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Socio-demographic, lifestyle, and dietary determinants of essential and possibly-essential trace element levels in adipose tissue from an adult cohort☆

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ABSTRACT

There is increasing evidence linking levels of trace elements (TEs) in adipose tissue with certain chronic conditions (e.g., diabetes or obesity). The objectives of this study were to assess concentrations of a selection of nine essential and possibly-essential TEs in adipose tissue samples from an adult cohort and to explore their socio-demographic, dietary, and lifestyle determinants. Adipose tissue samples were intraoperatively collected from 226 volunteers recruited in two public hospitals from Granada province. Trace elements (Co, Cr, Cu, Fe, Mn, Mo, Se, V, and Zn) were analyzed in adipose tissue by high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). Data were collected on socio-demographic characteristics, lifestyle, diet, and health status by face-to-face interview. Predictors of TE concentrations were assessed by using multivariable linear and logistic regression. All TEs were detected in all samples with the exception of Se (53.50%). Iron, zinc, and copper showed the highest concentrations (42.60 mg/kg, 9.80 mg/kg, and 0.68 mg/kg, respectively). Diet was the main predictor of Cr, Fe, Mo, and Se concentrations. Body mass index was negatively associated with all TEs (β coefficients = −0.018 to −0.593, p = 0.001–0.090) except for Mn and V. Age showed a borderline-significant positive correlation with Cu (β = 0.004, p = 0.089). Residence in a rural or semi-rural area was associated with increased Co, Cr, Fe, Mo, Mn, V and Zn concentrations and with β coefficients ranging from 0.196 to 0.544 ($p < 0.05$). Furthermore, individuals with higher educational level showed increased Cr, Co, Fe and V concentrations (β coefficients = 0.276–0.368, p = 0.022–0.071). This is the first report on the distribution of these TEs in adipose tissue and on their determinants in a human cohort and might serve as an initial step in the elucidation of their clinical relevance.
We identified socio-demographic, lifestyle, and dietary determinants of trace element concentrations in adipose tissue from an adult cohort (n = 226), and shed light on the potential clinical relevance of the accumulation of these elements.

1. Introduction

Trace elements (TEs) refer to “elements that occurs in natural and perturbed environments in small amounts and that, when present in sufficient bioavailable concentrations might be toxic to living organism” (Wada, 2004). TEs are required in small amounts (usually ≤ 100 mg/day) to ensure decisive functions to maintain human health (Fraga, 2005). They are usually present at very low concentrations in human tissues, representing approximately 0.02% of the weight of a human adult (Kulkarni et al., 2014). Although several classifications have been proposed, three groups of TEs can be defined according to their role in the human body: 1) nutritionally essential, e.g., cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn); 2) possibly essential, e.g., chromium (Cr) or vanadium (V); or 3) non-essential, e.g., arsenic (As), cadmium (Cd), or lead (Pb) (Prashanth et al., 2015).

Trace elements are widely distributed in the environment, and their concentrations in the human body are influenced by several factors such as gender, age, percentage element retention, nutritional status, chemical form, and binding sites (Caroli, 2007). While anions such as Cr, I, Mo, or Se are readily absorbed, and whole-body home-

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ostasis is mediated mainly by renal excretion, cations such as Cu, Fe, Mn or Zn need specific pathways for absorption, and their homeostasis is affected by gastrointestinal and biliary secretion (Aggett, 1985). Cobalt is indirectly related to the formation of red blood cells because it is part of vitamin B-12, but moderate long-term intake can induce chronic toxicity in several organs and tissues, including the thyroid gland, lungs, skin, or immune system (Simonsen et al., 2012). Copper is a cofactor of many redox enzymes and is involved in several biological processes, including antioxidant defense, neuropeptide synthesis, and immune function (Bost et al., 2016), and acute or chronic copper toxicity is relatively rare (Gaetke and Chow, 2003). Manganese is essential for many ubiquitous enzymatic reactions, including the synthesis of amino acids, lipids, proteins, and carbohydrates, while the excessive accumulation of manganese can result in neurobehavioral deficits in humans (Chen et al., 2006). The physiological need for V is currently controversial, and its elevated accumulation has been implicated in the pathogenesis of certain neurological disorders and cardiovascular diseases. However, a positive role has been proposed for Cr (III), V (IV) components e.g. oxovanadium or vanadyl, and Zn in the prevention of obesity-related metabolic disturbances (Mukherjee et al., 2004; Tinkov et al., 2015).

In humans, diet is considered the main source of internal concentrations of TEs, which can differentially accumulate in organs and tissues after their absorption (Prashanth et al., 2015). The accumulation of TEs in the body is governed not only by environmental exposure but also by mechanisms involved in their absorption, distribution, metabolism, and elimination (Ng et al., 2015). The liver has been reported as the main deposition site for Co, Cu, and Mn (Rahil-Khazen et al., 2002), while the highest concentrations of Mo have been reported in the liver, kidney, and small intestine (Sardesai, 1993). Mo and V are also stored in bones (Rehder, 2013). Zn is mostly accumulated in muscle, bone, skin and hair (Plant et al., 2012), whereas there is no evidence of a specific storage site for Se in the human body.

