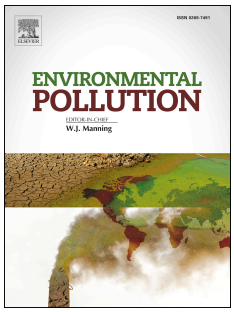


Trace elements concentration in adipose tissue and the risk of incident type 2 diabetes in a prospective adult cohort

Celia Rodríguez-Pérez, Celia Gómez Peña, Francisco M. Pérez-Carrascosa, Petra Vrhovnik, Ruth Echeverría, Inmaculada Salcedo-Bellido, Vicente Mustieles, Fiket Željka, Juan Pedro Arrebola

PII: S0269-7491(21)01078-2

DOI: https://doi.org/10.1016/j.envpol.2021.117496

Reference: ENPO 117496

To appear in: *Environmental Pollution*

Received Date: 21 October 2020

Revised Date: 23 April 2021

Accepted Date: 28 May 2021

Please cite this article as: Rodríguez-Pérez, C., Peña, Celia.Gó., Pérez-Carrascosa, F.M., Vrhovnik, P., Echeverría, R., Salcedo-Bellido, I., Mustieles, V., Željka, F., Arrebola, J.P., Trace elements concentration in adipose tissue and the risk of incident type 2 diabetes in a prospective adult cohort, *Environmental Pollution* (2021), doi: https://doi.org/10.1016/j.envpol.2021.117496.

Author statement

All authors: study design, data collection and analysis; **Celia Gómez Peña, Francisco M. Pérez-Carrascosa and Juan Pedro Arrebola:** acquisition of data; **Petra Vrhovnik and Fiket Željka**: chemical analysis of the data; **Celia Rodríguez Pérez, Vicente Mustieles and Juan Pedro Arrebola:** analysis and interpretation of data and led the data analysis; **Celia Rodríguez Pérez:** Writing- Original draft preparation; **Juan Pedro Arrebola**: Supervision; **All authors**: Reviewing and Editing.

Trace elements in adipose tissue and type-2 diabetes incidence risk

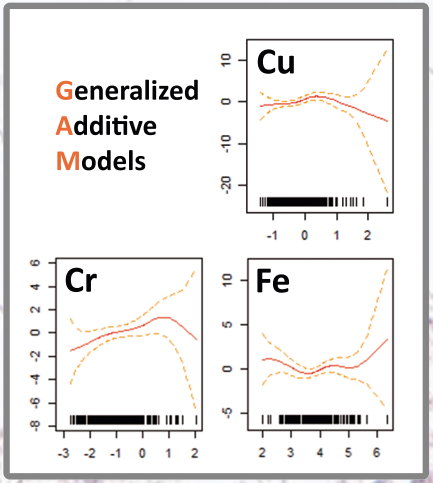
GraMo cohort
N= 221 adults
2003-2019 follow-up

Chromium
Copper
Iron

Positive association

Non-significant trends

Vanadium	Negative
Zinc	Negative



Trace elements concentration in adipose tissue and the risk of incident type 2 diabetes in a prospective adult cohort

Celia Rodríguez-Pérez[1,2,3,4*], Celia Gómez Peña[4,5], Francisco M. Pérez-Carrascosa[4,6], Petra Vrhovnik[7], Ruth Echeverría[3], Inmaculada Salcedo-Bellido[3,4,8], Vicente Mustieles,[3,4,8]. Fiket Željka[9], Juan Pedro Arrebola[3,4,8]

[1]*Departmento de Nutrición y Bromatología. Universidad de Granada. Campus de Melilla, Spain.*
[2]*I Instituto de Nutrición y Tecnología de los Alimentos 'José Mataix', Universidad de Granada, Granada, Spain*
[3]*Departamento de Medicina Preventiva y Salud Pública. Universidad de Granada, Granada, Spain.*
[4]*Instituto de Investigación Biosanitaria de Granada ibs.GRANADA, Spain*
[5] *Unidad de Gestión Clínica de Farmacia Hospitalaria, Hospital Universitario San Cecilio, Granada, Spain*
[6]*Oncology Unit, Hospital Universitario Virgen de las Nieves, Granada, Spain*
[7]*Slovenian National Building and Civil Engineering Institute (ZAG), Ljubjana, Slovenia*
[8]*CIBER de Epidemiología y Salud Pública (CIBERESP), Spain*
[9] *Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia*

Corresponding author: Dr. Celia Rodríguez-Pérez.
Department of Nutrition and Food Science. University of Granada (Spain). Campus of Melilla, 52071 Melilla, Spain.
Email: celiarp@ugr.es

Abstract

The aim of this work is to study the associations of adipose tissue TE concentrations with type 2 diabetes (T2DM) incidence over a 16-year follow-up period in an adult cohort from Southern Spain. Clinical information, including 16-year T2DM incidence, was gathered from hospital records. Chemical analyses of Cr, V, Zn, Fe, Cu and Se in adipose tissue were performed using inductively coupled plasma mass spectrometry. Multivariable Cox-regression models were used. Adipose tissue concentrations of individual TEs showed that Fe (HR= 1.59, 95% CI: 0.99 to 2.56, p=0.057), Cr (HR= 1.58, 95% CI: 1.07-2.33, p=0.022) and Cu (HR= 1.61, 95% CI: 1.01- 2.58, p=0.046) were positively associated with T2DM incidence. The Cr-association was maintained in multi-TEs analysis (HR=1.68, 95% CI: 1.02-2.76, p=0.041). Furthermore, adipose tissue V (β=0.283, p=0.004) and Zn (β=0.217, p=0.028) concentrations were positively associated with β-pancreatic cell function (HOMA-β), while Se showed an inverse association (β= -0.049, p=0.027). Although further research is warranted on the potential mechanisms of action, our results suggest that adipose tissue concentrations of certain trace elements (particularly Fe, Cr and Cu) are associated with the risk of incident T2DM, while V and Zn might have a protective effect. These biomarkers might complement prediction algorithms and contribute to identify patients with an increased risk of T2DM.

Keywords: adipose tissue, chromium, diabetes, iron, copper, trace elements.

Capsule

Adipose tissue trace elements such as Fe, Cr and Cu were associated with the risk of incident type 2 diabetes mellitus in an adult cohort.

Abbreviations: AMPK: AMP-activated protein kinase; BMI: Body mass index; CP: calculated using glucose and C-peptide levels; Cr: Chromium; Cu: Copper; FDA: Food and Drug Administration; GAM: Generalized additive models; HOMA2_ β: β-cell function; HOMA2-IS: insulin sensitivity; HOMA2-IR: insulin resistance; I: calculated using glucose and insulin levels; HRs: Hazard ratios; Iron: Fe; ROS: reactive oxygen species; Se: Selenium; T2DM: type 2 diabetes mellitus; TEs: Trace elements; V: Vanadium; WHO: World Health Organization; ZFP36: Zn finger protein 36; Zn: Zinc.

1. Introduction

Type 2 diabetes (T2DM) is characterized by increased blood glucose levels caused by a reduced insulin secretion from pancreatic β-cells and/or insulin resistance (Franks and McCarthy, 2016). In addition, T2DM is closely related to highly-prevalent comorbidities such as cardiovascular disease, hypertension, obesity or dyslipidemia (Petrie et al., 2018). The World Health Organization (WHO) estimated that 422 million adults worldwide (nearly 9%) suffered from diabetes (WHO, 2016).

Although far from been completely understood, the etiology of T2DM is considered a complex mixture of internal and external risk factors, many of them susceptible of modification e.g., sedentary lifestyle or unbalanced diet (Zheng et al., 2018). In this regard, most studies have focused their research on the macronutrient's status e.g., increasing the intake of complex carbohydrates to the detriment of simple sugars as a strategy to prevent T2DM. However, micronutrients and, specifically, some trace elements (TEs) have been postulated as determinants of T2DM risk (Pasula and Sameera, 2013; Tinkov et al., 2015). TEs are present at very low concentrations in natural and perturbed environments, and are required at low amounts by humans (usually $\leq$ 100 mg/day). However, TEs are essential for a number of physiological processes, mainly involved in immunity and metabolism, since they serve as cofactors for multiple enzyme systems (Wiernsperger and Rapin, 2010; Dubey et al. 2020). Specifically, the imbalance of Chromium (Cr), vanadium (V), zinc (Zn), copper (Cu), iron (Fe) and selenium (Se) seems to be linked to T2DM development and progression, as well as to T2DM-derived complications (Kazi et al., 2008; Moreno-Navarrete et al., 2014; Wiernsperger and Rapin, 2010). It has been proposed that both TE deficiencies or overload could be associated with oxidative stress, which is closely related to insulin resistance and diabetes (Dubey et al. 2020). In addition, Cr, Zn, Cu, Fe and Se exert antioxidant effects and, therefore, might ultimately enhance insulin action by the activation of insulin receptor sites or increment of insulin sensitivity (Hussain et al., 2009).

