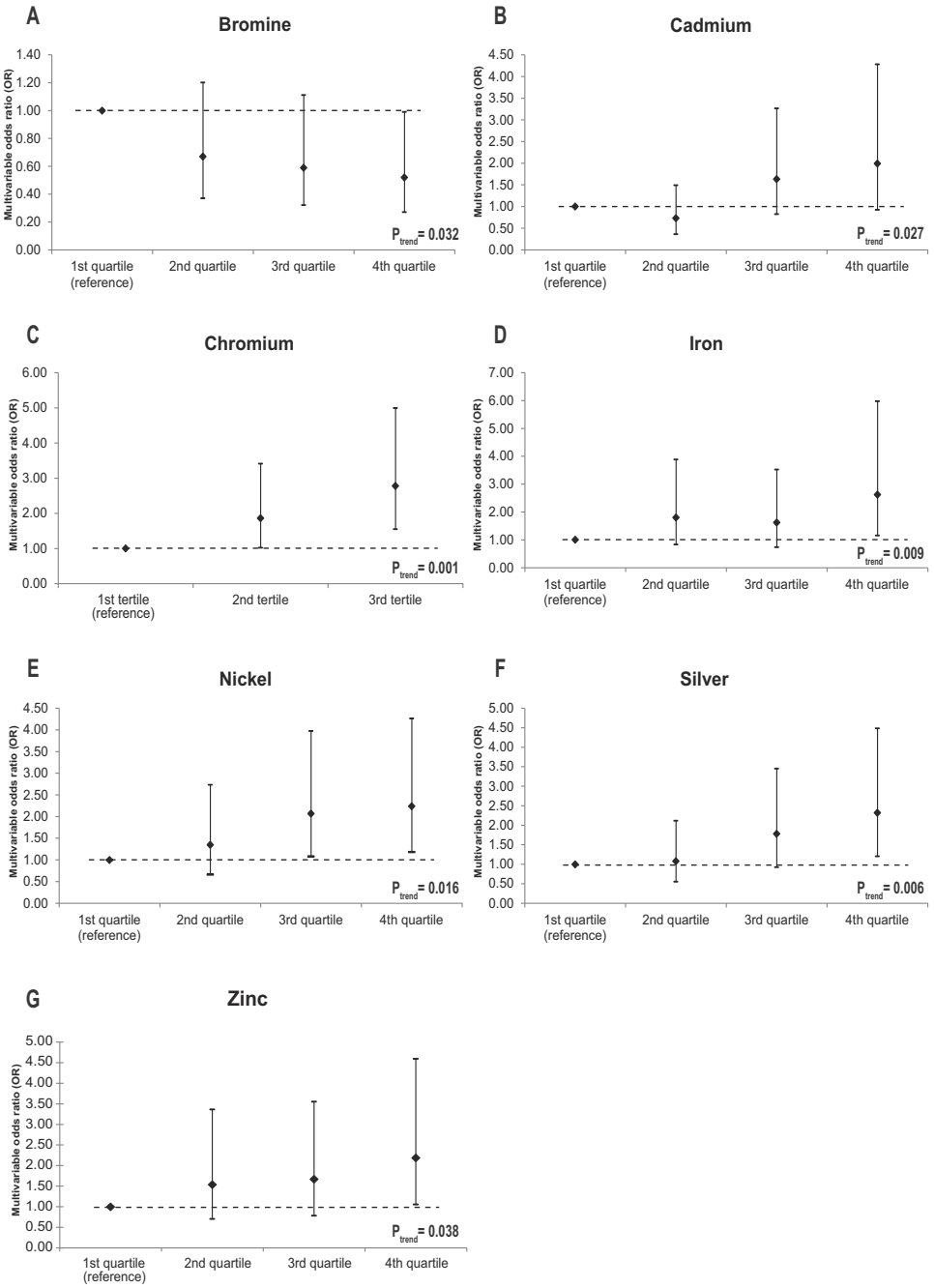

Fig. 2. Trace elements with significant associations for previously undiagnosed, screening detected type 2 diabetes. Multivariable adjusted odds ratios (OR) for previously undiagnosed, screening-detected type 2 diabetes with increasing concentrations of trace elements with significant associations over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

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.

4. 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, measurement of iron levels in blood is not a very reliable method to assess the iron status of an individual, and ferritin and transferrin are better markers for iron status. Increased ferritin levels have 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 GADA, IA-2A 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.

5. 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.

Competing financial interests

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

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.

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