The elemental composition of body tissues is considered an indicator of the nutritional and pathological status of humans (Abdulla and Chmielnicka, 1989). Most published studies suggest that the main determinants of the body burden of most TEs (such as Co, Mn or Se) are socio-demographic and environmental factors (Fraga, 2005). The vast majority of the available literature is focused on the study of TE concentrations in certain biological matrices, such as urine, plasma, nail or hair (Błażewicz et al., 2013; Glorennec et al., 2016). However, although these matrices are relatively easy to obtain, their TE concentrations might not always correlate with total body storage in humans (Bogden and Klevay, 2000). Thus, urine and blood TE concentrations are considered indicators of recent intake (Navarro-Alarcon and Cabrera-Vique, 2008) but not always of intracellular concentrations (Beneš et al., 2000). There is increasing evidence that TE concentrations in other more stable tissues (e.g. liver, kidney or bone) might better reflect long-term intake (Beneš et al., 2000). In particular, inadequate attention has been paid to their content in adipose tissue or to the factors influencing their accumulation in this matrix (Tinkov et al., 2015). This is of particular relevance because adipose tissue has recently been highlighted as a key organ in which trace elements perform their physiological functions; therefore, it represents the ideal matrix for quantification of the biological availability of these elements (Hubler et al., 2015; Tinkov et al., 2015). Specifically, a reduced adipose tissue content of some TEs (e.g., Cr, V, and Zn) was recently reported to impair intra-adipocyte insulin signaling, leading to adipose tissue insulin resistance (Tinkov et al., 2015), while iron-overload in adipose tissue was found to induce insulin resistance and hypertriglyceridemia (Hubler et al., 2015). New data also indicate that the metabolism of some elements (e.g., iron) may be regulated at adipose tissue level, suggesting that iron-overload should not be measured solely in serum (Hubler et al., 2015). Hence, further research is warranted on adipose tissue concentrations of TEs and on the biological role of their accumulation in this matrix.

This study represents a first step towards evaluation of the metabolic implications of TE accumulation in adipose tissue. The objectives were to determine the concentrations of nine essential and possibly-essential TEs in adipose tissue samples from an adult cohort and to explore their socio-demographic, dietary, and lifestyle determinants using a multivariable approach.

2. Material and methods

2.1. Study area, design, and characteristics of participants

This research is part of a wider investigation designed to study and identify environmental factors affecting the development of chronic disease in an adult cohort from Southern Spain (GraMo cohort). The recruitment of the population has been extensively described elsewhere (Arrebola et al., 2009; Arrebola et al., 2010). In brief, study subjects came from two public hospitals in Granada province: San Cecilio University Hospital in the city of Granada (240,000 inhabitants, urban area) and Santa Ana Hospital in the town of Motril (50,000 inhabitants, semi-rural area). Participants were recruited between July 2003 and June 2004 from patients undergoing non-cancer-related surgery (hernias (41%), gallbladder diseases (21%), varicose veins (12%), and other conditions (26%). Inclusion criteria were: age over 16 years, absence of cancer, non-receipt of hormonal therapy, and residence in one of the study areas for at least 10 years. The exclusion criteria were the following: volunteers who have suffered or suffer malignant tumor pathology in the period of recruitment or those who had any hormonal disease related to hypothalamic axis. All subjects signed their informed consent to participate in the study, which was approved by the ethics committees of both hospitals. Out of the 409 individuals contacted, 387 (95%) agreed to participate and were included in the initial cohort (used for cross-sectional analyses in the present study), obtaining adequate adipose tissue samples from 226 (58%) of these. No statistically significant differences in sex or age distribution were found between participants and non-participants (data not shown in tables). Main characteristics of the participants are summarized in Table 1.

Two large-scale geological units can be differentiated in Granada province: the Betic Cordillera and the Neogene Basin. Betic Cordillera is mainly composed of sedimentary and metamorphic rocks, while Neogen Basin largely comprises Miocene silts and marls, continental and lacustrine deposits of diverse composition, and sediments deposited at the bottom of brackish lakes (Díez et al., 2009). According to Díez (2006), soils from Granada province present an average of 10 mg/kg of As and Co, ∼20 mg/kg of Cu, Ni, and Pb, ∼40 mg/kg of Cr and Zn, and ∼3.1 mg/kg of Mo (Díez et al., 2009).

2.2. Independent variables

Data were gathered on socio-demographic characteristics, lifestyle, diet, and health status in face-to-face interviews conducted by trained personnel at the time of recruitment during the hospital stay. Questionnaires and research procedures were standardized and validated in a pilot study with 50 subjects. The questionnaire was designed and validated in a previous investigation (Buckland et al., 2009; González and Riboli, 2010).