According to Kruse-Jarres and Rükgauer (2000), the most accurate information on the potential contribution of TEs to disease prevention/onset comes from the tissues that reflect the immediate biochemical processes. Nevertheless, most of the researches have been focused on TE concentrations in blood (plasma or serum) and urine. In this regard, adipose tissue has been overlooked, despite being considered a metabolically

active tissue closely related to T2DM onset. Adipose tissue also intervenes in metabolic homeostasis through the synthesis of biologically active substances, sending and responding to signals that modulate energy intake or insulin sensitivity, among other functions (Abranches et al., 2015; Coelho et al., 2013). In fact, the adipose tissue dysfunction observed under obesity conditions can cause impaired insulin action or even insulin resistance (Abranches et al., 2015). Additionally, it has been hypothesized that an unbalance of some TE concentrations in adipose tissue could lead to insulin resistance and further metabolic disruption (Kazi et al., 2008; Tinkov et al., 2015), although there is scant epidemiological research in the field. Indeed, we evidenced negative associations of adipose tissue concentrations of Co, Cu, Mo and Se with the degree of obesity in adults from GraMo cohort (Rodríguez-Pérez et al., 2018). Thus, further studies are warranted to elucidate the potential influence of TEs in adipose tissue dysfunction.

Based on the foregoing, the present work, which is encompassed in a largely characterized adult cohort from Southern Spain, aims to study the associations of adipose tissue concentrations of Cr, Zn, Cu, V, Se and Fe with T2DM incidence over a 16-year follow-up period.

2. Materials and methods

2.1. Study area and GraMo cohort

This research is part of a wider investigation focused on identifying environmental factors affecting the development of several chronic diseases in an adult cohort from Southern Spain (GraMo cohort). The design and population recruitment have been described elsewhere (Arrebola et al., 2010, 2009; Rodríguez-Pérez et al., 2018). Briefly, study participants were recruited in two public hospitals in Granada province: San Cecilio University Hospital in the city of Granada (considered urban area, 240,000 inhabitants) and Santa Ana Hospital in the town of Motril (considered semi-rural area, 50,000 inhabitants). Participants were recruited from patients undergoing non-cancer-related surgery. Main surgery reasons were: hernias (41%), gallbladder diseases (21%), varicose veins (12%) and other conditions (26%). Inclusion criteria were: age over 16 years, residence in one of the study areas for at least 10 years, absence of cancer, and non-receipt of hormonal therapy. Of 409 individuals initially contacted, 387 (95%) agreed to participate and were included in the initial cohort. From these, TE

concentrations in adipose tissue samples were available for 226 (58%) participants. After exclusion of type 2 diabetics at recruitment (n=12), 214 participants were included in the longitudinal analyses. From these, a total of 132 participants additionally had glucose biomarkers measured in serum samples and were included in the complementary cross-sectional analyses. All participants included in the study were requested to sign an informed consent. The study was approved by the Ethics Committee of Granada (Comité de Ética de la Investigación Provincial de Granada, 8/2016).

2.2. Sampling and trace element analysis

Sampling and TE analyses have been detailed elsewhere (Rodríguez-Pérez et al., 2018). In brief, adipose tissue samples were freeze-dried in lyophilizer (for at least 72 hours) until a plateau weight was reached, subsamples of 0.1 g adipose tissue were totally digested in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria) with a mixture of 7 mL HNO_3 and 0.1 mL HF. After digestion, indium (1 µg/L) was added to each sample as internal standard. High-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) using an Element 2 instrument (Thermo, Bremen, Germany) was used for multi-element analyses of adipose tissue samples, following the analytical conditions previously published (Cukrov et al., 2008; Fiket et al., 2007). Standards were prepared by appropriate dilution of a multi-element reference solution (Analytika, Prague, Czech Republic) containing Co, Cr, Cu, Fe, Mo, Mn, Se, V, and Zn. Concentrations <LOD were substituted with the LOD/square root of 2.

A simultaneous analysis of the blank and certified reference materials (Mussel NCS DC 78005, Scallop (Pecten maximus) IAEA 452) was performed as quality control of the analytical procedure. For all elements, good agreement between analyzed and certified concentrations was obtained (within analytical uncertainty ± 10%).

2.3. Outcome assessment

Data on incident cases of T2DM were retrieved from the DIRAYA clinical records database between September and October 2019, as previously described (Salcedo-Bellido et al., 2021).

A participant was considered as incident type-2 diabetic when: 1) T2DM diagnosis had been registered during the follow-up period in his/her DIRAYA records,

and 2) regular prescription of antidiabetics had been registered in the prescription database. There were 26 patients with inconsistencies between the two databases. This was further elucidated by performing a thorough review of the individual clinical history sheets, consultation reports, laboratory data and other medical reports.

In order to assess potential early subclinical effects of TE exposure, a subsample of the study population with available serum samples (n=132) was included in the cross-sectional evaluation of glucose homeostasis markers in those participants free of T2DM at recruitment. Biomarkers of glucose homeostasis [i.e., serum glucose, immunoreactive insulin (I) and C-reactive protein (CP)] were analyzed in serum samples collected at recruitment under 12-h fasting conditions. Glucose concentrations were analyzed by means of a validated enzymatic method by using a Cobas c311 bioanalyzer (Roche) (Wu, 2006). Insulin and C-peptide were quantified by using validated *in vitro* immunological tests performed on a Cobas e-411 bioanalyzer (Roche) (Clark, 1999; Sapin, 2003). By using the abovementioned markers, the following set of indicators of β-cell function and insulin resistance/sensitivity were calculated -homeostasis model assessment (HOMA2)-: HOMA2-IR (insulin resistance), HOMA2-IS (insulin sensitivity) and HOMA2-β (β-cell function). These indicators were calculated by using the tool available at http://www.dtu.ox.ac.uk/homacalculator/index.phpStatistical, based on the updated HOMA2 formula (Levy et al., 1998).

2.4. Covariates

Data on socio-demographic characteristics, lifestyle, and health status, including sex, age, residence, education, occupational class, smoking habit, alcohol consumption, adipose tissue origin, and body mass index (BMI), were collected by face-to-face interviews conducted by trained personnel at the time of recruitment during the hospital stay. The questionnaire was previously validated (Riboli et al., 2002).

Smokers were defined as those with daily tobacco consumption ≥ 1 cig., while alcohol consumption was considered at a weekly consumption ≥ 1 drink. Residence in the area of Granada at the time of the surgery was considered “urban” and residence in the area of Motril was considered “semi-rural”. A participant was considered hypertensive at recruitment when the diagnoses and/or chronic consumption of anti-hypertensive medication were registered in his/her clinical records.

2.5. Statistical analyses

Descriptive analyses included the calculation of medians and 25-75th percentiles for the interval variables and percentages for the categorical variables. Spearman correlation tests were employed to evaluate the relationship between pairs of TE concentrations.

The longitudinal associations of TE concentrations with incident T2DM risk were explored by means of Cox-regression models, with time-to-events as the time variable. Therefore, hazard ratios with their corresponding 95% confidence intervals (CIs) were calculated as risk estimators. Data on participants who died before the observation of a study outcome were censored; therefore, only their disease-free time was considered in the analyses. Generalized additive models (GAM) were used for the evaluation of the functional form of the associations. The cox.zph function, implemented in the R survival package, was used for performing a test for the proportional hazards assumption, obtaining satisfactory results.

Cross-sectional associations of TE concentrations with serum glucose biomarkers were analyzed using linear regression models, for which beta coefficients and 95% CIs were reported.

TE concentrations were log-transformed in order to reduce the skewness of the distribution. Models were adjusted for *a priori* identified potential predictors of the exposure (Rodríguez-Pérez et al., 2018) or the disease. Additional covariates included variables whose inclusion produced changes > 10% in beta coefficients and/or those reported as relevant confounders in previous studies, such as sex (male/female), age (years), residence (urban/semi-rural), education (primary schooling not completed/primary or higher), occupational class (manual workers / non-manual workers, as defined by Regidor et al., 2001), hypertension diagnosis at recruitment, body mass index (BMI), smoking habit (smoker/ex-smoker), and alcohol consumption (consumer/non-consumer). Sensitivity analyses were performed by removing BMI and hypertension from the covariates. Rationale for these analyses is further described in the results and discussion section.

The associations of TE concentrations with glucose homeostasis markers were explored by means of multivariable linear regression models, entering ln-transformed

biomarkers of glucose homeostasis as dependent variables. Models were adjusted for the same covariates as Cox-regression models.

Data were stored and processed using the R statistical computing environment v3.2.3 (http://www.r-project.org/). We used the following packages: "survival" for cox regression analyses (Therneau, 2021), "gamlss" for GAM models (Rigby and Stasinopoulos, 2005), and CoxR2 for computing R^2 (Hyeri and Ronghui, 2020). The significance level was set at $p \leq 0.05$ and $p \leq 0.10$ as borderline significant, and all tests were two-tailed.

3. Results

Main characteristics of the participants, including a comparison between those who developed T2DM during follow-up *vs* T2DM-free, are summarized in Table 1. Out of 214 participants, 25 males (64.1%) and 14 females (35.9%) developed T2DM during the follow-up. Compared to participants free of incident T2DM, those that developed T2DM over follow-up cases were more likely to have primary or higher education (59.0%), be manual workers (76.9%), and be smokers or ex-smokers at recruitment (71.8%). Noteworthy, those participants who developed T2DM during the follow-up had significantly higher BMI [median (25th-75th percentile): 29.7 (25.9-32.5) *vs.* 26.6 (23.6-29.4) kg/m^2] and were older [median (25th-75th percentile): 59 (53-70) *vs.* 48 (34-64) years old] than those who did not.

A detailed description of this subcohort including adipose tissue TE concentrations has been reported elsewhere (Rodríguez-Pérez et al., 2018). In brief, the TEs under study were detected in all the adipose tissue samples except Se, which was found in concentrations above the LOD in only 53.5% of them (data not shown in tables). Fe, Zn and Cu showed the highest median adipose tissue concentrations, followed by Cr > Se > V. The 39 participants that developed T2DM over the follow-up showed higher median adipose tissue concentrations of Cr (462 *vs* 373, p=0.163), Fe (59500 *vs* 41600, p=0.171), Cu (996 *vs* 647, p=0.026) and lower Zn (9350 *vs* 9900, p=0.404) and Se (9.3 *vs* 23, p=0.231).

