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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<sup>☆</sup>

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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 ( $\beta$  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 ( $\beta = 0.004$ ,  $p = 0.089$ ). Residence in a rural or semi-rural area was associated with increased Co, Cr, Fe, Mo, Mn, V and Zn concentrations and with  $\beta$  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 ( $\beta$  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.

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## 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  $\leq 100$  mg/day) to ensure decisive functions to maintain human health (Fraga, 2005). They are usually present at very low

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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 homeostasis 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 Neogene 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,

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