Body mass index (BMI) was expressed as weight/height squared (Kg/m^2). Participants were considered smokers or alcohol consumers

Table 1
Characteristics of the study population.

		n	%
Sociodemographic characteristics	**Sex**		
	Women	99	43,80
	Men	127	56,20
	Residence		
	Urban (Granada)	109	48,20
	Rural/Semi-rural (Motril)	117	51,80
	Education		
	Up to primary schooling	71	31,40
	Secondary/University	155	68,60
Occupation	**Manual worker**	176	77,90
	Non-manual worker	50	22,10
	Occupation during the last 10 years		
	Industry	32	14,20
	Building	36	15,90
	Hand-farming	97	42,90
Lifestyle and diet	**Smoker**	68	30,10
	Alcohol consumption	114	50,40
	Cheese consumer	210	92,90
	Cheese consumption		
	< 2 portions/week	106	47,10
	2–6 portions/week	70	31,10
	> 6 portions/week	49	21,80
	Egg consumer	215	95,10
	Meat consumer	136	60,20
	Meat consumption		
	≤ 1 portions/week	22	9,80
	= 2 portions/week	63	28,10
	> 2 portions/week	139	62,10
	Chiken consumer	175	77,40
	Processed meat consumer	201	88,90
	Vegetables consumption		
	< 1 portions/week	60	26,90
	= 2 portions/week	61	27,40
	> 2 portions/week	102	45,70
	Legumes consumption		
	< 2 portions/week	51	22,80
	≥ 2 portions/week	173	77,20
	Fatty fish consumer	155	68,60
	Organic food consumer	77	34,10

	Mean	SD	Percentiles	
			25th	75th
Age (yrs)	53,60	11,80	47,00	62,00
BMI (kg/m^2)	26,70	4,40	23,42	29,33
Beer consumption (glasses/week)	2,39	4,46	0,00	3,00
How many cups of wine/week	1,98	3,91	0,00	2,00
Spirit consumption (glasses/week)	0,89	2,86	0,00	0,00
Water consumption (glasses/week)	5,83	4,20	3,00	8,00
Milk consumption (glassess/day)	1,56	1,17	1,00	2,00

SD: Standard Deviation.

at any level of daily tobacco (≥1 cig/day) or weekly alcohol (≥1 drink/week) consumption. Residence in the city of Granada at the time of the surgery was considered "urban" and residence in the area of Motril was considered "semi-rural".

The dietary section comprised a food frequency questionnaire that included the following food groups: meat, cold meats, fats, fish, eggs, milk, cheese, vegetables, legumes, fruit, bread, and pasta. The type of milk predominantly drunk (skimmed/semi-skimmed/whole) and the types of fish (white/blue) and meat (white/red) consumed were also recorded.

2.3. Sampling and trace elements analysis

Samples of 5–10 g of adipose tissue were intra-operatively collected and immediately coded and stored at −80 °C until chemical analysis. The adipose tissue samples were freeze-dried in liophilizator (for at least 72 h) until a plateu weight was reached. Samples were kept in deep-freeze (−80 °C) until the analysis. The digestion and multielement analyses of samples were performed in 2015 at the Laboratory for inorganic environmental geochemistry and chemodynamics of nanoparticles, Ruđer Bošković Institute, Zagreb, Croatia. Before the analysis, subsamples (0.1 g) of adipose tissue underwent total digestion in the microwave oven (Multiwave 3000, Anton Paar, Graz, Austria) with a mixture of 7 mL HNO_3 and 0.1 mL HF. Indium (1 μg/L) was added to the digested samples as internal standard. Multi-element analysis of prepared samples was performed by high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) using an Element 2 instrument (Thermo, Bremen, Germany). The instrument conditions and measurement parameters have been reported elsewhere (Fiket et al. 2007, Cukrov et al., 2008). Standards were prepared by appropriate dilution of a multi-element reference solution (Analytika, Prague, Czech Republic) containing Co, Cr, Cu, Fe, Mn, Mo, Se, V, and Zn. Quality control of the analytical procedure was performed by simultaneous analysis of the blank and certified reference materials, Mussel NCS ZC 78,005, also known as GBW 08571, China National Analysis Center for Iron and Steel, Beijing, China and Scallop (*Pecten maximus*) IAEA 452, IAEA, Vienna, Austria).

All samples were analyzed for total concentrations of nine elements (Co, Cr, Cu, Fe, Mn, Mo, Se, V, and Zn). Good agreement between analyzed and certified concentrations, within their analytical uncertainty, was obtained for all elements. For NCS ZC78005 the average recoveries per element were as follows: Co (99%), Cr (95%), Cu (94%), Fe (93%), Mn (95%), Mo (92%), Se (90%) and Zn (92%). For IAEA 452 the average recoveries per element were as follows: Co (92%), Cr (93%), Cu (104%), Fe (94%), Mn (103%), Se (89%), V (98%) and Zn (93%).

2.4. Statistical analysis

TE concentrations were expressed as means, standard deviations, medians, and 25th and 75th percentiles. Because ANOVA assumptions were not always fulfilled, the non-parametric Mann–Whitney *U* test and the Kruskal–Wallis test were used to compare subgroups with the total cohort in bivariate analyses. Spearman correlation tests were employed to evaluate the relationship between pairs of TE concentrations.

First, the shapes of the relationships between continuous predictors and TE concentrations were visually evaluated through locally weighted scatterplot smoothing (LOWESS), a non-parametric local regression method. Next, predictors of TE concentrations were examined in multivariable linear regression models, using a combination of backward and forward stepwise techniques. Interactions were explored by entering the product term of the two variables in the equation. The level of statistical significance was set at $p \leq 0.050$ ($p \leq 0.100$ as borderline significant). TE concentrations were always entered in models as continuous variables and log-transformed in order to minimize the influence of extreme values. In the case of Se (% > LOD = 53.5%), the variable was dichotomized (⟨LOD/⟩LOD) and unconditional logistic regression was performed.