Results of adjusted Cox regression analyses of the associations of individual natural log-transformed adipose tissue concentrations of TEs with T2DM risk are displayed in Table 2 and in Figure 1 (the latter using quartiles of TE concentrations). In

addition, GAM models assessing the shape of the relationships of the associations are shown in Figure 2. Although not all are entirely significant at the traditional $p<0.05$ level, we evidenced positive individual associations of continuous log-transformed V (HR= 1.19, 95% CI: 0.60-2.39, p=0.615), Cr (HR= 1.58, 95% CI: 1.07-2.33, p=0.022), Fe (HR=1.59, 95% CI: 0.99-2.58, p=0.057) and Cu (HR= 1.61, 95% CI: 1.01-2.58, p=0.046) with T2DM risk. Those significant associations showed a seemingly dose-response pattern, as displayed in Figure 1. Furthermore, negative but non-statistically associations with T2DM risk were found for Se (HR=0.97, 95% CI: 0.84-1.12 and Zn (HR=0.95, 95% CI: 0.48-1.90). When all TEs were entered simultaneously in a global model, the results were similar to those found in individual models (Table S1). A Cox-model fitted only with the covariates is shown as supplementary material (Table S2).

Sensitivity analyses (BMI- and hypertension- unadjusted models) did not substantially change the magnitude of the associations found (Tables S1 and S2). Further adjustment for type of surgery at recruitment (hernia, gall bladder, varicose veins, others) produced no relevant changes in model coefficients (data not shown).

Tables 3 and S3 summarize the results from multivariable linear regression models assessing the cross-sectional associations of adipose tissue TEs with log-transformed biomarkers of glucose homeostasis. We observed that increased concentrations of V (β= 0.283, p=0.004) and Zn (β= 0.217, p=0.028), as well as lower Se concentrations (β= -0.049, p=0.027), were associated with increased β-cell function. In addition, higher adipose tissue concentrations of Cu were related to increased HOMA2-IS estimated from fasting glucose and insulin levels (β= 0.209, p=0.015).

In order to shed light on the relationship of our longitudinal results with the complementary cross-sectional analyses, we explored the associations of HOMA2 indices with T2DM risk (Table S4). Although not statistically significant, HOMA2_β (CP and I) and HOMA2_IS (CP and I) were negatively associated with T2DM incidence, (HR= 0.59, 95% CI: 0.32-1.13, p=0.112; HR= 0.69, 95% CI: 0.38-1.25, p=0.215 and HR=0.47, 95% CI: 0.21-1.05, p=0.064; HR=0.62, 95% CI: 0.35-1.15, p=0.104, respectively). Additionally, a positive association between insulin resistance calculated using glucose and C-peptide levels -HOMA2_IR (CP)- with T2DM risk (HR=2.14, 95% CI: 0.95- 4.80, p=0.065) evidenced that higher insulin resistance at baseline is associated with future T2DM risk.

Figure S1 shows the Spearman correlations between pairs of adipose tissue TE concentrations. Positive correlations were found between all pairs of TEs except for Zn-Se and V-Se, that were not significantly associated. Further discussion of correlations between TE concentrations has been shown elsewhere (Rodríguez-Pérez et al., 2018).

Finally, sensitivity analyses were performed by testing the associations in BMI- and hypertension-unadjusted models (Tables S5 and S6).

4. Discussion

This is the first epidemiologic study addressing the associations of TEs accumulated in adipose tissue with the risk of T2DM, which appears to be of relevance for the ethology of insulin resistance and other metabolic disturbances, as previously suggested elsewhere (Tinkov et al., 2015). We evidenced positive associations of adipose tissue Fe, Cr and Cu with T2DM incidence.

Laboratory studies have evidenced diabetogenic and obesogenic effects of low adipose tissue Cr concentrations (Tinkov et al., 2015). However, no epidemiological studies have verified these effects in humans. The importance of Cr in glucometabolic disorders has been observed in clinical stages of relatively severe Cr deficiency but, on the contrary, its supplementation could have no effect in healthy individuals (Wiernsperger and Rapin, 2010). *In vivo* and *in vitro* studies have identified several potential mechanisms of action of Cr to enhance the insulin signalling pathway and AMP-activated protein kinase (AMPK) activity, possibly acting as antioxidant agent or up-regulating cellular glucose uptake (Hua et al., 2012). Nevertheless, there are conflicting results from different case-controls studies, in which salivary or plasma/serum Cr concentrations have been measured in individuals with T2DM compared to controls (Chen et al., 2017; Martínez et al., 2018). However, it is worth to mention that saliva and blood TE concentrations commonly reflect recent intakes while adipose tissue concentrations have been suggested as a potential proxy of the biological availability of TEs (Kizalaite et al., 2019).

It is well known that T2DM is frequently developed together with other comorbidities, e.g. obesity (Frayn et al., 2007). In fact, the prevalence of obesity among T2 diabetics can reach 90% (Bhupathiraju and Hu, 2016). Furthermore, adipose tissue dysfunction has been pointed out as a very probable link between diabetes and obesity

(Tsave et al., 2016). In fact, in GraMo cohort, BMI (kg/m^2) at recruitment was positively associated with the risk of incident T2DM in a multivariable model with only covariates (HR:1.11, 95%CI: 1.05-1.16, Supplementary Material Table S2). In this context, we hypothesized a potential modifying effect of obesity in the associations between Cr adipose tissue concentrations and T2DM incidence, which was tested by entering the product term of BMI (at recruitment) * Cr concentrations as well as by stratifying the models by median BMI (data not shown). Although we found no significant interaction, it is important to highlight that we only used BMI at recruitment. Therefore, a potential confounding effect of weight changes over the follow-up cannot be discarded.

It is remarkable that both Cu and Fe are essential metals implicated in oxidative stress status. Uriu-Adams and Keen (2005) reported a potential bi-directional relationship between Cu metabolism and T2DM. Indeed, we found increased adipose tissue Cu concentrations at recruitment in those participants that developed T2DM over the follow-up, and Cu concentrations were positively associated with incident T2DM risk. Conversely, in our complementary cross-sectional analyses, Cu was positively associated HOMA2_IS (I), which could be understood as a protective effect, since HOMA2-IS (I) was negatively related to T2DM risk. We need to bear in mind that cross-sectional analyses are more prone to biases such as reversed causality, which might explain the divergent associations. Our findings emphasize the need for assessing the implications of environmental factors by using complementary designs.

Interestingly, Tinkov et al. (2012) reported an increase in the oxidative stress and inflammation status in adipose tissue of Wistar rats following Cu administration. The same authors reported an enhancement of Fe absorption during Cu repletion, which could explain the positive association between Fe adipose tissue concentration and T2DM risk. Fe also participates in the formation of reactive hydroxyl radicals, including reactive oxygen species (ROS), which play an important role in insulin resistance and T2DM (Zabłocka-Słowińska and Grajeta, 2017). Several biomarkers of body Fe stores e.g., serum ferritin concentrations have been proposed and have been shown to be associated with increased risk of T2DM in adult volunteers (Chen et al., 2018; Fernandez-Real et al., 2002). Earlier studies have linked the total body Fe stores with insulin resistance in the general population (Fernandez-Real et al., 1998). Whilst overall adipose tissue Fe concentrations were not found to differ between metabolically healthy

obese *vs* metabolic syndrome individuals (Kizalaite et al., 2019), significantly higher Fe concentrations in upper subcutaneous adipose tissue of those with metabolic syndrome were found (Kizalaite et al., 2019). In addition, Wlazlo et al. (2013) observed correlations between markers of Fe metabolism (e.g. ferritin and transferrin) and adipocyte insulin resistance in 574 adults from the Diabetes and Atherosclerosis Maastricht (CODAM) cohort, suggesting that Fe adipose tissue stores could be involved in the development of insulin resistance. Moreover, insulin is known to stimulate iron uptake by adipocytes and hepatocytes (Davis et al., 1986). Data from the adipose tissue of 4 different Caucasian cohorts showed a direct relationship between elevated body Fe storages and certain metabolic disorders by leading the accumulation of intracellular iron in adipocyte, decreasing *ADIPOQ* gene and protein expression and insulin action (Moreno-Navarrete et al., 2014). Interestingly, in our study we observed a U-shaped relationship between Fe adipose tissue concentration and the risk of T2DM in the GAM models (Figure 2). This trend was also found after quartile-stratification (Figure 1). These results suggest that not only Fe overload but also Fe depletion in adipose tissue could be associated with T2DM risk. Further validation on larger and more diverse populations is necessary to confirm these novel findings.