All models were diagnosed to ensure goodness-of-fit and the fulfillment of implementation conditions. Generalized standard-error inflation factors were used to verify the absence of collinearity between independent variables, while the homoscedasticity was tested by plotting residual against fitted values. The linearity of quantitative independent variables was checked with partial regression plots, and the normality of errors was verified using normal QQ plots with 95% confidence intervals.

A double-blinded procedure was implemented so that neither chemical analysts nor statisticians were aware of the identity or characteristics of any participant. The R statistical computing environment v3.0 (http://www.r-project.org/) was used for data analyses.

3. Results and discussion

3.1. Adipose tissue trace element concentrations in the study population

Table 2 shows the adipose tissue concentrations of the TEs under study. All were detected in 100% of analyzed samples with the exception of Se, which was found in 53.5%. This is the first study to report adipose tissue concentrations of these TEs, hampering comparisons with other studies. This is important, because the relationship between plasma concentrations of TEs and their accumulated concentrations in body tissue is not well established. In fact, marked differences in TE concentrations have been found between body tissues (adipose tissue, liver, and kidney) and plasma or serum (Bogden and Klevay, 2000; Tinkov et al., 2017). Strikingly, 20% of absorbed Co was found at one month, and 10% still remained at one year post-absorption and might even be permanently retained (Simonsen et al., 2012). This finding supports our hypothesis of a major accumulation of TEs in adipose tissue, where transient fluctuations would be lower in comparison to body fluids (e.g., plasma or urine), suggesting that adipose tissue concentrations would be more representative of long-term exposure. It is noteworthy that TEs tend to bind covalently to organic groups after their absorption, thereby forming lipophilic compounds and ions that are readily absorbed by fatty tissues (Wiernsperger and Rapin, 2010).

Table 2
Distribution of adipose tissue TE concentrations (mg/kg).

	% > LOD	Mean ± SD	Percentiles		
			25th	50th	75th
Cr	100	0.59 ± 0.81	0,20	0,38	0,62
V	100	0.016 ± 0.011	0,01	0,01	0,62
Fe	100	59.4 ± 56.7	28,40	42,60	70,87
Zn	100	11.98 ± 9.83	6,97	9,80	13,78
Mo	100	0.017 ± 0.018	0,01	0,01	0,02
Se	53,50	0.073 ± 0.058	0,04	0,06	0,09
Co	100	0.011 ± 0.02	0,00	0,01	0,01
Mn	100	0.228 ± 0.178	0,13	0,17	0,28
Cu	100	1.031 ± 1.184	0,47	0,68	1,21

LOD: Limit of Detection; SD: Standard Deviation.

Among the elements quantified in the present study, the highest concentrations were observed for Fe, Zn, and Cu, with median concentrations of 42.6 mg/kg, 9.8 mg/kg, and 0.68 mg/kg, respectively. These TEs were found in similar proportions by Rahil-Khazen et al. (2002) in other biological matrices (liver, spleen, pancreas, heart, kidney, brain, and cerebellum) from 30 adult autopsies (Rahil-Khazen et al., 2002).

Fig. 1 depicts the correlation coefficients between pairs of TEs in adipose tissue. All pairs showed positive correlations, which were statistically significant in most cases. The strongest associations were found between Zn and Fe (0.61, $p < 0.001$), V and Mn (0.73, $p < 0.001$), and Cr and Mo (0.70, $p < 0.001$). Non-significant correlations were observed between Cu and Mn ($p = 0.187$), Cu and Mo ($p = 0.098$), Cu and Cr ($p = 0.253$), and Zn and C ($p = 0.054$). The

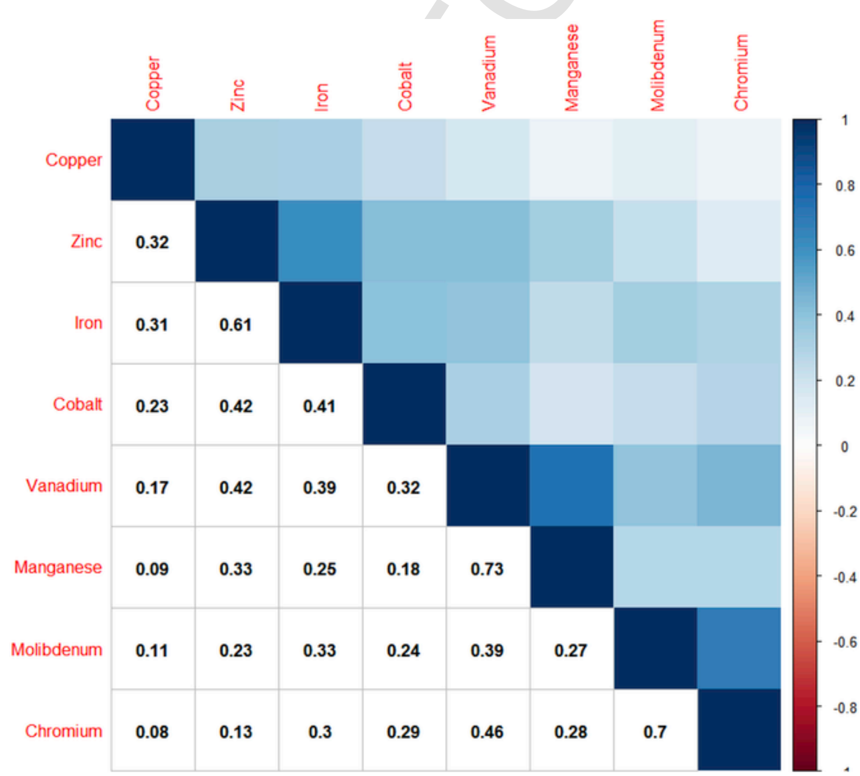

Fig. 1. Spearman correlations between pairs of adipose tissue TE concentrations.

positive correlations observed may be related to common factors affecting TE homeostasis (e.g., total diet composition or health and nutritional status) (Fairweather-Tait, 1992), especially the diet, which is acknowledged to be the main source of these elements (Prashanth et al., 2015). On the other hand, the concentrations of certain TEs are strongly interdependent, i.e., high intakes of some TEs interfere with the utilization and tissue storage of others, while low concentrations of dietary TEs can enhance the absorption of others (Tapiero et al., 2003). One of the most frequently documented antagonisms is between Cu and Zn, due to their competition for certain binding sites (Rahil-Khazen et al., 2002). Nevertheless, August et al. (1989) suggested that modestly increased intakes of Cu do not interfere with Zn absorption when Zn intake is satisfactory (August et al., 1989), which might support our findings of positive correlations between these TEs. Contrary to our results, Rahil-Khazen et al. (2002) found a negative correlation between Fe and Zn concentrations in spleen, although this association was not observed in liver, pancreas, heart, kidney, brain, or cerebellum (Rahil-Khazen et al., 2002). In this context, some authors reported competition between Zn and Fe for the divalent metal transporter-1 in the enterocytes of the small intestine (Kordas and Stoltzfus, 2004), although the present results suggest that this might not influence their proportional accumulation in adipose tissue.

3.2. Predictors of adipose tissue trace element concentrations

Tables 3 and 4 displays the results of multivariable analyses to identify predictors of TE concentrations. In the present population, a borderline-significant positive correlation was found between age and Cu concentrations ($\beta = 0.004$, $p = 0.090$), consistent with the report by Kouremenou-Dona et al. (2006) of an increase in serum Cu concentrations with higher age in 506 healthy Greek adults (Kouremenou-Dona et al., 2006). In a recent study of adult rats, Fu et al. (2015) observed a rise in Cu concentrations in the subventricular zone along the wall of brain ventricles with age, probably caused by the age-dependent expression of different markers, e.g., Cu storage protein Mt1a (Fu et al., 2015).

Residency in the semi-rural area was associated with increases of 22–72% in adipose tissue concentrations of Co, Cr, Fe, Mn, Mo, V, and Zn. Higher serum TE concentrations were previously observed between dwellers in rural and urban or industrial areas (Mohmand et al., 2015). The greater use of fertilizers, organic manures and irrigation in the countryside might contribute to a higher accumulation of TE in the agroecosystem (He et al., 2005). In another study, increased Mn concentrations was found in air from rural *versus* urban areas (Howe et al., 2004), which may explain the positive association between Mn accumulation and rural or semi-rural residence in the present study. In fact, it has also been reported that land in rural and semi-rural areas in the Southern region of Granada province is richer in Co, Cr, Cu, and Zn in comparison to land in Granada city (Díez et al., 2009) (Supplementary Material, Fig. S1).

No gender differences in TE concentrations were observed in the present study population. Skalnaya et al. (2016) observed sex-related differences in TE circulating concentrations among individuals aged from 10 to 59 years, but the statistical significance of this difference was lost among individuals older than 40 years (Skalnaya et al., 2016). The mean age of the present participants was 53 years, and no significant gender differences were observed in any sub-group after stratification by age (data not shown in tables).

Adipose tissue concentrations of Co, Cr, Fe, Se, and V were increased in participants with higher *versus* lower educational level, widely considered as an indicator of socioeconomic status (McLaren, 2007). Because individuals with high socioeconomic status are expected to have easier access to sources of nutritious food, their dietary patterns are likely to be more diverse and healthy (Dubois and Girard, 2001). In a study of 1134 adults in the UK, lower plasma Se concentrations were observed in those receiving state benefits or having a lower educational level (Bates et al., 2002). Another factor that might enhance TEs adipose tissue storage is the consumption of vitamin/mineral supplements, which has increased over recent years and tends to be more common among individuals with higher socioeconomic status (Darmon and Drewnowski, 2008).

Adipose tissue Cu concentrations were 22% higher in participants employed in agriculture *versus* other areas of work, likely attributable to the use of organic cupric salts, which can be assimilated by the human body through the epidermis (Georgopoulos et al., 2001). Adipose tissue Zn concentrations were lower for manual workers, although the association was only borderline-significant ($\beta = -0.158$, $p = 0.082$), while significantly increased concentrations of Zn were found in participants engaged in manual farming ($\beta = 0.161$, $p = 0.050$).