Regarding our cross-sectional findings, Zn and V concentrations were positively associated with β-cell function, exerting a potentially protective effect, since this biomarker was eventually was linked to decreased T2DM risk, although with limited statistical significance. Zn is involved in the synthesis, storage and secretion of insulin by pancreatic β-cells (González-Villalva et al., 2016; Shan et al. 2014), and Zn supplementation has shown to improve insulin sensitivity in obese diabetic patients (Cruz et al., 2017). A process of obesity-related chronic inflammation can alter the metallothionein and Zip-14 zinc transporter protein, producing unbalanced Zn content in organs and tissues, such as adipose tissue, thus inducing hypozincemia in obese individuals (Noh et al., 2014). In agreement, Tinkov et al. (2016) found a significant 34% decrease in Zn adipose tissue concentration in high fat fed rodents compared to controls, that also exerted lower insulin levels and HOMA2-IR values (Tinkov et al., 2016). However, we did not achieve similar results in the present study. Bouchard et al (2007) found that the expression of Zn finger protein 36 (*ZFP36*) in adipose tissue was negatively correlated with fasting insulin levels, the insulin resistance index, and 2-h post-glucose insulinemia in women, thus likely protecting against T2DM. In addition, adequate Zn delivery by Zn transporters as mediators of pathogenesis of T2DM should

not be discarded (Fukunaka and Fujitani, 2018), especially when previous studies have found changes in the expression profile of Zn transporters in adipose tissue from diabetic and non-diabetic rats (Maxel et al., 2015). Thus, looking at our results and the previous findings from *in vivo* studies, we hypothesize that Zn concentrations in adipose tissue might not be as determinant as the impairment of Zn transporters for the development of T2DM. In addition, β-cell induction might be an early mechanism of action of Zn to prevent insulin resistance. Congruently, we evidenced a lower incident T2DM risk in those individuals in Q2-Q3 of Zn concentrations compared to those in Q1, although this statement is somewhat speculative, since the association was not statistically significant (Figure 1). A similar trend was reported by Shan et al. (2014) in a case-control study among Chinese participants, in which higher plasma Zn concentrations were associated with a lower T2DM risk in a dose-dependent manner.

Little is known about the potential diabetogenic effect of V. Previous *in vivo* studies have shown that V exposure can regulate glucose homeostasis and improve insulin sensitivity in animal models (Mehdi et al., 2006). Additionally, V treatment significantly increased total β-cell number and total islets volume in diabetic adult male Sprague-Dawley rats (Pirmoradi et al., 2016). A protective effect of V in pancreatic β-cells from palmitate-induced apoptosis have also found *in vitro* (Gao et al., 2011). Congruently, in our population, V adipose tissue concentrations were significantly and positively associated with HOMA2__β calculated using both glucose and C-peptide levels or glucose and insulin levels, which could suggest a protective effect of T2DM development. However, we did not find a significant relationship between HOMA2_β and T2DM in the complementary cross-sectional analyses. V has shown to activate glucose uptake in 3T3-L1 differentiated adipocytes *in vitro* and *in vivo*, thus acting as an insulin enhancer (Mehdi et al., 2006). Early studies suggested the enhancement of glucose transport as a potential mechanism of action of V (Mohammad et al., 2006). Despite most of the few previous *in vivo* researches in adipose tissue report a negative relationship between V adipose tissue concentration and the onset of T2DM, they are in general inconclusive. On the one hand, GLUT4 expression in adipose tissue was significantly decreased in Streptozotocin-diabetic rats compared to controls, and normal expression was restored after V treatment. Thus, the researchers suggested that V might contribute to glucose homeostasis *in vivo* (Cam et al., 2001). On the other hand, Tinkov et al. (2015) found a 33% decrease in V adipose tissue concentration in female Wistar rats fed with a high-fat diet compared to controls together with an inverse correlation

between V adipose tissue concentrations and markers of insulin resistance i.e. insulin and HOMA2-IR values. High-fat foods are poor sources of V, thus, an adequate dietary intake of V by including good V dietary sources, such as grain and grain products, seem to be necessary to maintain an adequate adipose tissue V balance, which could improve glucose homeostasis. Further than the above-mentioned research, no epidemiological studies have been performed in human adipose tissue with or without T2DM and there are still several gaps of knowledge on the mechanisms underlying the potential protective effect of V on T2DM (Domingo and Gómez, 2016). Further research is warranted on its clinical relevance in adipose tissue microenvironment.

Se is another TE involved in the antioxidant system (Tinggi 2008) and, noteworthy, oxidative stress has been highlighted as a factor reducing insulin secretion and increasing insulin resistance (Bleys, J. et al. 2007). However, we did not find any significant association between Se adipose tissue concentrations and T2DM risk. Similarly, De Vega et al. (2016) did not find any associations among total serum Se in healthy and diabetic patients (de Vega et al. 2016). A recent intervention study concluded that plasma Se concentrations in newly diagnosed T2DM patients did not significantly differ from the control group (Binti Othman et al. 2017). Contrarily, highly significant decrease of blood serum Se concentration in T2DM patients (n=40) compared to healthy controls (n=36) was reported by Badran et al. (2016). Accordingly, Yadav et al. (2016) observed lower serum Se in 35 pre-diabetic individuals compared to the healthy control group. Moreover, they observed a significantly negative associations of Se serum concentrations with insulin resistance. Contrarily, we also found a significant negative association between Se adipose tissue concentrations and HOMA2_β, which might indeed be related to a chance finding, residual confounding or even reversed causality. In contrast, a positive association between serum Se concentrations and incidence of diabetes has been found in a hospital-based case–control study of 847 adults in Northern Taiwan (Lu et al. 2016), and similar results were found in a cross-sectional analysis of 8,876 U.S. adults (Bleys et al. 2007). Higher plasma Se levels were also related to a higher risk of T2DM in the Dongfeng-Tongji cohort from China (Yuan et al. 2018). Interestingly, a systematic review of observational studies concluded that there may exist a U-shaped non-linear dose–response relationship between serum Se and T2DM risk, on the basis of the dissimilar findings according the magnitude of Se concentrations (Wang et al. 2015).

It is notable that a multivariable model fitted only with the covariates produced a R-squared of 0.571 (Supplementary Material, Table S2), which increased to 0.637 after adjustment for Cr, Cu and Fe concentrations (Data not shown). Although this was not a large increase, our findings suggest that TE adipose tissue concentrations might help to improve predictive algorithms. Despite that adipose tissue cannot not be easily reached, those patients undergoing routine abdominal or inguinal surgery (from which many of them are precisely under increased risk of metabolic diseases) could benefit from these biomarkers.

Strengths of our study include the longitudinal design with a 16-year follow-up and complementary longitudinal and cross-sectional studies. The use of adipose tissue as a biological matrix for the quantification of TE concentrations in an epidemiological setting was based on our previous work (Rodríguez-Pérez et al., 2018) and represents a novel approach which, as abovementioned, might provide mechanistic information of *in situ* processes that could be relevant at a systemic level. However, more research is warranted on the clinical implications of the presence of these TEs in the adipose tissue. Sample size in the present study was limited, although enough for yielding suggestive associations, which were screened using different statistical approaches. Nevertheless, caution should be taken when extrapolating our results to other populations with different sociodemographic characteristics, TE concentrations and T2DM risk. Furthermore, and despite we performed a thorough clinical follow up using clinical databases, we cannot rule out a degree of underdiagnoses, since T2DM diagnoses over follow-up were based on clinical records and not on biomarkers (Soriguer et al., 2012). Another limitation is related to potential unmeasured changes in the covariates and/or TE levels during the follow-up. In addition, the potential selection bias caused by the hospital-based recruitment cannot be discarded, although we included a wide variety of conditions that would, if so, reduce the risk estimates but not produce false positive associations. Finally, sensitivity analyses were performed by testing the associations in BMI- and hypertension-unadjusted models, given that these covariates might also be in the causal pathway between TE and T2DM development. Further research on the speciation status is planned in GraMo cohort that will shed light on which chemical forms are mainly responsible for the observed effects.

5. Conclusions

To date, this is the first study exploring the relationship between levels of TEs in adipose tissue and incidence of T2DM in an adult cohort. Our findings suggest a potential role of Fe, Cr and Cu as potential risk factors for T2DM, as well as a protective role of V and Zn for the prevention of T2DM. Furthermore, our results emphasize the relevance of adipose tissue as a matrix for the assessment of the metabolic implications of certain TEs, which could complement current prediction algorithms of T2DM risk. The results from this study could be particularly relevant for individuals routinely undergoing abdominal surgery, in which adipose tissue samples could be easily obtained.

Acknowledgments

We would like to acknowledge the collaboration of the patients taking part in it.

Funding

Dr. JP Arrebola is under contract within the Ramón y Cajal Program (Ministerio de Economía, Industria y Competitividad, Spain). This study was supported by research grants from CIBER de Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III, Junta de Andalucía and European Regional Development Fund – FEDER (PI16/01858, PI18/01573, PI20/01568). This work was supported by the Ministry of Higher Education, Science and Technology of the Republic of Slovenia (P2-0273).

Contribution statement.

All authors: study design, data collection and analysis; **Celia Gómez Peña, Francisco M. Pérez-Carrascosa and Juan Pedro Arrebola:** acquisition of data; **Petra Vrhovnik and Fiket Željka**: chemical analysis of the data; **Celia Rodríguez Pérez, Vicente Mustieles and Juan Pedro Arrebola:** analysis and interpretation of data and led the data analysis; **Celia Rodríguez Pérez:** Writing- Original draft preparation; **Juan Pedro Arrebola**: Supervision; **All authors**: Reviewing and Editing.

References

Abranches, M.V., de Oliveira, F.C.E., da Conceição, L.L., Peluzio, M. do C.G., 2015. Obesity and diabetes: the link between adipose tissue dysfunction and glucose homeostasis. Nutr. Res. Rev. 28, 121–132.

Arrebola, J.P., Fernandez, M.F., Porta, M., Rosell, J., de la Ossa, R.M., Olea, N.,

519 Martin-Olmedo, P., 2010. Multivariate models to predict human adipose tissue
520 PCB concentrations in Southern Spain. Environ. Int. 36(7), 705-713.