Adipose tissue Se concentrations were higher in manual *versus* non-manual workers ($\beta = 1.002$, $p = 0.010$). Interestingly, participants who were working or had worked in industrial activities showed increased adipose tissue concentrations of V, Co, and Fe, although only borderline significance was obtained for Fe ($p = 0.085$). Workers in mining, foundries, smelters, and other metal-based industrial operations are known to have an increased risk of exposure to certain metals (Annangi et al., 2016). V is frequently used in the manufacture of fertilizers as well as in the steel and iron–steel industry (e.g. manufacture of automobiles and ships) and is highly persistent in the environment and in living organisms (Imtiaz et al., 2015). Li et al. (2004) found Fe concentrations to be two-fold higher in 500 welders than in controls (Li et al., 2004). Co is mainly used in alloys with Fe, Ni, and other metals to produce both corrosion-resistant products and abrasion-resistant steels. As previously reported (Albanese et al., 2015; Díez et al., 2009), Co is one of the main mineralization deposits in the soils of Southern Spain (Fig. S1, Supplementary material). Princivalle et al. (2017) recently reported increased urinary and plasma Co concentrations in 34 adults during their employment in a hard metal plant where metallic Co and Co oxide were frequently manufactured (Princivalle et al., 2017). In contrast to the present results, Dawson et al. (2000) found lower Co concentrations in sperm from 50 men who worked in a refinery or smelters in comparison to controls (Dawson et al., 2000). Some of the associations observed between TE concentrations and occupational class or educational level might also be explained, at least in part, by the dietary patterns of specific population subgroups.

Diet has been highlighted as the most important TE exposure route in the general population (Caroli, 2007), and the presence of TEs in foods is often influenced by the availability of metals in the soil in which they were grown (Kabata-Pendias, 2004). Thus, vegetable intake was positively associated with adipose tissue Co, Cr and Se concentrations in the present study. Vegetables, especially crucifers (e.g. broccoli or cauliflower) and alliums, are considered a good source of Se (Keck and Finley, 2004), and their consumption is reflected in the Se content of human tissue and body fluids (Navarro-Alarcon and Cabrera-Vique, 2008). Vegetables in general are a source of Cr and, to a lesser extent, Co (Kumar and Soni, 2007; Lendinez et al., 2001).

Cheese consumption was negatively associated with adipose tissue Cr, Mo, and Se concentrations. An *in vivo* study found that a high-fat diet diminishes the accumulation of Cr in adipose tissue among other insulin-sensitive tissues (Tinkov et al., 2014), although

Table 3
Determinants of adipose tissue TE concentrations (mg/kg). Multivariable linear regression analyses.

		Cr					V					Fe					Zn					Mo
		beta	exp(beta)	95%CI		p	beta	exp(beta)	95%CI		p	beta	exp(beta)	95%CI		p	beta	exp(beta)	95%CI		p	beta
				Lower	Upper				Lower	Upper				Lower	Upper				Lower	Upper		
Sociodemographic characteristics	**(Intercept)**	0.479	1.614	-3.836	-2.714	0.668	-4.661	0.009	-5.189	-4.132	<0.001	4.106	60.709	3.051	5.266	<0.001	2.447	11.549	2.043	3.100	<0.001	-3.33
	Age (years)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Sex = **man**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	BMI (Kg/m^2)	-0.593	0.553	-1.243	0.053	0.072*	–	–	–	–	–	-0.018	0.982	-0.037	-0.001	0.048*	-0.010	0.990	-0.024	0.003	0.090*	-0.02
	Residence = rural/ semi-rural[a]	0.416	1.516	0.080	0.753	0.016*	0.329	1.390	0.119	0.540	0.002*	0.263	1.301	-0.129	0.592	0.048*	0.223	1.250	0.034	0.450	0.031*	0.19
Occupation	**Ocuppation in industry over the last 10 years** = yes	–	–	–	–	–	0.262	1.300	-0.470	-0.054	0.010*	–	–	–	–	–	–	–	–	–	–	–
	Ocuppation in construction over the last 10 years = yes	–	–	–	–	–	–	–	–	–	–	0.251	1.286	-0.038	0.535	0.090*	–	–	–	–	–	–
	Manual worker[b]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	-0.158	0.854	-0.291	0.060	0.082*	–
	Current work = Hand-farming	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.161	1.174	0.054	0.365	0.05*	–
	Educaton[c] ≥ Primary	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Education[c]																					
	Primary	0.125	1.133	-0.172	0.423	0.314	0.110	1.116	-0.071	0.290	0.328	0.014	1.014	-0.023	0.255	0.995	–	–	–	–	–	–
	≥ Secondary	0.361	1.435	-0.031	0.754	0.071*	0.276	1.318	0.041	0.511	0.022*	0.368	1.444	0.022	0.658	0.023*	–	–	–	–	–	–
Lifestyle and diet	**Cheese consumer** = yes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	-0.37
	Cheese consumption[d]																					
	2-6 portions/week	-0.164	0.849	-0.431	0.103	0.228	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	> 6 portions/week	-0.298	0.742	-0.601	0.005	0.054*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Cheese consumption[d] ≥ 2 portions/week	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Egg consumer = yes	–	–	–	–	–	–	–	–	–	–	-0.556	0.573	-1.002	-0.036	0.023*	–	–	–	–	–	–
	Meat consumer = yes	–	–	–	–	–	–	–	–	–	–	-0.194	0.824	-0.402	0.047	0.084*	–	–	–	–	–	–
	Meat consumption[e] > 1 portion/week	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Chiken consumer = yes	–	–	–	–	–	–	–	–	–	–	-0.213	0.808	-0.463	0.042	0.097*	–	–	–	–	–	0.15
	Processed meat consumer = yes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Vegetables consumption[d] ≥ 2 portions/week	0.292	1.339	0.029	0.555	0.030*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Legumes consumption[d] ≥ 2 portions/week	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	-0.18
	Blue fish consumer = yes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Organic foods consumer =	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.161	1.174	-0.268	0.037	0.049*	–
	Alcohol consumer = yes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Current																					

Table 4
Determinants of adipose tissue TE concentrations (mg/kg). Multivariable linear regression analyses.

		Se					Co					Mn					Cu				
		OR	exp(beta)	95%CI		p	beta	exp(beta)	95%CI		p	beta	exp(beta)	95%CI		p	beta	exp(beta)	95%CI		p
				Lower	Upper				Lower	Upper				Lower	Upper				Lower	Upper	
Sociodemographic characteristics	**(Intercept)**	1.070	2.916	0.011	106.629	0.977	-6.001	0.002	-7.577	-4.243	<0.001	-1.651	0.192	-2.505	-0.699	<0.001	1.019	2.770	-0.567	2.604	0.207
	Age (years)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.004	1.004	-0.001	0.009	0.089*
	Sex = man	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	BMI (Kg/m2)	0.926	2.524	0.864	0.986	0.022*	-0.035	0.965	-0.059	-0.014	0.002*	–	–	–	–	–	-0.480	0.619	-0.959	-0.002	0.049*
	Residence = rural/ semi-rurala	0.122	1.129	0.041	0.335	0.000*	0.544	1.723	0.075	0.974	0.001*	0.260	1.296	0.009	0.563	0.011*	-0.371	0.690	-0.543	-0.200	<0.001*
Occupation	**Ocuppation in industry over the last 10 years** = yes	–	–	–	–	–	0.465	1.592	-0.853	-0.157	0.008*	–	–	–	–	–	0.197	1.217	0.022	0.371	0.027*
	Ocuppation in construction over the last 10 years = yes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Manual workerb	2.724	15.241	1.278	6.000	0.011*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Current work = Hand-farming	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Educatonc ≥ Primary	2.372	–	1.091	5.292	0.031*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Educationc																				
	Primary	–	–	–	–	–	0.010	1.010	-0.285	0.306	0.874	–	–	–	–	–	–	–	–	–	–
	≥ Secondary	–	–	–	–	–	0.355	1.426	-0.057	0.717	0.070*	–	–	–	–	–	–	–	–	–	–
Lifestyle and diet	**Cheese consumer** = yes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Cheese consumptiond																				
	2-6 portions/week	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	> 6 portions/week	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Cheese consumptiond ≥ 2 portions/week	0.461	1.586	0.239	0.872	0.019*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Egg consumer = yes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Meat consumer = yes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Meat consumptione > 1 portion/week	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.271	1.311	-0.018	0.560	0.066*
	Chiken consumer = yes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Processed meat consumer = yes	3.880	–	1.287	13.059	0.020*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Vegetables consumptiond ≥ 2 portions/week	1.962	–	0.922	4.252	0.083*	0.326	1.386	-0.018	0.770	0.09*	–	–	–	–	–	–	–	–	–	–
	Legumes consumptiond ≥ 2 portions/week	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Blue fish consumer = yes	0.461	–	0.239	0.872	0.042*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Organic foods consumer = yes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Alcohol consumer = yes	0.456	–	0.213	0.946	0.038*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Current smoker = yes	–	–	–	–	–	–	–	–	–	–	-0.162	0.851	-0.343	0.009	0.071*	–	–	–	–	–

the mechanism underlying this effect has not been fully elucidated (Tinkov et al., 2015). In the same line, a negative correlation was reported between plasma concentrations of Se-containing enzymes (e.g. glutathione peroxidase) in chickens on a high-fat diet, suggesting that dietary fat could reduce the intestinal transport of Se (Mutanen and Mykkanen, 1984). In addition, fatty fish has been described as an important source of highly bioavailable Se (Fox et al., 2004).

It is well documented that animal tissues can induce iron absorption (Hurrell and Egli, 2010), but meat and chicken intake showed a borderline-negative association with adipose tissue Fe concentrations in the present study. Similar findings were reported by Hunt et al. (1995), who observed decreased levels of ferritin, iron-capacity, and transferrin saturation in individuals following a high animal-protein diet, suggesting a reduced iron status (Hunt et al., 1995). On the other hand, chicken and meat intakes showed positive associations with adipose tissue concentrations of Mo ($\beta = 0.155$, $p = 0.070$) and Cu ($\beta = 0.271$, $p = 0.066$), respectively. Meat is considered a relatively good source of Mo (Lonnerdal, 1996), whose concentrations are two-fold higher in chicken than in mammalian meats (Gerber et al., 2009).

A negative correlation was also observed between egg consumption and adipose tissue Fe concentrations, consistent with the reported inhibition of iron absorption by egg proteins (Cook and Monsen, 1976), which has been related to the formation of an insoluble phosvitin-iron complex (Ishikawa et al., 2007).

In the present study, water consumption was not associated with adipose tissue TE concentrations in either the urban or semi-rural setting, consistent with previous reports that human agricultural activities do not significantly affect concentrations of Zn or Se in drinking water from our study area (Díaz et al., 1996; Terrés-Martos et al., 2002).