521 Arrebola, J.P., Martin-Olmedo, P., Fernandez, M.F., Sanchez-Cantalejo, E., Jimenez-
522 Rios, J.A., Torne, P., Porta, M., Olea, N., 2009. Predictors of concentrations of
523 hexachlorobenzene in human adipose tissue: A multivariate analysis by gender in
524 Southern Spain. Environ. Int. 35(10), 27-32.

525 Arrebola, J.P., Fernández, M.F., Martin-Olmedo, P., Bondee, J.P., Martín-Rodriguez,
526 J.L., Expósito, J., Rubio-Domínguez, A., Olea, A., 2015. Historical exposure to
527 persistent organic pollutants and risk of incident hypertension. Environ. Res. 138,
528 217-223.

529 Salcedo-Bellido, I., Gómez-Peña, C., Pérez-Carrascosa, F. M., Vrhovnik, P., Mustieles,
530 V., Echeverría, R., ... & Arrebola, J. P., 2021. Adipose tissue cadmium
531 concentrations as a potential risk factor for insulin resistance and future type 2
532 diabetes mellitus in GraMo adult cohort. Sci. Total Environ. 780, 146359.

533 Bhupathiraju, S.N., Hu, F.B., 2016. Epidemiology of Obesity and Diabetes and Their
534 Cardiovascular Complications. Circ. Res. 118, 1723–1735.

535 binti Othman, F., bin Jan Mohamed, H. J., Sirajudeen, K. N. S., Rajab, N. F., 2017. The
536 influence of selenium status on body composition, oxidative DNA damage and total
537 antioxidant capacity in newly diagnosed type 2 diabetes mellitus: A case-control
538 study. J. Trace Elem. Med. Biol. 43, 106–112.

539 Bouchard, L., Vohl, M.C., Deshaies, Y., Rhéaume, C., Daris, M., Tchernof, A., 2007.
540 Visceral adipose tissue zinc finger protein 36 mRNA levels are correlated with
541 insulin, insulin resistance index, and adiponectinemia in women. Eur. J.
542 Endocrinol. 157(4), 451-457.

543 Cam, M.C., Brownsey, R.W., Rodrigues, B., McNeill, J.H., 2001. Lack of in vivo effect
544 of vanadium on GLUT4 translocation in white adipose tissue of streptozotocin-
545 diabetic rats. Metabolism. 50, 674–680.

546 Chen, L., Li, Y., Zhang, F., Zhang, S., Zhou, X., Ji, L., 2018. Elevated serum ferritin
547 concentration is associated with incident type 2 diabetes mellitus in a Chinese
548 population: A prospective cohort study. Diabetes Res. Clin. Pract. 139, 155–162.

549 Chen, S., Jin, X., Shan, Z., Li, S., Yin, J., Sun, T., Luo, C., Yang, W., Yao, P., Yu, K.,
550 Zhang, Y., Cheng, Q., Cheng, J., Bao, W., Liu, L., 2017. Inverse association of
551 plasma chromium levels with newly diagnosed type 2 diabetes: A case-control
552 study. Nutrients. 9(3), 294.

553 Clark, P.M., 1999. Assays for insulin, proinsulin(s) and C-peptide. Ann. Clin. Biochem.
554 36(5), 541-564.

555 Coelho, M., Oliveira, T., Fernandes, R., 2013. Biochemistry of adipose tissue: An
556 endocrine organ. Arch. Med. Sci. 9(2), 191-200.

557 Cruz, K.J.C., Morais, J.B.S., de Oliveira, A.R.S., Severo, J.S., do Nascimento Marreiro,
558 D., 2017. The effect of zinc supplementation on insulin resistance in obese
559 subjects: A systematic review. Biol. Trace Elem. Res. 176, 239–243.

560 Cukrov, N., Frančišković-Bilinski, S., Mikac, N., Roje, V., 2008. Natural and
561 anthropogenic influences recorded in sediments from the Krka river estuary
562 (Eastern Adriatic coast), evaluated by statistical methods. Fresenius Environ. Bull.
563 17(7a), 855-863.

564 Davis, R.J., Corvera, S., Czech, M.P., 1986. Insulin stimulates cellular iron uptake and
565 causes the redistribution of intracellular transferrin receptors to the plasma
566 membrane. J. Biol. Chem. 261, 8708–8711.

567 de Vega, R. G., Fernández-Sánchez, M. L., Fernández, J. C., Menéndez, F. V. Á., Sanz-
568 Medel, A., 2016. Selenium levels and glutathione peroxidase activity in the plasma
569 of patients with type II diabetes mellitus. J. Trace Elem. Med. Biol. 37, 44–49

570 Domingo, J.L., Gómez, M., 2016. Vanadium compounds for the treatment of human
571 diabetes mellitus: A scientific curiosity? A review of thirty years of research. Food
572 Chem. Toxicol. 95, 137-141.

573 Dubey, P., Thakur, V., Chattopadhyay, M., 2020. Role of Minerals and Trace Elements
574 in Diabetes and Insulin Resistance. Nutrients. 12(6), 1864.

575 Fernandez-Real, J.M., Lopez-Bermejo, A., Ricart, W., 2002. Cross-talk between iron
576 metabolism and diabetes. Diabetes 51, 2348–2354.

577 Fernandez-Real, J.M., Ricart-Engel, W., Arroyo, E., Balanca, R., Casamitjana-Abella,

R., Cabrero, D., Fernandez-Castaner, M., Soler, J., 1998. Serum ferritin as a component of the insulin resistance syndrome. Diabetes Care 21, 62–68.

Fiket, Ž., Roje, V., Mikac, N., Kniewald, G., 2007. Determination of arsenic and other trace elements in bottled waters by high resolution inductively coupled plasma mass spectrometry. Croat. Chem. Acta. 80(1), 91-100.

Fraga, C.G., 2005. Relevance, essentiality and toxicity of trace elements in human health. Mol. Aspects Med. 26, 268-298.

Franks, P.W., McCarthy, M.I., 2016. Exposing the exposures responsible for type 2 diabetes and obesity. Science 354, 69–73.

Frayn, K.N., Tan, C.D., Karpe, F., 2007. Adipose tissue: A key target for diabetes pathophysiology and treatment?, in: Hormone and Metabolic Research. 39(10), 739-742.

Fukunaka, A., Fujitani, Y., 2018. Role of zinc homeostasis in the pathogenesis of diabetes and obesity. Int. J. Mol. Sci. 19(2), E476.

Gao, Z., Zhang, C., Yu, S., Yang, X., Wang, K., 2011. Vanadyl bisacetylacetonate protects β cells from palmitate-induced cell death through the unfolded protein response pathway. J. Biol. Inorg. Chem. 16(5), 789-798.

González-Villalva, A., Colín-Barenque, L., Bizarro-Nevares, P., Rojas-Lemus, M., Rodríguez-Lara, V., García-Pelaez, I., Ustarroz-Cano, M., López-Valdez, N., Albarrán-Alonso, J.C., Fortoul, T.I., 2016. Pollution by metals: Is there a relationship in glycemic control? Environ. Toxicol. Pharmacol. 46, 337–343.

Hua, Y., Clark, S., Ren, J., Sreejayan, N., 2012. Molecular mechanisms of chromium in alleviating insulin resistance. J. Nutr. Biochem. 23, 313–319.

Hussain, F., Maan, M.A., Sheikh, M.A., Nawaz, H., Jamil, A., 2009. Trace elements status in type 2 diabetes. Bangladesh J. Med. Sci. 8, 52.

Hyeri Y., Ronghui, X., 2020. CoxR2: R-Squared Measure Based on Partial LR Statistic, for the Cox PH Regression Model. R package version 1.0. Available online at: https://CRAN.R-project.org/package=CoxR2

Kazi, T.G., Afridi, H.I., Kazi, N., Jamali, M.K., Arain, M.B., Jalbani, N., Kandhro,

G.A., 2008. Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biol. Trace Elem. Res. 122, 1–18.

Kizalaite, A., Brimiene, V., Brimas, G., Kiuberis, J., Tautkus, S., Zarkov, A., Kareiva, A., 2019. Determination of Trace Elements in Adipose Tissue of Obese People by Microwave-Assisted Digestion and Inductively Coupled Plasma Optical Emission Spectrometry. Biol. Trace Elem. Res. 189(1), 10-17.

Kruse-Jarres, J.D., Rükgauer, M., 2000. Trace elements in diabetes mellitus. Peculiarities and clinical validity of determinations in blood cells. J. trace Elem. Med. Biol. 14, 21–27.

Levy, J.C., Matthews, D.R., Hermans, M.P., 1998. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care. 21(12), 2191-2192.

Lowe, J., Taveira- da- Silva, R., Hilário- Souza, E., 2017. Dissecting copper homeostasis in diabetes mellitus. IUBMB Life 69, 255–262.

Lu, C.W., Chang, H.H., Yang, K.C., Kuo, C.S., Lee, L.T., & Huang, K.C., 2016. High serum selenium levels are associated with increased risk for diabetes mellitus independent of central obesity and insulin resistance. BMJ Open Diabetes Res. Care. 4(1), e000253.

Martínez, L.M., Pagán, D.M., Jornet, P.L., 2018. Trace Elements in Saliva as Markers of Type 2 Diabetes Mellitus. Biol. Trace Elem. Res. 1–7.

Maxel, T., Smidt, K., Larsen, A., Bennetzen, M., Cullberg, K., Fjeldborg, K., Lund, S., Pedersen, S.B., Rungby, J., 2015. Gene expression of the zinc transporter ZIP14 (SLC39a14) is affected by weight loss and metabolic status and associates with PPARγ in human adipose tissue and 3T3-L1 pre-adipocytes. BMC Obes. 2, 46.