Although legumes are considered a relevant source of Mo (Novotny, 2011), their consumption was negatively associated with Mo concentrations in the present study, possibly because a higher intake of Mo may increase its transfer from plasma to urine, thereby reducing its accumulation in tissues, as suggested by Novotny (2011) (Novotny, 2011). Unfortunately, little information is available on the influence of other trace elements or dietary components, such as proteins, on Mo absorption (Burguera and Burguera, 2007).

Lower adipose tissue Se concentrations were found in alcohol consumers than in non-consumers ($\beta = -0.786$, $p = 0.038$), in agreement with the findings of (Bergheim et al., 2003). The biological mechanism underlying this phenomenon has not been fully characterized, but it may be related to unbalanced dietary habits of alcohol consumers, their reduced hepatic storage capacity, alcohol-induced alterations in Se metabolism, or abnormal Se excretion in their urine or feces (Luty-Frąckiewicz et al., 2002).

A significant inverse association was observed between the consumption of organic food and adipose tissue concentrations of Zn. This contrasts with the recent study by Mark et al., who found that an organic diet had no effect on Zn intake and absorption (determined by fecal excretion of stable enriched isotopes) in 17 healthy men consuming organic and conventional diets in a double-blinded, cross-over, intervention trial (Mark et al., 2013). However, circulating Zn levels only serve as an estimation of dietary uptake and cannot be comparable with tissue accumulation (King et al., 2000).

The degree of obesity (measured as BMI) showed a negative correlation with adipose tissue concentrations of Co ($\beta = -0.035$, $p = 0.002$), Cr ($\beta = -0.595$, $p = 0.072$), Cu ($\beta = -0.480$, $p = 0.049$), Fe ($\beta = -0.018$, $p = 0.075$), Mo ($\beta = -0.302$, p= < 0.001), Se ($\beta = -0.077$, $p = 0.021$), and Zn ($\beta = -0.081$, $p = 0.090$), although some of these associations were only borderline significant. Sánchez et al. (2010) also reported a negative association of serum Fe and Cu with adiposity in Andalusian adults aged between 25 and 60 years (Sánchez et al., 2010). Increased obesity has frequently been associated with high-fat diets and, therefore, is considered a proxy of diet quality (Kopelman, 2000). In this regard, several *in vivo* studies have observed decreased Zn adipose tissue concentrations in high-fat fed mice (Tallman and Taylor, 2003). This may be caused by an obesity-related disruption of Zn-transporting proteins in obese individuals, as shown by Smidt et al. (2007), who found differences in Zn expression between 12 obese and 12 non-obese participants and suggested that Zn metabolism in adipocytes is actively controlled by Zn-transporters (Smidt et al., 2007). It was recently hypothesized that hypercaloric diets reduce the Cr content of adipocytes (Tinkov et al., 2015), but the mechanisms responsible for this action remain under investigation; however, recent clinical trials showed that Cr supplementation significantly reduces body weight (Onakpoya et al., 2013). Furthermore, a cross-sectional representative survey of the US population ($n = 15,945$) showed a significant negative correlation between BMI and Se serum concentrations (Kimmons et al., 2006). Little is known about the potential association of internal concentrations of Mo or Co with BMI. Yoshida et al. (2006) found no relationship between Mo serum content and BMI in 70 healthy Japanese adults (Yoshida et al., 2006). The association between BMI and plasma Co concentrations was positive in obese Polish boys but negative in the control group of non-obese girls (Błażewicz et al., 2013). In agreement with the present findings, Padilla et al. (2010) reported a significant negative association between blood Co concentrations and BMI (Padilla et al., 2010).

Finally, a negative relationship was found between smoking habit and Mn concentrations in the present population, in agreement with previous findings by Takser et al. (2004) in pregnant women (Takser et al., 2004). However, no significant association was observed between manganese in whole blood concentrations and smoking habit in a cross-sectional study of 276 individuals in the USA (Kim et al., 2015), and Díaz et al. found no correlation between serum Mn concentrations and smoking habit in 368 Spanish individuals (Díaz et al., 2001). On the other hand, a higher expression of Mn-superoxide dismutase (Mn-SOD) was reported in the central bronchial and alveolar epithelia of smokers in comparison to non-smokers (Harju et al., 2004), which may be explained by the increased oxidative status of the former. Thus, tissue accumulation of Mn might be inhibited by higher Mn-SOD activity resulting from a smoking-induced increase in the generation of reactive oxygen species.

Our hospital-based population might not be entirely representative of the general population of the area, and further research in different geographic areas and population groups is required to verify these findings. Considering that this is a cross-sectional exploratory study with an alpha error set at 5%, there is always a relatively small chance of false positive associations. However, the majority of them are biologically plausible and therefore worthy of further investigation. To our knowledge, this is the first published report on the distribution of these TEs in adipose tissue and its determinants in a human cohort. These results shed light on the potential clinical relevance of adipose tissue TE concentrations and may have important public health implications, given increasing scientific evidence on the role of TEs in insulin resistance and other metabolic disruptions in obesity (Tinkov et al., 2015).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.09.093.

Conflict of interest

The authors declare no conflict of interest.

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