Mehdi, M.Z., Pandey, S.K., Théberge, J.-F., Srivastava, A.K., 2006. Insulin signal mimicry as a mechanism for the insulin-like effects of vanadium. Cell Biochem. Biophys. 44, 73–81.

Mohammad, S., Taha, A., Bamezai, R.N.K., Baquer, N.Z., 2006. Modulation of glucose transporter (GLUT4) by vanadate and Trigonella in alloxan-diabetic rats. Life Sci. 78, 820–824.

637 Moreno-Navarrete, J.M., Novelle, M.G., Catalan, V., Ortega, F., Moreno, M., Gomez-
638 Ambrosi, J., Xifra, G., Serrano, M., Guerra, E., Ricart, W., Fruhbeck, G., Dieguez,
639 C., Fernandez-Real, J.M., 2014. Insulin resistance modulates iron-related proteins
640 in adipose tissue. Diabetes Care 37, 1092–1100.

641 Noh, H., Paik, H.Y., Kim, J., Chung, J., 2014. The alteration of zinc transporter gene
642 expression is associated with inflammatory markers in obese women. Biol. Trace
643 Elem. Res. 158, 1–8.

644 World Health Organization (WHO), 2016. Global Report on Diabetes. Available from:
645 https://www.who.int/diabetes/global-report/en/. Accessed 30 September 2020.

646 Petrie, J.R., Guzik, T.J., Touyz, R.M., 2018. Diabetes, Hypertension, and
647 Cardiovascular Disease: Clinical Insights and Vascular Mechanisms. Can. J.
648 Cardiol. 34(5), 575-584.

649 Pirmoradi, L., Noorafshan, A., Safaee, A., Dehghani, G.A., 2016. Quantitative
650 assessment of proliferative effects of oral vanadium on pancreatic islet volumes
651 and beta cell numbers of diabetic rats. Iran. Biomed. J. 20(1), 18-25.

652 Sapin R., 2003. Insulin assays: Previously known and new analytical features. Clin.
653 Lab. 49, 113-121.

654 Regidor, E., 2001. The Goldthorpe Social Class Classification: reference framework for
655 a proposal for the measurement of social class by the Working Group of the
656 Spanish Society of Epidemiology. Rev. Esp. Salud Publica. 75, 13-22.

657 Riboli, E., Hunt, K., Slimani, N., Ferrari, P., Norat, T., Fahey, M., Charrondière, U.,
658 Hémon, B., Casagrande, C., Vignat, J., Overvad, K., Tjønneland, A., Clavel-
659 Chapelon, F., Thiébaut, A., Wahrendorf, J., Boeing, H., Trichopoulos, D.,
660 Trichopoulou, A., Vineis, P., Palli, D., Bueno-de-Mesquita, H., Peeters, P., Lund,
661 E., Engeset, D., González, C., Barricarte, A., Berglund, G., Hallmans, G., Day, N.,
662 Key, T., Kaaks, R., Saracci, R., 2002. European Prospective Investigation into
663 Cancer and Nutrition (EPIC): study populations and data collection. Public Health
664 Nutr. 5(6B), 1113-1124.

665 Rigby, R.A., Stasinopoulos, D.M., 2005. Generalized additive models for location, scale
666 and shape. J R Stat Soc Ser C Appl Stat. 54, 507–554.

667 Rodríguez-Pérez, C., Vrhovnik, P., González-Alzaga, B., Fernández, M.F., Martin-
668 Olmedo, P., Olea, N., Fiket, Ž., Kniewald, G., Arrebola, J.P., 2018. Socio-
669 demographic, lifestyle, and dietary determinants of essential and possibly-essential
670 trace element levels in adipose tissue from an adult cohort. Environ. Pollut. 236,
671 705-713.

672 Pasula, S., Sameera, K., 2013. Trace elements in diabetes mellitus. J. Clin. Diagn. Res.
673 7, 1863–1865.

674 Shan, Z., Bao, W., Zhang, Y., Rong, Y., Wang, X., Jin, Y., ... & Liu, L. 2014.
675 Interactions between zinc transporter-8 gene (SLC30A8) and plasma zinc
676 concentrations for impaired glucose regulation and type 2 diabetes. Diabetes. 63(5),
677 1796-1803.

678 Soriguer, F., Goday, A., Bosch-Comas, A., Bordiú, E., Calle-Pascual, A., Carmena, R.,
679 Casamitjana, R., Castaño, L., Castell, C., Catalá, M., 2012. Prevalence of diabetes
680 mellitus and impaired glucose regulation in Spain: the Di@ bet. es Study.
681 Diabetologia 55, 88–93.

682 Therneau, T., 2021. A Package for Survival Analysis in R. R package version 3.2-10.
683 Available online at https://CRAN.R-project.org/package=survival

684 Tinkov, A.A., Ajsuvakova, O.P., Shehtman, A.M., Boev, V.M., Nikonorov, A.A., 2012.
685 Influence of iron and copper consumption on weight gain and oxidative stress in
686 adipose tissue of Wistar rats. Interdiscip. Toxicol. 5, 127–132.

687 Tinkov, A.A., Popova, E. V, Gatiatulina, E.R., Skalnaya, A.A., Yakovenko, E.N.,
688 Alchinova, I.B., Karganov, M.Y., Skalny, A. V, Nikonorov, A.A., 2016. Decreased
689 adipose tissue zinc content is associated with metabolic parameters in high fat fed
690 wistar rats. Acta Sci. Pol. Technol. Aliment. 15, 99–105.

691 Tinkov, A.A., Popova, E. V, Polyakova, V.S., Kwan, O. V, Skalny, A. V, Nikonorov,
692 A.A., 2015. Adipose tissue chromium and vanadium disbalance in high-fat fed
693 Wistar rats. J. Trace Elem. Med. Biol. 29, 176–181.

694 Tsave, O., Yavropoulou, M.P., Kafantari, M., Gabriel, C., Yovos, J.G., Salifoglou, A.,
695 2016. The adipogenic potential of Cr(III). A molecular approach exemplifying
696 metal-induced enhancement of insulin mimesis in diabetes mellitus II. J. Inorg.

Biochem. 163, 323-331.

Uriu-Adams, J.Y., Keen, C.L., 2005. Copper, oxidative stress, and human health. Mol. Aspects Med. 26, 268–298.

Wang, Xin-liang, Yang, T., Wei, J., Lei, G., & Zeng, C., 2015. Association between serum selenium level and type 2 diabetes mellitus: a non-linear dose–response meta-analysis of observational studies. Nutr. J. 15(1), 48.

Wiernsperger, N., Rapin, J., 2010. Trace elements in glucometabolic disorders: an update. Diabetol. Metab. Syndr. 2, 70.

Wlazlo, N., van Greevenbroek, M.M., Ferreira, I., Jansen, E.H., Feskens, E.J., van der Kallen, C.J., Schalkwijk, C.G., Bravenboer, B., Stehouwer, C.D., 2013. Iron metabolism is associated with adipocyte insulin resistance and plasma adiponectin: the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study. Diabetes Care 36, 309–315.

Wu, A. 2006. Tietz Clinical Guide to Laboratory Tests. 4th ed. WB Saunders Co. Philadelphia.

Yadav, C., Manjrekar, P. A., Agarwal, A., Ahmad, A., Hegde, A., & Srikantiah, R. M., 2017. Association of Serum Selenium, zinc and magnesium levels with glycaemic indices and insulin resistance in pre-diabetes: a cross-sectional study from South India. Biol. Trace Elem. Res. 175(1), 65–71.

Yuan, Y., Xiao, Y., Yu, Y., Liu, Y., Feng, W., Qiu, G., ... & Wu, T., 2018. Associations of multiple plasma metals with incident type 2 diabetes in Chinese adults: The Dongfeng-Tongji Cohort. Environ. Pollut. 237, 917-925.

Zabłocka-Słowińska, K., Grajeta, H., 2017. Selenium and copper in type 2 diabetes mellitus-more doubt than certainty. J. Elem. 22. 365-376.

Zheng, Y., Ley, S.H., Hu, F.B., 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat. Rev. Endocrinol. 14, 88.

Figure captions

Figure 1. Quartiles of trace elements concentrations and type 2 diabetes risk.

Figure 2. TE concentrations and diabetes risk. Generalized Additive Models.

Table 1. Characteristics of the studied population comparing those who developed type 2 diabetes mellitus (T2DM) during follow-up vs diabetes-free during follow up.

	Free of T2DM during follow-up (N=175)	**Incident T2DM during follow-up (N=39)**	**Total (N=214)**	p-value
Sex				0.377
Male	98 (44%)	25 (64.1%)	123 (57.5%)	
Female	77 (56%)	14 (35.9%)	91 (42.5%)	
Residence				0.486
Urban	83 (47.4%)	21 (53.9%)	104 (48.6%)	
Semi-rural	92 (52.6%)	18 (46.1%)	110 (51.4%)	
Education				0.123
Primary uncompleted	48 (27.4%)	16 (41.0%)	64 (29.9%)	
Primary or higher	127 (72.6%)	23 (59.0%)	150 (70.1%)	
Occupational class				0.999
Non-manual worker	40 (22.9%)	9 (23.1%)	49 (22.9%)	
Manual worker	135 (77.1%)	30 (76.9%)	165 (77.1%)	
Smoking habit				0.999
Non-smoker	71 (40.6%)	11 (28.2%)	82 (38.3%)	
Smoker or ex-smoker	104 (59.4%)	28 (71.8%)	132 (61.7%)	
Alcohol consumption				0.999
No habitual consumer	83 (47.4%)	19 (48.7%)	102 (47.7%)	
Habitual consumer	92 (52.6%)	20 (51.3%)	112 (52.3%)	
Adipose tissue origin				0.981
Hernia	81 (46.3%)	20 (51.3%)	101 (47.2%)	
Gall Bladder	34 (19.4%)	7 (18.0%)	41 (19.2%)	
Varicose Veins	8 (4.6%)	1 (2.6%)	9 (4.2%)	
Other	52 (29.7%)	11 (28.2%)	63 (29.4%)	
BMI (kg/m^2)[1]	26.6 (23.6 - 29.4)	29.7 (25.9 -35.2)	27.2 (23.9 - 29.9)	0.000*
Age (years)[1]	48 (34 - 64)	59 (53 -70)	52.5 (36 - 66)	0.003*
Chromium (µg /kg)[1]	373 (201 - 609)	462 (232 - 655)	382 (204 - 618)	0.163
Iron (µg/kg)[1]	41600 (27400 - 69800)	59500 (34700 - 79800)	43700 (29900 - 70700)	0.171
Vanadium (µg/kg)[1]	12 (9 - 19)	14 (10 - 19)	13 (9 - 19)	0.468
Copper (µg/kg)[1]	647 (470 - 1040)	996.5 (554 - 1466)	677 (475 - 1150)	0.026*
Zinc (µg/kg)[1]	9900 (7100 - 13700)	9350 (6600 - 12700)	9800 (7100 - 13600)	0.404
Selenium (µg/kg)[1]	23 (0.5 - 63)	9.3 (0.5 - 51)	18 (0.5 - 60)	0.231

[1] Data expressed as median (25th - 75th percentile)
* p<0.05 level

730

731

732

733

Table 2. Log-transformed individual TE concentrations (µg/kg) and T2DM risk. Cox-regression models

	HR	95% Confidence Interval		p-value
		Lower	Upper	
Vanadium	1.19	0.60	2.39	0.615
Chromium	1.58	1.07	2.33	0.022
Selenium	0.97	0.84	1.12	0.681
Iron	1.97	0.99	2.58	0.057
Copper	1.61	1.01	2.58	0.046
Zinc	0.95	0.48	1.90	0.879

HR: Hazard Ratio. Models were adjusted by sex (male/female), age, residence (urban/semi-rural), education (primary schooling not completed/primary or higher), occupational class i.e., manual workers (social classes I+II+III) and non-manual workers (social classes IV+V), hypertension at recruitment, BMI, smoking habit (smoker/ex-smoker), alcohol consumption (consumer/non-consumer).

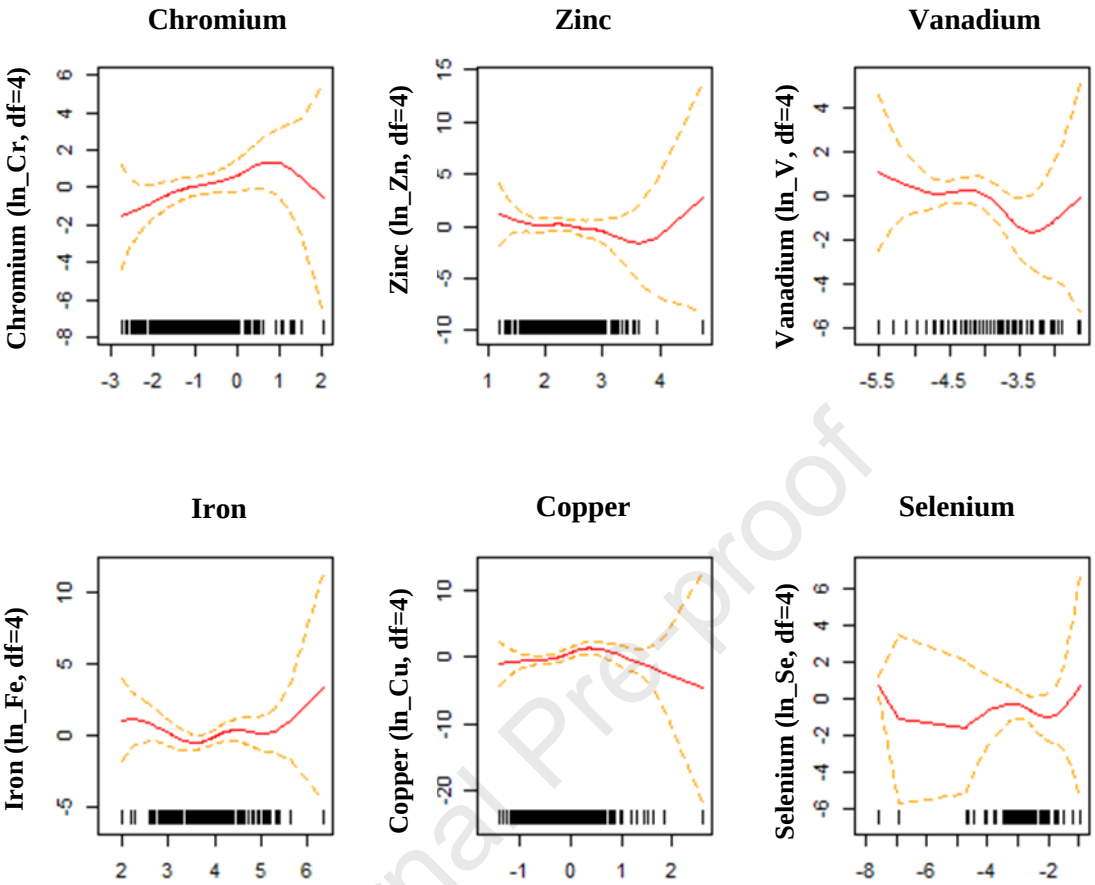
734

Table 3. Trace elements adipose tissue concentrations and log-transformed biomarkers of glucose homeostasis. Linear regression models.

	Cr			V			Zn			Fe			Cu			Se		
	Beta	SE	p-value	Beta	SE	p-value	Beta	SE	p-value	Beta	SE	p-value	Beta	SE	p-value	Beta	SE	p-value
HOMA2_ β (CP)	0.056	0.060	0.351	0.283	0.097	**0.004**	0.217	0.098	**0.028**	-0.019	0.073	0.796	0.095	0.086	0.272	-0.049	0.022	**0.027**
HOMA2_ β (I)	0.057	0.063	0.366	0.256	0.102	**0.014**	0.152	0.103	0.141	-0.005	0.076	0.953	0.008	0.090	0.928	-0.045	0.023	**0.050**
HOMA2_IS (CP)	0.001	0.043	0.979	-0.099	0.071	0.169	-0.104	0.070	0.141	-0.027	0.052	0.603	-0.037	0.061	0.542	0.013	0.016	0.414
HOMA2_IS (I)	-0.004	0.061	0.951	-0.085	0.101	0.404	-0.038	0.100	0.703	-0.029	0.074	0.695	0.209	0.085	**0.015**	0.002	0.022	0.940
HOMA2_IR (CP)	-0.001	0.043	0.979	0.099	0.071	0.169	0.104	0.070	0.142	0.027	0.052	0.608	0.037	0.061	0.544	-0.013	0.016	0.418
HOMA2_IR (I)	-0.064	0.067	0.344	0.012	0.113	0.918	0.034	0.112	0.764	-0.026	0.082	0.752	-0.094	0.096	0.329	-0.007	0.025	0.773

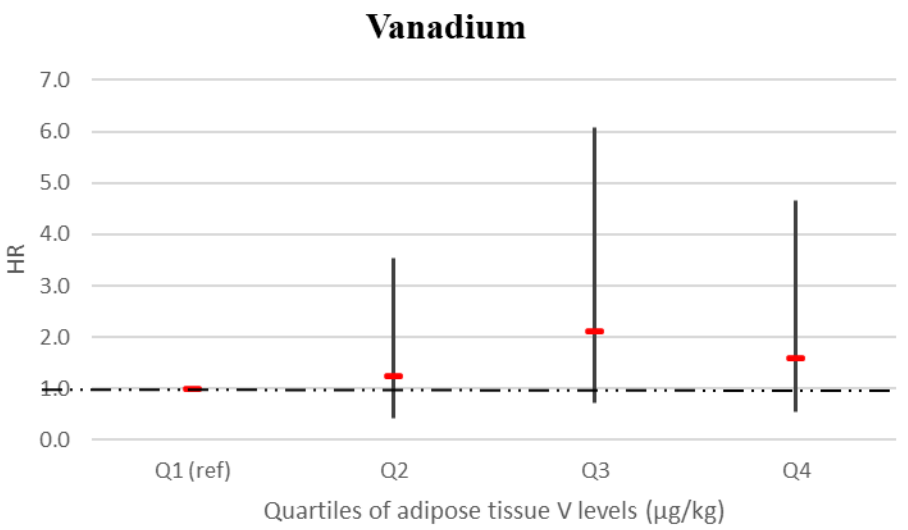
HOMA2_ β, β-cell function; HOMA2-IS, insulin sensitivity; HOMA2-IR, insulin resistance; CP: calculated using glucose and C-peptide levels; I, calculated using glucose and insulin levels SE, standard error. Models were adjusted by sex (male/female), age, residence (urban/semi-rural), education (primary schooling not completed/primary or higher), occupational class i.e., manual workers (social classes I+II+III) and non-manual workers (social classes IV+V), hypertension at recruitment, BMI, smoking habit (smoker/ex-smoker), alcohol consumption (consumer/non-consumer).

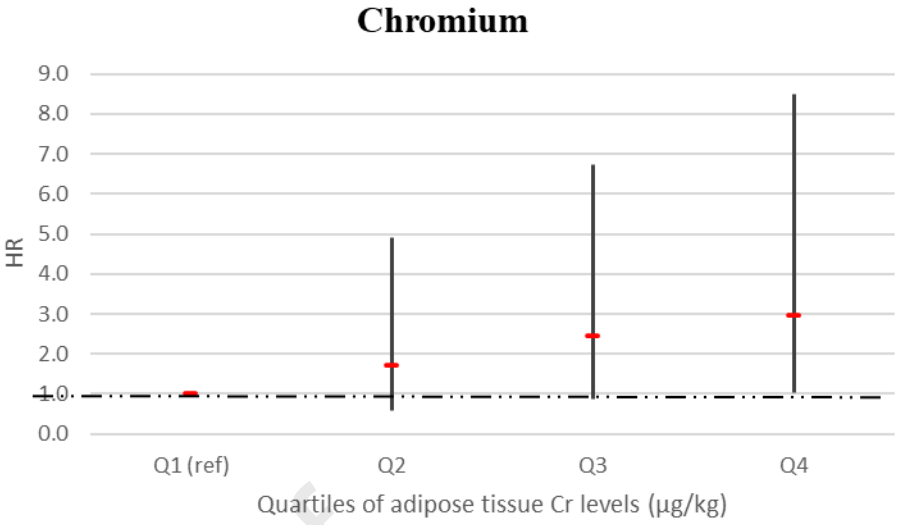
Figure 2. Adipose tissue TE concentrations and incident diabetes risk. Generalized Additive Models.

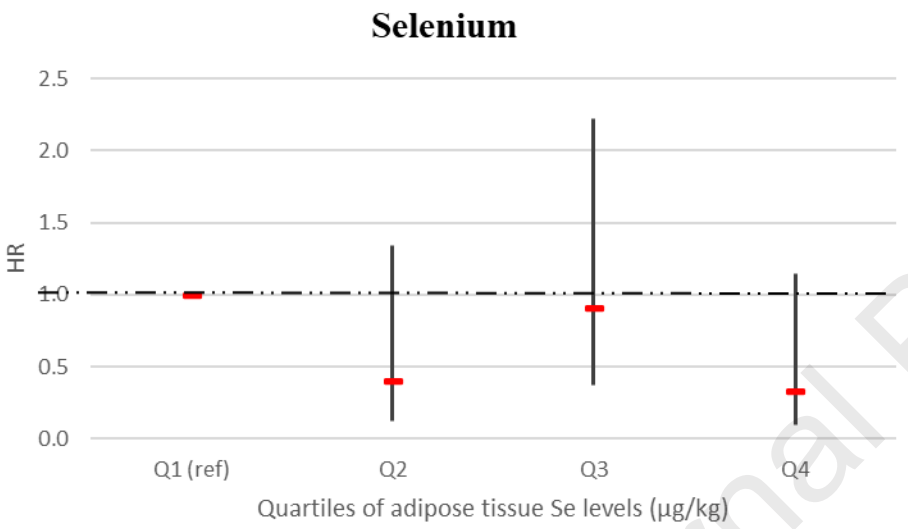


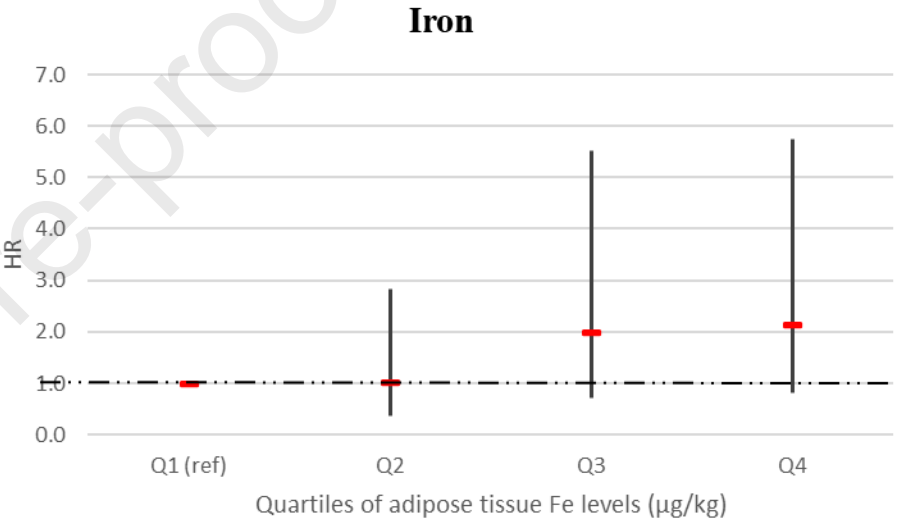
GAM models were adjusted for sex, age, residence, education, occupational class, smoking habit, alcohol consumption, adipose tissue origin, and body mass index (BMI). df: degrees of freedom.

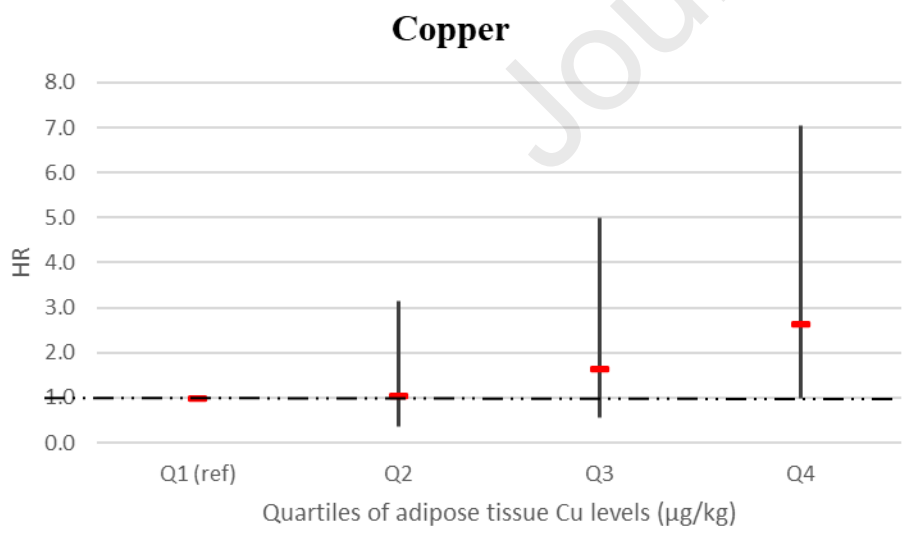
Figure 1. Quartiles of trace elements concentrations and T2DM risk.

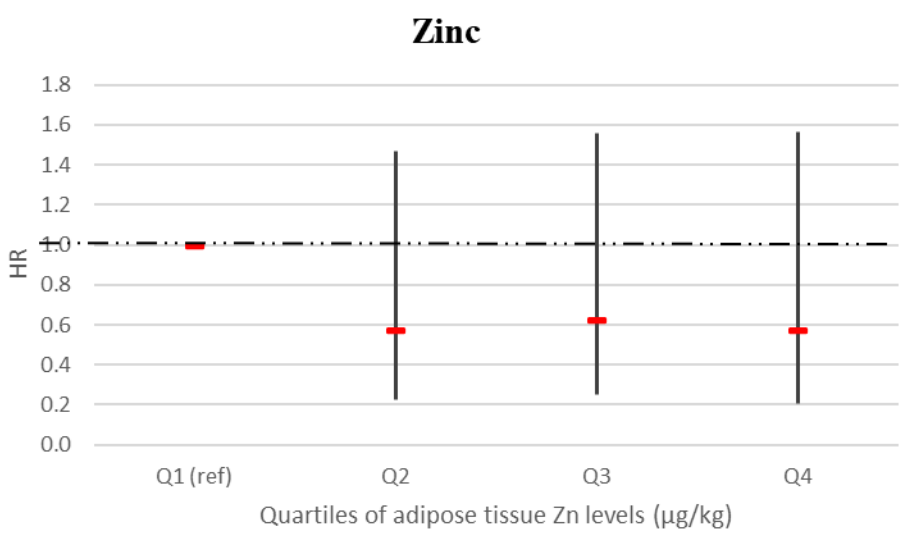












HR: Hazard Ratio; Q: Quartile; for each quartile, the hazard ratio with its corresponding 95% confidence interval is displayed; ref, reference category. Models were adjusted by sex (male/female), age, residence (urban/semi-rural), education (primary schooling not completed/primary or higher), occupational class i.e. manual workers (social classes I+II+III) and non-manual workers (social classes IV+V), hypertension at recruitment, BMI, smoking habit (smoker/ex-smoker), alcohol consumption (consumer/non-consumer) and diabetes.

Highlights

- ~~Adipose tissue Cr, V, Zn, Fe, Cu and Se concentrations were measured in an adult cohort~~
- ~~Association between TEs and T2DM in a 16-year follow-up period cohort was studied~~
- Fe, Cu and Cr adipose tissue concentrations were positively associated with T2DM
- Adipose tissue Cr, Cu and Fe levels might be indicative of an increased risk of T2DM
- V and Zn concentrations were positively associated with β-pancreatic cell function
- V and Zn adipose tissue levels might have a protective effect on T2DM incidence

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: