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Toenail zinc as a biomarker: Relationship with sources of environmental exposure and with genetic variability in MCC-Spain study

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ABSTRACT

Background: Toenails are commonly used as biomarkers of exposure to zinc (Zn), but there is scarce information about their relationship with sources of exposure to Zn.
Objectives: To investigate the main determinants of toenail Zn, including selected sources of environmental exposure to Zn and individual genetic variability in Zn metabolism.
Methods: We determined toenail Zn by inductively coupled plasma mass spectrometry in 3,448 general population controls from the MultiCase-Control study MCC-Spain. We assessed dietary and supplement Zn intake using food frequency questionnaires, residential proximity to Zn-emitting industries and residential topsoil Zn levels through interpolation methods. We constructed a polygenic score of genetic variability based on 81 single nucleotide polymorphisms in genes involved in Zn metabolism. Geometric mean ratios of toenail Zn across categories of each determinant were estimated from multivariate linear regression models on log-transformed toenail Zn.
Results: Geometric mean toenail Zn was 104.1 μg/g in men and 100.3 μg/g in women. Geometric mean toenail Zn levels were 7 % lower (95 % confidence interval 1–13 %) in men older than 69 years and those in the upper tertile of fibre intake, and 9 % higher (3–16 %) in smoking men. Women residing within 3 km from Zn-emitting industries had 4 % higher geometric mean toenail Zn levels (0–9 %). Dietary Zn intake and polygenic score were unrelated to toenail Zn. Overall, the available determinants only explained 9.3 % of toenail Zn variability in men and 4.8 % in women.
Discussion: Sociodemographic factors, lifestyle, diet, and environmental exposure explained little of the individual variability of toenail Zn in the study population. The available genetic variants related to Zn metabolism were not associated with toenail Zn.

1. Introduction

Zinc (Zn) is an essential element for the human body, which plays an important role in biological processes as a structural, catalytic, and intracellular and intercellular signalling component (World Health Organization, 1996; Kambe et al., 2015). As Zn homeostasis is tightly controlled, in order to maintain metabolic functions over a wide range of Zn intakes, it is difficult to assess deficiency or excess of this element, which can be associated with health effects (King et al., 2016; Plum et al., 2010) Zn deficiency clinically affects central nervous, epidermal, gastrointestinal, immune, reproductive and skeletal systems (Roohani et al., 2013). On the other hand, toxicity symptoms (nausea, vomiting, epigastric pain, lethargy, and fatigue) can also appear in case of very high exposures to Zn (Agnew and Slesinger, 2022). In addition, occupational exposure to zinc compounds or fumes is known to be associated to specific short-term health effects, while their long-term consequences are not still well known (Zinc, 2022; Chuang et al., 2014).

Diet and dietary supplements are the main sources of Zn for humans (approximately 90–95 %) (Simon-Hettich et al., 2001) Zn content of foods differs widely; oysters and meat are some of the products with higher amounts of Zn, although whole grain cereals, legumes and nuts can be important sources for people under vegetarian diets (Sandstead, 2015). There are other possible sources of exposure, either by ingestion (i.e. drinking water) or combined with inhalation (Zn in air due to industrial emission, dust or occupational exposure to Zn fumes), although their contribution to Zn body burden, at least in the general population, remains uncertain (Simon-Hettich et al., 2001; Sandstead, 2015).

As the most common reason to try to evaluate Zn status is to address Zn deficiency, researchers usually rely on estimations of dietary Zn intake (King et al., 2016). However, assessment of exposure to Zn from an environmental research point of view needs to consider the integrated exposure from all the different sources, an approach that can be achieved using biomarkers. In this sense, Zn exposure researchers have used different biological matrices, like whole blood, plasma –the most commonly used (King et al., 2016)-, serum, urine, scalp hair or nails, which reflect different time-windows of exposure (Simon-Hettich et al., 2001; Who, 1996). Usually, serum, plasma and urine are more sensitive to short-term changes, while toenail clippings are generally considered to give an estimation of longer-term exposure (6–9 months), which would make them a suitable matrix in the study of chronic diseases (King et al., 2016; Gutiérrez-González et al., 2019). Nails have additional logistic advantages: they are easy to collect and store, and toenails have the advantage over fingernails that are less exposed to external contamination (Esteban and Castaño, 2009). Besides, several circumstances and conditions like fever or infections may alter Zn concentrations on some biological matrices like plasma, thus affecting the stability of Zn levels (King et al., 2016; Who, 1996), while elements once deposited in toenails remain unchanged (Hopps, 1977; Sukumar et al., 2006). However, nowadays, the information on the real value of Zn in toenails as biomarker of exposure is still scarce and unclear (Gutiérrez-González et al., 2019; Jaramillo Ortiz et al., 2022); additional data are needed in regard to its relationship with possible sources of exposure to Zn and the factors that may modulate Zn toenail levels.

Previous research has described differences in Zn concentrations or in Zn metabolism by basic epidemiological variables, such as age or gender in other matrices such as urine and serum (Berglund et al., 2011; Tubek, 2006), but data for toenail Zn are unclear (Gutiérrez-González et al., 2019). On the other hand, differences in processes involved in Zn homeostasis regulation can affect toenail Zn concentrations, in which metallothioneins (MTs) and two Zn transporters families (ZIP [SLC39A]) and ZnT [SLC30A]) have a crucial role (Kambe et al., 2015). MTs are metal-binding proteins that, under physiological conditions, bind Zn, although they also have high affinity for toxic metals. ZnT transporters have a role as cation diffusion proteins, while ZIP transporters mobilize Zn to the cytosol from intracellular organelles or the extracellular space (Kimura and Kambe, 2016). To the best of our knowledge, whether genetic differences (i.e., single nucleotide polymorphisms (SNPs)) in genes that codify for these proteins may play a role on toenail Zn concentrations has not been studied.

Our aim in this study is to investigate which factors determine toenail Zn concentrations in male and female controls from general population in Spain by exploring their association with sociodemographic, anthropometric, lifestyle factors and with Zn exposure from different sources (i.e., diet, supplements, tobacco, soil, industrial emissions) and evaluating its possible relationship with individual genetic variability in Zn metabolism and transportation, as estimated through a specifically constructed polygenic score (PS_{Zn}).

2. Methods

2.1. Study population and design

MCC-Spain (https://www.mccspain.org) is a population-based multicase-control study designed to explore environmental and genetic factors associated with common cancers or tumours with peculiar epidemiological features in Spain (Castaño-Vinyals et al., 2015). We recruited participants living in 12 different provinces of Spain (Supplementary Fig. 1) from 2008 to 2013. Inclusion criteria for participants were to be 20 to 85 years old, be able to answer the questionnaire and reside for at least 6 months in the study areas. Cases had histological confirmed incident tumours (breast, colorectal, prostate, stomach and chronic lymphocytic leukaemia). Controls were randomly selected from Primary Health centres belonging to the catchment's areas of those hospitals where cancers cases were recruited, and were frequency-matched to cases by sex, age (five-year intervals) and study area (province). We invited controls to participate in the study by telephone on behalf of their General Practitioner, obtaining a mean participation rate of 53 %. The Ethics Committee of all participating centres approved the study protocol, and all participants provided an informed consent before their enrolment. For this study, which aims to describe toenail Zn in the general population, we only included controls with available Zn toenail concentrations ($n = 3,448$). Among them, 2,351 participants had also available genetic data (Supplementary Fig. 2). We have summarized baseline characteristics of the total sample and the sample with genetic data ("genetic sample") in Supplementary Table 1.

2.2. Data collection

We collected information on sociodemographic characteristics, anthropometric measures (one year before recruitment), physical activity over the previous 5 years, occupational and medical history, drug intake or smoking status (one year before recruitment) through a structured questionnaire in a face-to-face interview. Data on diet and alcohol consumption habits during the previous year, that is the approximate time-window of exposure reflected by toenail (Gutiérrez-González et al., 2019), were gathered using a validated semiquantitative food frequency questionnaire (FFQ) (Martin-moreno et al., 1993). This FFQ collected information on >140 food items, which was used to estimate the daily intake of different elements, including Zn, by applying the Spanish CESNID food composition tables (Farrán et al., 2003). We also asked participants if they had regularly used vitamins or dietary supplements in the preceding year, as well as the brand, to identify those including Zn. We also collected information on current address of residence, which was geocoded into Universal Transverse Mercator (UTM) ED50 zone 30 N coordinates using Google Earth Pro and double-checked with the National Cadastre and the "street-view" application of Google Earth Pro.

2.3. Toenail sampling, laboratory analyses and calibration

Toenail clippings from all toes of both feet were collected with stainless steel nail clippers, either at recruitment by research personnel or by the participant within the following two weeks, and were stored in paper envelopes at room temperature until sent to the laboratory. Samples were cleaned twice by washing samples for 5 min in Triton-X 100 5 % (w/v) aqueous solution, Mili-Q water and acetone using an ultrasonic bath. Subsequently, toenail samples were digested using a 4:1 (v/v) solution of nitric acid and hydrogen peroxide into a microwave digestion system and then made up to 5 ml using MiliQ water.

We determined toenail Zn, along with other 17 metals, by inductively coupled plasma mass spectrometry (ICP-MS) (XSeries 2, Thermo Scientific) at the Environmental Bioanalytical Chemistry Unit of the University of Huelva (Spain). We adjusted the concentration measured by the equipment taking into account the dilution factor and sample weight, according to the following formula: [Real](ng/g) = [Equipment] (ng/g) (dilution factor (g))/(sample weight(g)). The limit of detection for Zn was 0.27 ng g^{-1}, obtained from the calibration curve (Harris, 2020).

Quality control of the analyses included: a) analysis of hair reference material NSC DC73347a (LGC Standards), in each sample batch with a medium accuracy of 90 %, which value was maintained along the time ± 5 %; b) monitoring of the ICP-MS response along the time by measurement of control concentrations of the different elements at a point on the calibration curve (5 ng ml^{-1}), every 20 analysed samples; c) instrumental drift correction by addition of rhodium (Rh) (100 ng ml^{-1}), as internal standard, to all the samples and calibrants used, the samples whose response differs ± 10 % with respect to the internal standard were measured again; d) analysis every 5 samples of reagents blanks containing 5 % HNO_3 (Suprapur quality) and 100 ng ml^{-1} of Rh; e) analysis of duplicate samples every 2.5 h of the sequence; f) spiked sample analysis, spiking the reference materials with the analytes under study (50 ng ml^{-1}).

We also performed, reproducibility analyses of toenail samples from non-eligible participants from the MCC-Spain study in two different laboratories (Environmental Bioanalytical Chemistry Unit of the University of Huelva and Mass Spectrometry Unit of the University of Oviedo, Spain), obtaining an intraclass correlation coefficient for Zn of 0.983 (95 % CI: 0.973–0.989) (Cervantes et al., 2015).

Our study included many small toenail samples (median toenail mass: 20.6 mg), which may suppose a challenge for ICP-MS as their signal can be out of the optimal measurement range of the calibration line (Harris, 2020; Skoog et al., 2017). In preliminary analyses, we observed a systematic bias associated with toenail sample weight, as Zn geometric mean (GM) concentrations were higher in toenail samples with very small mass. A similar bias has been previously described in a few studies, while in others the sample weight was taken into account when determining the levels of metals (Gutiérrez-González et al., 2019). Also, measured metal concentrations varied across laboratory batches. Therefore, we calibrated toenail Zn concentrations for sample mass heterogeneity and between-batch variability using a heteroscedastic spline mixed model (Pinheiro and Bates, 2000), with fixed effects for the average bias in log-transformed Zn concentrations as a spline function of log-transformed toenail sample mass, random effects for between-batch variation in this mass-related bias, and heterogeneous within-batch error variance in log-transformed Zn concentrations as a spline function of log-transformed mass. From this model, we derived the calibrated Zn concentrations that would have been observed had all toenail specimens been analysed in the same average batch and sample masses been set to the GM for all participants, conditional on sex, five-year age group, and province.

2.4. Zinc in topsoil

We obtained the estimation of Zn concentration in topsoil (upper soil horizon) from the Geochemical Atlas of Spain, which includes 13,317 soil sample points from mainland Spain. More information about the sample-collection procedures and the chemical-analysis techniques used have been previously published (Locutura-Rupérez, 2012). In brief, soil samples were analysed by ICP-MS after crushing, pulverizing and partial digestion. Topsoil was chosen for this study since this determination is closer to the bioavailable metal/metalloid content of soil and tends to display the highest association with pollution. For the analysis, each participant's geocoded address of residence was assigned to estimated levels for Zn using an interpolation method from soil sample points (Núñez et al., 2017).

2.5. Proximity to zinc-emitting industrial facilities

We identified industries in Spain releasing Zn to air included in the Spanish Pollution Release and Transfer Register (PRTR-Spain) (PRTR España, 2022) corresponding to 2009, from the Spanish Ministry for the Ecological Transition. The geographic coordinates of these industrial facilities, geocoded into UTM ED50 zone 30 N, have been previously validated (García-Pérez et al., 2019). We classified participants as exposed to industrial Zn if there were one or more Zn-emitting industries within a 3-km radius from their place of residence, and as unexposed otherwise.

2.6. Genetic variability in Zn metabolism and transportation: PS_{Zn}

Peripheral blood was collected from participants and its cellular fraction was separated for DNA extraction and stored at −80 °C. We used Infinium Human Exome BeadChip (Illumina, San Diego, USA) to genotype >200,000 coding markers, as well as 6000 additional custom SNPs on several pathways of interest. We first identified 40 different genes involved in Zn metabolism and transportation through a literature search (Supplementary Table 2), and then selected the 510 SNPS in the genotyping array that were at these genes.

Following standard quality control procedures, we excluded SNPs that were monomorphic or with minor allele frequencies below 5 % as well as those with unknown genotypes in study participants, leaving a total of 81 SNPs from 31 genes in the present analysis (Supplementary Tables 2 and 3).

We constructed a polygenic score for toenail Zn (PS_{Zn}) to combine the effect of the 81 available SNPs linked to Zn metabolism in 2,351 of the 3,448 study participants (68.2 %) with known genotypes for all SNPs (genetic sample). We first fitted separate logistic regression models relating the number of minor alleles for each SNP (continuously coded as 0 for major-allele homozygous, 1 for heterozygous, and 2 for minor-allele homozygous genotypes) with the log-transformed toenail Zn concentration, adjusting for other SNPs at the same gene, sex, age groups, and province indicators. For logistic regression models, we categorized toenail Zn as high, if toenail Zn was higher than the median, and as low, otherwise. Then we calculated the PS_{Zn} for each participant as the weighted sum of minor alleles for each SNP, with weights equal to their estimated coefficients from the above logistic regression models. The estimated coefficients per minor allele for each SNP are shown in Supplementary Table 3.

To avoid the potential overfitting induced by assessing the relation of the PS_{Zn} with toenail Zn on the same data used in its development, we performed a leave one out cross-validation. We calculated an alternative PS ($PS_{Zn\ 1\text{-out}}$) for each participant based on the regression coefficients for each SNP estimated from the rest of participants. We repeated this procedure sequentially for all the participants and combined over the entire genetic sample to obtain a nearly unbiased estimate of the expected association between $PS_{Zn\ 1\text{-out}}$ and toenail Zn in an independent sample from the same population (Efron and Tibshirani, 1994).

2.7. Statistical analysis

Toenail Zn concentrations were right-skewed and log-transformed for the analyses. To allow for sex-specific determinants of toenail Zn, we performed all analyses separately in men and women. We calculated geometric mean toenail Zn concentrations and 95 % confidence intervals (CIs) for pre-specified categories or tertiles of sociodemographic characteristics (age, ethnicity, educational level, and province), lifestyle factors (body mass index (BMI), recreational physical activity, and smoking status), Zn intake (diet and supplements), residential topsoil Zn, proximity to Zn-emitting industries, genetic variants for Zn metabolism and transportation (PS_{Zn} and $PS_{Zn\ 1\text{-out}}$), and season of toenail sample collection. To further explore the role of diet on toenail Zn, we also estimated GM of toenail Zn for tertiles of specific dietary components and patterns (Mediterranean diet).

We estimated geometric mean ratios of toenail Zn concentrations and their 95 % CIs comparing categories of the above determinants by exponentiating coefficients from linear regression models on log-transformed toenail Zn. We fitted a first model for each independent variable, adjusted for sociodemographic factors (age groups, educational level, and province indicators). Afterwards, we fitted a single multivariate model, including sociodemographic factors, and also smoking status, other potentially correlated Zn sources (categories of diet and supplement Zn intake, topsoil Zn, and industrial Zn exposure), season of toenail collection, genetic variability (tertiles of $PS_{Zn\ 1\text{-out}}$), and factors potentially interfering Zn absorption (tertiles of food groups that are known sources of dietary phytate: cereals, vegetables and legumes, and nuts) (Bel-Serrat et al., 2014). We performed tests for log-linear trend in adjusted geometric mean toenail Zn concentrations across categories of ordinal factors. Also, specifically for dietary variables, a sensitivity analyses was performed, fitting the same models, but adding total energy intake among adjustment variables (Willett et al., 1997).

To further explore the shape of the dose–response relations between dietary Zn intake and toenail Zn concentrations, we included natural cubic splines of Zn intake with two internal knots at the 33th and 67th percentiles and boundary knots at the 1st and 99th percentiles in fully-adjusted linear regression models on log-transformed toenail Zn (Durrleman and Simon, 1989). Natural cubic splines allow for different cubic trends at either side of internal knots and linear trends beyond boundary knots, and hence they can accommodate a wide variety of smooth dose–response curves, while avoiding implausible shapes at the tails of Zn intake distribution.

Finally, to evaluate whether genetic variants might modulate the relationship between toenail Zn concentrations and different sources of Zn exposure (smoking, diet and supplement intake, topsoil, and industrial exposure), we included interaction terms between categories of these Zn sources and tertiles of the $PS_{Zn\ 1\text{-out}}$ in fully-adjusted linear regression models on log-transformed toenail Zn. We estimated different geometric mean ratios of toenail Zn within each $PS_{Zn\ 1\text{-out}}$ tertile and tested for homogeneity across tertiles using joint Wald tests of interaction coefficients. We performed all statistical analyses with Stata, version 16 (Stata Corp).

3. Results

The main characteristics of the participants ($n = 3,448$) are shown in Table 1. Almost all controls were European, and there were no differences in the level of education according to sex. Compared to men, women were younger, had a lower BMI, lower tobacco and alcohol consumption and were physically less active in their free time. Women had a higher adherence to Mediterranean diet, but lower caloric and dietary Zn intake, and the weight of their nail samples was slightly lower than in men. The use of dietary supplements was infrequent, including those containing Zn, although it was more common among women. With respect to dietary Zn, men, younger participants and those with university studies had higher Zn intakes (Supplementary Table 4). Finally, there were no differences in $PS_{Zn\ 1\text{-out}}$ values by sex, age or level of education, although $PS_{Zn\ 1\text{-out}}$ differed by region (Supplementary Table 5).

Tables 2 and 3 present GM toenail Zn levels and adjusted GM ratios (model 1 & 2) by sociodemographic and diet-related variables, respectively. Further descriptive parameters of toenail Zn can be found in Supplementary Tables 6 and 7. All participants had Zn levels above the limit of detection. Mean Zn levels in toenails were higher in men (GM 104.1; CI 95 % 102.0–106.3 μg g^{-1}) than in women (GM 100.3; CI 95 % 98.9–101.8 μg g^{-1}), although sex was not a predictor of toenail Zn in fully adjusted models (GM ratio: 1.01; 95 % CI: 0.96–1.05). In the first multivariate model, only in men Zn levels decreased with age, and increased with tobacco consumption. There were no differences regarding menopausal status in women. There were differences among regions in both sexes: Zn levels in men from Barcelona and Murcia, and women from Madrid were higher than the global sex-specific mean, while those from Cantabria –both, men and women- or Gipuzkoa –only women- had lower toenail Zn concentrations. In regard to the explored sources of exposure, we did not observe any association between toenail Zn and dietary Zn intake or supplement intake in either men or women. The dose–response analysis between dietary zinc intake and toenail Zn (Fig. 1), did not show a clear association between both variables; in any case, it might suggest an inverse relationship in men. Regarding soil Zn, we found an increase of toenail Zn with soil Zn levels in men. Instead, we

Table 1
Main characteristics of controls from MCC-Spain Study eligible for the toenail Zn determinants analysis (n = 3,448).

Variable	Total	Men	Women	p-value
Participants	3448 (100 %)	1707 (49.5 %)	1741 (50.5 %)	
Sociodemographic characteristics				
Age (mean ± SD)	62.5 ± 12.1	66.1 ± 9.7	58.9 ± 13.1	<0.01
Age (categorized)				<0.01
<56 years	954 (27.7 %)	217 (12.7 %)	737 (42.3 %)	
56–69 years	1387 (40.2 %)	826 (48.4 %)	561 (32.2 %)	
>69 years	1107 (32.1 %)	664 (38.9 %)	443 (25.4 %)	
Education				0.15
Primary	1668 (48.4 %)	848 (49.7 %)	820 (47.1 %)	
Secondary	1037 (30.1 %)	488 (28.6 %)	549 (31.5 %)	
University	743 (21.5 %)	371 (21.7 %)	372 (21.4 %)	
Ethnicity				<0.01
Non-European	51 (1.5 %)	12 (0.7 %)	39 (2.2 %)	
European	3394 (98.4 %)	1693 (99.2 %)	1701 (97.7 %)	
Unknown	3 (0.1 %)	2 (0.1 %)	1 (0.1 %)	
Province				<0.01
Madrid	655 (19.0 %)	313 (18.3 %)	342 (19.6 %)	
Barcelona	729 (21.2 %)	454 (26.6 %)	275 (15.8 %)	
Navarra	243 (7.0 %)	74 (4.3 %)	169 (9.7 %)	
Gipuzkoa	347 (10.1 %)	89 (5.2 %)	258 (14.8 %)	
Leon	420 (12.2 %)	223 (13.1 %)	197 (11.3 %)	
Asturias	227 (6.6 %)	104 (6.1 %)	123 (7.1 %)	
Murcia	36 (1.0 %)	25 (1.5 %)	11 (0.6 %)	
Huelva	101 (2.9 %)	58 (3.4 %)	43 (2.5 %)	
Cantabria	329 (9.5 %)	166 (9.7 %)	163 (9.4 %)	
Valencia	124 (3.6 %)	68 (4.0 %)	56 (3.2 %)	
Granada	160 (4.6 %)	109 (6.4 %)	51 (2.9 %)	
Girona	77 (2.2 %)	24 (1.4 %)	53 (3.0 %)	
Anthropometry and habits				
BMI (kg/m^2, mean ± SD)	26.6 ± 4.3	27.3 ± 3.7	25.8 ± 4.8	<0.01
BMI (kg/m^2, categorized)				<0.01
<25	1259 (36.5 %)	456 (26.7 %)	803 (46.1 %)	
25–30	1308 (37.9 %)	816 (47.8 %)	492 (28.3 %)	
>30	636 (18.5 %)	364 (21.4 %)	272 (15.6 %)	
Unknown	245 (7.1 %)	71 (4.1 %)	174 (10.0 %)	
Smoking status				
Never	1534 (44.5 %)	499 (29.2 %)	1035 (59.4 %)	<0.01
Former smoker	1170 (33.9 %)	825 (48.3 %)	345 (19.8 %)	
Current smoker	732 (21.3 %)	373 (21.9 %)	359 (20.6 %)	
Unknown	12 (0.4 %)	10 (0.6 %)	2 (0.1 %)	
N. cigarettes/day current smokers (mean ± SD)	18.1 ± 11.9	21.4 ± 13.0	15.2 ± 9.9	<0.01
Physical activity (METs/week)				
0 METs /week	1374 (39.9 %)	650 (38.1 %)	724 (41.6 %)	<0.01
<8 METs /week	282 (8.2 %)	103 (6.0 %)	179 (10.3 %)	
8–15.9 METs /week	366 (10.6 %)	150 (8.8 %)	216 (12.4 %)	
>=16 METs /week	1390 (40.3 %)	782 (45.8 %)	608 (34.9 %)	
Unknown	36 (1.0 %)	22 (1.3 %)	14 (0.8 %)	
Diet				
Adherence to Mediterranean diet				0.01
Low	2461 (71.4 %)	1263 (74.0 %)	1198 (68.8 %)	
High	708 (20.5 %)	314 (18.4 %)	394 (22.6 %)	
Unknown	279 (8.1 %)	130 (7.6 %)	149 (8.6 %)	
Total energy intake (kcal/d, mean ± SD)	1897 ± 629	2034 ± 663	1763 ± 562	<0.01
Ethanol intake (g/d, mean ± SD)	10.8 ± 15.3	16.6 ± 18.3	5.0 ± 8.3	<0.01
Fibre intake (g/d, mean ± SD)	22.5 ± 9.7	22.8 ± 9.7	22.2 ± 9.7	0.03
Zinc intake (mg/d, mean ± SD)	9.3 ± 2.9	9.8 ± 3.0	8.8 ± 2.7	<0.01
Supplement intake				
No	2806 (81.4 %)	1443 (84.5 %)	1363 (78.3 %)	<0.01
Yes, not specified	137 (4.0 %)	45 (2.6 %)	92 (5.3 %)	
Yes, containing zinc	135 (3.9 %)	39 (2.3 %)	96 (5.5 %)	
Unknown	370 (10.7 %)	180 (10.6 %)	190 (10.9 %)	
Other environmental sources				
Zn in soil (mg/kg,mean ± SD)	4.36 ± 0.4	4.38 ± 0.4	4.35 ± 0.4	
Any Zn-emitting industry within 3 km				<0.01
No	2312 (67.1 %)	1079 (63.2 %)	1233 (70.8 %)	
Yes	1128 (32.7 %)	622 (36.4 %)	506 (29.1 %)	
Unknown	8 (0.2 %)	6 (0.4 %)	2 (0.1 %)	
Toenail samples				
Samples' weight (g, mean ± SD)	0.026 ± 0.023	0.026 ± 0.020	0.025 ± 0.025	<0.01
Season of collection				0.03
Winter	997 (28.9 %)	475 (27.8 %)	522 (30.0 %)	
Spring	1033 (29.9 %)	499 (29.2 %)	534 (30.7 %)	
Summer	420 (12.2 %)	230 (13.5 %)	190 (10.9 %)	
Autumn	607 (17.6 %)	291 (17.0 %)	316 (18.1 %)	
Unknown	391 (11.4 %)	212 (12.5 %)	179 (10.3 %)	

Note: MCC-Spain is a population-based multicase-control study (2008–2013) designed to explore environmental factors associated with five types of cancer. n = 3,448 is the group of controls included in this work with available toenail Zn data. Data are n (%) or mean ± SD. p-value obtained using one-way ANOVA or Kruskal-Wallis for continuous variables or Pearson chi-square test for categorical variables. BMI: Body mass index; N.: number of participants; METs: metabolic equivalents of task; g/d: grams per day; mg/d: milligrams per day; SD: standard deviation.

observed a positive association of toenail Zn with the proximity to Zn industries (<3km) restricted to women (Table 2). Toenail Zn levels were lower in men whose samples had been collected in autumn, while there

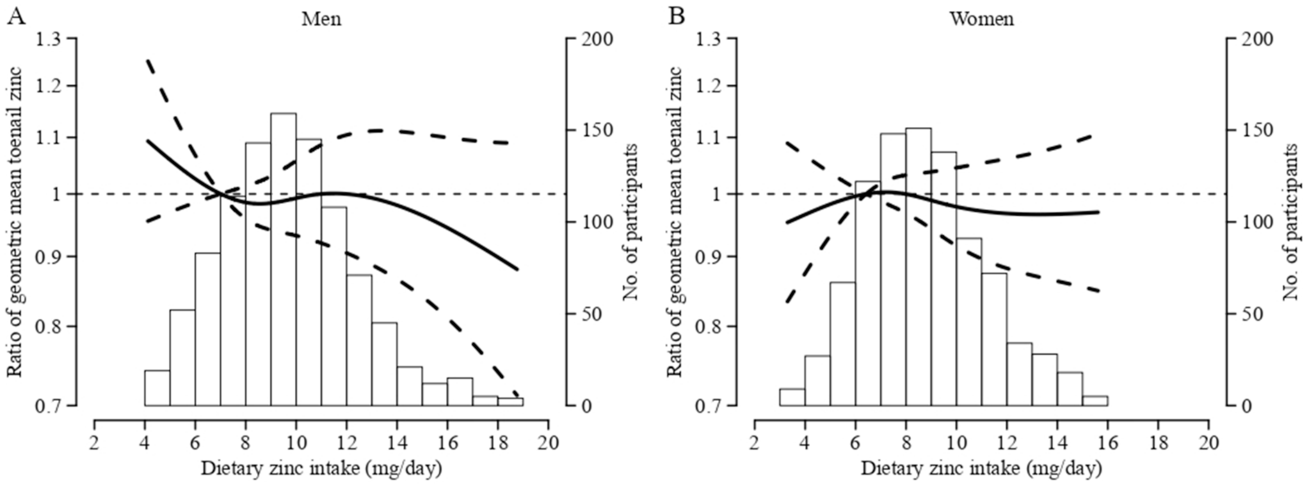

Fig. 1. Ratio of geometric mean toenail zinc concentrations as a smooth function of dietary zinc intake among control men (A) and women (B) from MCC-Spain Study. Curves represent adjusted geometric mean ratios (solid curves) and 95 % confidence intervals (dashed curves) based on natural cubic splines of dietary zinc intake with two internal knots at the 33th and 67th percentiles and boundary knots at the 1st and 99th percentiles. The reference value (geometric mean ratio = 1) was set at the 17th percentile of zinc intake distribution (7.01 mg/day for men and 6.42 mg/day for women). Geometric mean ratios were obtained from linear regression models on log-transformed toenail zinc concentrations adjusted for age group, educational level, province of residence, smoking status, supplement intake, topsoil zinc (tertiles), industrial zinc exposure, season of toenail collection, genetic variability (tertiles of PSzn 1-out), and tertiles of food groups that are known sources of dietary phytate (tertiles of cereals, vegetables and legumes, and nuts intake). Histograms represent dietary zinc intake distributions among men and women.

were no differences depending on the season of collection in women. Regarding the genetic variability in Zn metabolism, we observed a positive relationship between the PS_{Zn} and toenail Zn in both sexes, while we did not find this association with $Ps_{Zn\ 1\text{-out}}$, with the exception of men in the second tertile.

For specific food groups (Table 3), we identified an inverse relationship among Zn levels in men with dietary fibre intake as well as vegetables and legumes, nuts and eggs intake (limited to the second tertile). These results remained unchanged in sensitivity analyses, adjusting for total energy intake (Supplementary Table 8). In the second model, that included all the potential exposure sources and modulators of Zn as well as those food groups that could interfere with Zn absorption, smoking status remained as a determinant for higher toenail Zn in men, especially in former smokers, while the positive association of toenail Zn with $PS_{Zn\ 1\text{-out}}$ only remained in men in the second tertile. Geographical differences in toenail Zn in both sexes, as well as lower Zn in those toenails collected in autumn and in those men with higher intakes of vegetables, legumes and nuts were also still observed. The explored determinants (model 2) only explained 9.3 % and 4.8 % of toenail Zn variability in men and women, respectively.

Finally, we explored whether genetic variability in Zn metabolism could modulate the association between the different sources of Zn exposure and measured toenail Zinc levels (Table 4). The positive association between tobacco consumption and toenail Zn only remained for those men in the second tertile of $PS_{Zn\ 1\text{-out}}$ while in the case of women a positive association with supplements intake was observed for those in the second tertile of $PS_{Zn\ 1\text{-out}}$.

4. Discussion

Our aims in this work were to investigate the main determinants of toenail Zn, and to explore its relationship with selected sources of environmental exposure to Zn and individual genetic variability in Zn metabolism. For this purpose, we carried out a comprehensive evaluation of which factors were associated with toenail Zn levels in general population in Spain, and explored specifically the possible relationship of this biomarker with some of the major sources of exposure to Zn in humans, and whether individual genetic background related to Zn metabolism could modulate toenail Zn levels. Our results show that the relationship between toenail Zn and the main sources of exposure explored in this study is, in general, weak. Also, although genetic variability may play an important role in Zn metabolism and consequently modify toenail Zn concentrations, the PS_{Zn} built in our study to explore for the first time the association between SNPs of genes related to Zn metabolism and transportation with toenail Zn, failed to show a relationship.

Studies that report toenail Zn concentrations levels in the literature are scarce, and many of them have small sample sizes (Gutiérrez-González et al., 2019). In addition, there might be comparability problems among studies due to the effect of the mass of toenail sample on the measurement. Levels of the elements measured and their detection limits may vary according to the weight of the samples (Gutiérrez-González et al., 2019). For this work, since Zn levels were dependent on the mass of the samples, they were calibrated to avoid this bias, but this relevant issue is not considered in most reports, with only some exceptions (Brockman et al., 2009; Garland et al., 1993; Garland et al., 1996).

Aside from these considerations, Zn levels found in this study are similar to those from other studies performed in Spain (Martin-Moreno et al., 2003; Amaral et al., 2012; Sureda et al., 2017), as well as slightly above those found in France (Goulle et al., 2009) or Ireland (O'Rorke et al., 2012), and below those reported in Portugal (Coelho et al., 2014) or Italy (Bergomi et al., 2002). We also observed differences among Spanish provinces in our study, that were present even after adjusting for possible confounders and genetic factors, what suggests that there may be other determinants not identified yet.

At present, the available information on Zn determinants in toenails is, in general, scarce and inconclusive (Gutiérrez-González et al., 2019). In our study, toenail Zn in men was higher than in women except in the older group. However, the relationship of toenail Zn with age did not differ by sex in fully adjusted models. Although some authors have also described higher toenail levels in males (Matthews et al., 2019; Campos et al., 2007; Gonzalez et al., 2008), in most studies toenail Zn was similar in both sexes (Gutiérrez-González et al., 2019). However, men tend to have higher levels in other matrices like urine (Berglund et al., 2015; Canada, 2021), blood (Canada, 2021), plasma (Bales et al., 19901990), serum (Ghayour-Mobarhan et al., 2005; Fourth National Report on Human Exposure to Environmental Chemicals) or saliva (Bales et al., 1990). This might suggest a higher exposure to Zn in men, for instance

Table 2
Geometric mean (GM) toenail zinc levels (μg/g) by sex and sociodemographic and exposure-related variables and association with sociodemographic and exposure-related variables in MCC-Spain Study.

	Men				Women				
	N	Gmean (CI 95 %)	Gmean ratio Model 1 (CI 95 %)	Gmean ratio Model 2 (CI 95 %)	N	Gmean (CI 95 %)	Gmean ratio Model 1 (CI 95 %)	Gmean ratio Model 2 (CI 95 %)	p-int sex Model 2
Total	1707	104.1 (102.0,106.3)			1741	100.3 (98.9,101.8)			
Age									0.19
<56 years	217	108.7 (100.9,117.1)	Ref	Ref	737	98.9 (96.7,101.1)	Ref	Ref	
56–69 years	826	105.8 (102.9,108.8)	0.97 (0.91,1.04)	0.96 (0.88,1.06)	561	101.4 (98.9,103.9)	1.03 (0.99,1.07)	1.02 (0.97,1.08)	
>69 years	664	100.6 (97.4,103.8)	0.93 (0.87,0.99)	0.94 (0.85,1.03)	443	101.5 (98.3,104.9)	1.03 (0.99,1.07)	1.03 (0.96,1.10)	
p-trend			0.03	0.17			0.21	0.46	
Education									0.22
Primary	848	102.9 (99.9,106.1)	Ref	Ref	820	100.6 (98.4,102.9)	Ref	Ref	
Secondary	488	105.0 (100.8,109.3)	1.01 (0.96,1.06)	1.04 (0.97,1.11)	549	99.6 (97.0,102.2)	1.00 (0.96,1.03)	0.99 (0.94,1.05)	
University	371	105.6 (101.6,109.7)	1.01 (0.95,1.07)	1.02 (0.94,1.10)	372	100.9 (97.8,104.0)	1.01 (0.97,1.06)	1.01 (0.94,1.08)	
p-trend			0.77	0.50			0.54	0.91	
Ethnicity									
Non-European	1693	104.1 (101.9,106.2)	Ref		1701	100.4 (98.9,101.9)	Ref		
European	12	118.9 (89.8,157.4)	1.00 (0.87,1.42)		39	100.1 (87.8,114.1)	1.00 (0.89,1.10)		
Menopausal status									
Premenopausal					1231	100.7 (99.0,102.6)	Ref		
Postmenopausal					510	99.4 (96.8,102.1)	1.01 (0.96,1.06)		
BMI (kg/m2)									
<25	456	102.3 (97.8,107.0)	Ref		803	99.3 (97.2,101.4)	Ref		
25–30	816	104.3 (101.4,107.4)	1.02 (0.97,1.07)		492	100.7 (98.0,103.5)	1.01 (0.98,1.05)		
>30	364	104.7 (100.5,109.1)	1.02 (0.96,1.08)		272	103.1 (98.9,107.5)	1.03 (0.99,1.08)		
p-trend			0.110				0.20		
Recreational physical activity (METS, min/week)									
T1:0.00	650	105.9 (102.2,109.7)	Ref		724	100.2 (97.9,102.5)	Ref		
T2: Men 0.01–24.00; Women 0.01–17.09	485	104.7 (100.9,108.6)	0.99 (0.94,1.04)		427	100.3 (97.3,103.4)	1.00 (0.96,1.04)		
T3: Men > 24.00; Women > 17.09	550	101.6 (98.0,105.3)	0.98 (0.93,1.03)		576	100.6 (98.0,103.2)	1.01 (0.98,1.05)		
p-trend			0.37				0.78		
Smoking status									0.08
Never	499	97.9 (94.3,101.6)	Ref	Ref	1035	100.3 (98.4,102.3)	Ref	Ref	
Former smoker	825	106.3 (103.5,109.2)	1.07 (1.02,1.13)	1.09 (1.02,1.16)	345	99.2 (96.0,102.5)	0.99 (0.95,1.03)	0.99 (0.93,1.05)	
Current smoker	373	107.7 (102.1,113.7)	1.09 (1.03,1.16)	1.08 (0.99,1.17)	359	101.5 (98.2,105.0)	1.03 (0.99,1.07)	1.05 (0.99,1.11)	
p-trend			0.01	0.05			0.29	0.18	
Total Zn intake (mg/d)									0.96
T1: Men < 8.44; Women < 7.52	532	105.1 (101.1,109.3)	Ref	Ref	539	99.7 (96.9,102.6)	Ref	Ref	
T2: Men 8.44–10.62; Women 7.52–9.57	533	102.0 (98.2,105.9)	0.97 (0.92,1.02)	1.03 (0.95,1.11)	542	100.7 (98.2,103.2)	1.00 (0.96,1.04)	0.98 (0.93,1.04)	
T3: Men > 10.62; Women > 9.57	531	105.1 (101.5,108.9)	0.99 (0.94,1.05)	1.04 (0.94,1.14)	541	99.9 (97.4,102.6)	0.99 (0.94,1.04)	0.96 (0.89,1.03)	
p-trend			0.82	0.49			0.63	0.25	
Supplement intake									0.98
No	1443	103.9 (101.5,106.3)	Ref	Ref	1363	99.7 (98.1,101.4)	Ref	Ref	
Yes, not specified	45	102.3 (93.7,111.7)	0.97 (0.85,1.10)	1.04 (0.88,1.23)	92	105.0 (96.1,114.8)	1.05 (0.98,1.12)	1.05 (0.95,1.16)	
Yes, containing zinc	39	102.5 (92.5,113.7)	1.00 (0.87,1.15)	1.01 (0.85,1.19)	96	100.8 (95.9,105.9)	1.01 (0.94,1.07)	1.01 (0.92,1.11)	
Zn soil (mg/kg)									0.02

(*continued on next page*)

Table 2 (*continued*)

	Men				Women				
	N	Gmean (CI 95 %)	Gmean ratio Model 1 (CI 95 %)	Gmean ratio Model 2 (CI 95 %)	N	Gmean (CI 95 %)	Gmean ratio Model 1 (CI 95 %)	Gmean ratio Model 2 (CI 95 %)	p-int sex Model 2
T1: Men < 4.21; Women < 4.14	575	105.1 (101.6,108.7)	Ref	Ref	596	101.9 (99.3,104.6)	Ref	Ref	
T2: Men 4.21–4.63; Women 4.14–4.58	521	98.8 (94.9,102.9)	1.03 (0.94,1.13)	1.00 (0.89,1.12)	599	100.2 (97.9,102.7)	1.04 (0.97,1.10)	0.99 (0.91,1.09)	
T3: Men > 4.63; Women > 4.58	600	108.1 (104.4,111.8)	1.33 (0.95,1.88)	1.25 (0.85,1.84)	542	98.8 (96.0,101.6)	0.99 (0.88,1.11)	1.00 (0.86,1.16)	
p-trend			0.31	0.73			0.72	0.95	
Any Zn-emitting industry within 3 km									0.06
No	1079	105.8 (103.2,108.5)	Ref	Ref	1233	100.6 (98.8,102.4)	Ref	Ref	
Yes	622	101.1 (97.5,104.8)	1.00 (0.94,1.05)	0.98 (0.90,1.06)	506	99.7 (96.9,102.5)	1.04 (1.00,1.09)	1.03 (0.97,1.10)	
Season of collection									0.30
Winter	475	105.7 (101.2,110.3)	1.02 (0.98,1.06)	1.03 (0.98,1.08)	522	100.6 (97.9,103.4)	0.98 (0.96,1.01)	0.962 (0.92,1.01)	
Spring	499	103.4 (99.6,107.3)	1.00 (0.97,1.04)	0.98 (0.94,1.03)	534	101.1 (98.6,103.6)	1.01 (0.98,1.03)	1.004 (0.97,1.05)	
Summer	230	103.8 (98.6,109.3)	1.04 (0.99,1.10)	1.07 (1.00,0.1.38)	190	99.9 (94.6,105.6)	1.02 (0.98,1.06)	1.025 (0.97,1.08)	
Autumn	291	100.1 (95.3,105.1)	0.94 (0.90,0.98)	0.93 (0.87,0.99)	316	98.4 (95.0,102.0)	0.99 (0.96,1.02)	1.009 (0.97,1.06)	
Province									0.06
Madrid	313	107.6 (102.7,112.8)	1.04 (0.98,1.09)	1.18 (1.03,1.35)	342	106.3 (102.4,110.4)	1.06 (1.02,1.10)	1.10 (1.02,1.19)	
Barcelona	454	110.7 (107.1,114.5)	1.07 (1.02,1.12)	1.01 (0.77,1.34)	275	103.1 (99.1,107.1)	1.03 (0.98,1.07)	0.98 (0.87,1.10)	
Navarra	74	108.0 (97.5,119.6)	1.04 (0.95,1.14)	1.29 (1.07,1.53)	169	100.7 (96.1,105.4)	1.00 (0.95,1.05)	1.00 (0.89,1.12)	
Gipuzkoa	89	101.7 (89.4,115.6)	0.98 (0.90,1.07)	0.84 (0.65,1.10)	258	94.8 (90.9,99.0)	0.94 (0.91,0.99)	0.95 (0.86,1.04)	
Leon	223	100.1 (95.1,105.4)	0.97 (0.92,1.04)	1.14 (0.98,1.33)	197	100.4 (95.8,105.2)	1.00 (0.95,1.05)	0.98 (0.89,1.09)	
Asturias	104	108.8 (100.1,118.2)	1.06 (0.98,1.15)	1.11 (0.94,1.32)	123	104.8 (100.1,109.7)	1.05 (0.99,1.11)	1.04 (0.95,1.15)	
Murcia	25	122.1 (104.9,142.1)	1.17 (1.00,1.37)	–	11	109.4 (91.6,130.6)	1.09 (0.92,1.29)	–	
Huelva	58	112.2 (100.1,125.8)	1.09 (0.98,1.21)	1.11 (0.88,1.41)	43	102.5 (95.2,110.4)	1.02 (0.94,1.12)	1.09 (0.81,1.49)	
Cantabria	166	84.0 (78.4,90.1)	0.81 (0.75,0.86)	0.91 (0.79,1.06)	163	90.8 (87.7,94.0)	0.91 (0.86,0.95)	0.90 (0.82,0.98)	
Valencia	68	96.1 (85.1,108.5)	0.93 (0.85,1.03)	0.82 (0.60,1.06)	56	96.4 (90.6,102.6)	0.96 (0.89,1.04)	0.99 (0.82,1.21)	
Granada	109	104.2 (95.9,113.2)	1.01 (0.93,1.09)	1.14 (0.98,1.31)	51	98.6 (90.0,108.1)	0.98 (0.90,1.06)	0.96 (0.85,1.07)	
Girona	24	93.7 (68.6,128.2)	0.90 (0.77,1.05)	0.74 (0.55,1.00)	53	98.5 (92.9,104.6)	0.98 (0.91,1.07)	0.98 (0.74,1.30)	
Polygenic score (PS_{Zn})									
T1: <0.11	430	96.6 (92.9,100.3)	Ref		359	97.7 (94.1,101.3)	Ref		
T2: 0.11–0.47	417	105.6 (100.5,110.9)	1.10 (1.04,1.17)		363	97.1 (93.9,100.5)	0.99 (0.95,1.04)		
T3: >0.47	420	107.1 (102.9,111.5)	1.11 (1.04,1.17)		362	104.5 (101.3,107.9)	1.07 (1.02,1.12)		
p-trend			0.01				0.01		
Polygenic score leave one out ($PS_{Zn\ 1\text{-}out}$)									0.01
T1: <0.10	418	101.3 (97.5,105.2)	Ref	Ref	355	101.8 (98.1,105.6)	Ref	Ref	
T2: 0.10–0.47	434	106.9 (101.7,112.3)	1.06 (1.00,1.13)	1.11 (1.04,1.19)	364	98.7 (95.3,102.1)	0.97 (0.92,1.02)	0.96 (0.91,1.01)	
T3: >0.47	415	100.6 (96.6,104.6)	0.99 (0.93,1.05)	0.99 (0.92,1.06)	365	98.8 (95.8,102.0)	0.97 (0.93,1.02)	0.97 (0.92,1.02)	
p-trend			0.74	0.69			0.24	0.27	

Note: Ref: reference category; BMI: Body mass index; T: tertile; METs: metabolic equivalents of task; mg/d: milligrams per day; Gmean: Geometric mean; CI: confidence interval; PS_{Zn}: polygenic score for toenail Zn; $PS_{Zn1\text{-}out:}$ Cross validation (leave one out) of polygenic score for toenail Zn; -: no genetic data available. Model 1 adjusted for age groups, educational level, and province of residence. Model 2: adjusted by age groups, educational level, province of residence, smoking status, tertiles of dietary Zn, supplements intake, tertiles of topsoil Zn, industrial Zn exposure, season of toenail collection, genetic variability (tertiles of PSzn 1-out), and tertiles of food groups that are known sources of dietary phytate (tertiles of cereals, vegetables and legumes, and nuts intake). p-trend: tests for log-linear trend in adjusted geometric mean toenail Zn concentrations across categories of ordinal factors; p-int sex: Effect heterogeneity comparing the results of model 2 between men and women was assessed by Wald tests.

Table 3
Geometric mean (GM) toenail zinc levels (μg/g) by sex and diet-related variables and association with diet-related variables.

	Men				Women				
	N	Gmean (CI 95 %)	Gmean ratio Model 1 (CI 95 %)	Gmean ratio Model 2 (CI 95 %)	N	Gmean (CI 95 %)	Gmean ratio Model 1 (CI 95 %)	Gmean ratio Model 2 (CI 95 %)	p-int sex Model 2
Total	1707	104.1 (102.0,106.3)			1741	100.3 (98.9,101.8)			
Total energy intake (Kcal/ day)									
T1: Men < 1736; Women < 1500	532	103.4 (99.5,107.4)	Ref		540	100.3 (97.4,103.2)	Ref		
T2: Men 1736–2222; Women 1500–1913	532	105.3 (101.2,109.5)	1.02 (0.97,1.08)		541	98.9 (96.7,101.2)	0.98 (0.95,1.02)		
T3: Men > 2222; Women > 1913	532	103.5 (100.0,107.2)	0.99 (0.94,1.04)		541	101.2 (98.4,104.0)	1.01 (0.97,1.05)		
p-trend			0.62				0.65		
Ethanol intake (g/day)									
T1: Men < 4.60; Women 0.00	529	103.6 (99.8,107.5)	Ref		594	99.4 (97.0,101.9)	Ref		
T2: Men 4.60–20.13; Women 0.01–4.40	535	105.4 (101.3,109.8)	1.01 (0.96,1.06)		482	101.9 (98.8,105.1)	1.03 (0.99,1.07)		
T3: Men > 20.13; Women > 4.40	532	103.2 (99.7,106.7)	0.98 (0.93,1.04)		546	99.3 (96.9,101.8)	1.00 (0.96,1.05)		
p-trend			0.50				0.80		
Coffee intake (g/day)									
<50.00	355	101.9 (97.3,106.7)	Ref		360	100.5 (97.7,103.5)	Ref		
50.00–100.00	759	102.8 (99.8,105.9)	0.99 (0.93,1.04)		762	99.0 (96.8,101.3)	0.97 (0.94,1.01)		
>100.00	482	107.8 (103.4,112.3)	1.02 (0.96,1.09)		500	101.5 (98.6,104.4)	1.00 (0.96,1.05)		
p-trend			0.43				0.68		
Fibre intake (g/day)									
T1: Men < 18.20; Women < 17.87	532	109.1 (104.5,113.8)	Ref		541	99.7 (97.1,102.4)	Ref		
T2: Men 18.20–25.09; Women 17.87–24.15	532	100.4 (96.9,104.1)	0.92 (0.87,0.97)		540	100.9 (98.3,103.4)	1.00 (0.97,1.04)		
T3: Men > 25.09; Women > 24.15	532	102.9 (99.6,106.3)	0.94 (0.89,0.99)		541	99.8 (97.1,102.6)	0.99 (0.96,1.03)		
p-trend			0.02				0.66		
Meat intake (g/day)									
T1: Men < 72.72; Women < 54.36	532	102.8 (99.5,106.3)	Ref		540	99.2 (96.5,101.9)	Ref		
T2: Men 72.72–109.81; Women 54.36–82.47	532	104.0 (100.2,107.9)	1.00 (0.95,1.06)		541	99.9 (97.4,102.6)	1.00 (0.96,1.04)		
T3: Men > 109.81; Women > 82.47	532	105.4 (101.2,109.9)	1.00 (0.95,1.06)		541	101.3 (98.6,104.0)	1.02 (0.98,1.06)		
p-trend			0.89				0.35		
Fish intake (g/day)			Ref						
T1: Men < 49.73; Women < 43.03	531	106.0 (101.5,110.7)	Ref		540	101.0 (98.1,104.0)	Ref		
T2: Men 49.73–76.50; Women 43.03–66.96	534	103.3 (100.1,106.6)	0.97 (0.92,1.02)		541	100.0 (97.6,102.4)	0.98 (0.94,1.02)		
T3: Men > 76.50; Women > 66.96	531	102.9 (99.2,106.7)	0.96 (0.91,1.01)		541	99.4 (96.8,102.0)	0.97 (0.94,1.01)		
p-trend			0.09				0.13		
Vegetables and legumes (g/ day)									0.26
T1: Men < 230.12; Women < 243.65	532	106.7 (102.4,111.1)	Ref	Ref	540	98.6 (96.1,101.2)	Ref	Ref	
T2: Men 230.12–340.56; Women 243.65–344.87	533	102.1 (98.3,105.9)	0.94 (0.89,0.99)	0.93 (0.87,1.00)	541	99.0 (96.6,101.5)	0.99 (0.95,1.03)	1.00 (0.94,1.06)	
T3: Men > 340.56; Women > 344.87	531	103.5 (100.1,107.1)	0.95 (0.90,1.01)	0.93 (0.86,1.01)	541	102.7 (99.8,105.7)	1.03 (0.99,1.07)	1.05 (0.99,1.11)	
p-trend			0.10	0.09			0.16	0.14	
Fruits intake (g/day)									
T1: Men < 221.20; Women < 249.59	532	105.4 (101.5,109.6)	Ref		540	98.7 (96.3,101.2)	Ref		
T2: Men 221.20–390.74; Women 249.59–420.68	532	102.4 (98.6,106.2)	0.96 (0.91,1.02)		541	100.9 (98.3,103.6)	1.02 (0.98,1.06)		
T3: Men > 390.74; Women > 420.68	532	104.4 (100.7,108.3)	0.99 (0.94,1.04)		541	100.8 (97.9,103.7)	1.01 (0.97,1.05)		
p-trend			0.65				0.65		
Edible fats intake (g/day)									
<15.00	504		Ref		369		Ref		

(*continued on next page*)

Table 3 (*continued*)

	Men				Women				
	N	Gmean (CI 95 %)	Gmean ratio Model 1 (CI 95 %)	Gmean ratio Model 2 (CI 95 %)	N	Gmean (CI 95 %)	Gmean ratio Model 1 (CI 95 %)	Gmean ratio Model 2 (CI 95 %)	p-int sex Model 2
		104.3 (100.1,108.7)				98.1 (95.2,101.2)			
15.00, 37.50	848	103.8 (101.0,106.7)	0.99 (0.95,1.04)		864	101.7 (99.5,103.8)	1.03 (0.99,1.07)		
>37.50	244	104.6 (98.5,111.0)	0.98 (0.92,1.05)		389	98.6 (95.5,101.9)	1.00 (0.96,1.05)		
p-trend			0.58				0.91		
Nuts intake (frequency)									0.56
<=1–3 month	888	104.8 (101.6,108.1)	Ref	Ref	943	99.7 (97.7,101.7)	Ref	Ref	
1–4 week	475	99.6 (96.0,103.3)	0.94 (0.90,0.99)	0.93 (0.88,0.99)	450	99.9 (97.1,102.8)	1.00 (0.96,1.03)	0.99 (0.94,1.04)	
>=5–6 week	233	110.8 (105.3,116.6)	1.04 (0.98,1.11)	1.01 (0.94,1.11)	229	102.3 (98.0,106.7)	1.02 (0.97,1.07)	1.04 (0.96,1.11)	
p-trend			0.89	0.62			0.59	0.56	
Dairy products (g/day)									
T1: Men < 250.37; Women < 290.02	532	106.6 (102.5,110.9)	Ref		542	100.7 (98.0,103.5)	Ref		
T2: Men 250.37–415.23; Women 290.02–472.27	533	103.1 (99.4,106.8)	0.98 (0.93,1.04)		539	98.6 (96.3,101.0)	0.98 (0.95,1.02)		
T3: Men > 415.23; Women > 472.27	531	102.6 (98.9,106.4)	0.97 (0.92,1.03)		541	101.0 (98.2,103.9)	1.01 (0.97,1.05)		
p-trend			0.31				0.71		
Cereals intake (g/day)									0.28
T1: Men < 179.42; Women < 133.17	532	107.1 (103.3,111.1)	Ref	Ref	540	101.3 (98.5,104.2)	Ref	Ref	
T2: Men 179.42–248.02; Women 133.17–186.45	532	102.3 (98.6,106.1)	0.96 (0.91,1.01)	0.99 (0.92,1.06)	541	99.6 (97.2,102.1)	0.98 (0.95,1.02)	0.98 (0.92,1.03)	
T3: Men > 248.02; Women > 186.45	532	102.8 (98.9,106.9)	0.95 (0.90,1.00)	1.00 (0.93,1.08)	541	99.5 (96.9,102.1)	0.99 (0.95,1.03)	0.99 (0.93,1.05)	
p-trend			0.07	0.97			0.48	0.73	
Eggs intake (frequency)									
<=2–3 month	317	112.4 (106.8,118.3)	Ref		281	100.5 (96.4,104.8)	Ref		
1–2 week	872	100.5 (97.9,103.2)	0.90 (0.85,0.95)		880	98.9 (97.0,100.8)	0.98 (0.94,1.03)		
>=3–4 week	407	105.5 (100.6,110.7)	0.95 (0.89,1.01)		461	102.3 (99.3,105.5)	1.03 (0.99,1.08)		
p-trend			0.20				0.09		
Adherence Mediterranean diet									
Low	1263	104.5 (102.0,107.1)	1.00		1198	100.1 (98.3,101.9)	Ref		
High	314	102.9 (98.2,107.8)	0.98 (0.93,1.04)		394	100.5 (97.3,103.7)	0.99 (0.96,1.03)		

Note: Ref: reference category; T: tertile; g/d: grams per day; Gmean: Geometric mean; CI: confidence interval; Model 1 adjusted for age groups, educational level, and province of residence. Model 2: adjusted by age groups, educational level, province of residence, smoking status, tertiles of dietary Zn, supplements intake, tertiles of topsoil Zn, industrial Zn exposure, season of toenail collection, genetic variability (tertiles of PSzn 1-out), and tertiles of food groups that are known sources of dietary phytate (tertiles of cereals, vegetables and legumes, and nuts intake). p-trend: tests for log-linear trend in adjusted geometric mean toenail Zn concentrations across categories of ordinal factors; p-int sex: Effect heterogeneity comparing the results of model 2 between men and women was assessed by Wald tests.

through higher dietary intake (Ghayour-Mobarhan et al., 2005), although it could also be explained by sex-related biological factors (e.g. spermatogenesis) (Farag et al., 2021), men having higher Zn requirements than women (Institute of Medicine (US) Panel on. Zinc. National Academies Press (US), 2001). There may also be sex-related differences in the metabolism of Zn (differences in pharmacokinetics) (Poddalgoda et al., 2019), or in the expression of Zn transporters (Foster et al., 2011). Regarding age, available data are inconclusive, with some studies reporting positive (Garland et al., 1996; Martin-Moreno et al., 2003; Park et al., 2016), negative (Coelho et al., 2014; Marinho Reis et al., 2018; Rakovic et al., 1997) or no relationship with age (Campos et al., 2007; Gonzalez et al., 2008; Nouri et al., 2008; Hashemian et al., 2016), being age-related changes in the regulation of human Zn metabolism a possible explanation to these differences (Marinho Reis et al., 2018; Wastney et al., 1992). In our study, in adult population, we observed an inverse association with age only in men. Again, this might be related to the differences in Zn needs and functions between sexes.

Our exploration of the association of toenail Zn with possible sources of exposure to this element showed no association with dietary Zn or supplement intake and Zn in soil, although women had higher levels in the proximity to industries releasing Zn and, in men, levels were higher among smokers. Only one study found a positive relationship between total estimated dietary Zn and toenail Zn (Gonzalez et al., 2008) while others, in line with our results, did not find any association with diet (Milunsky et al., 1992; Graham et al., 1991), or supplement intake (Brockman et al., 2009; Gonzalez et al., 2008; Milunsky et al., 1992); unlike what occurs for other essential metals such as selenium (Gutiérrez-González et al., 2019). In regard to specific foods, some of them (i.e. meat and animal proteins, dairy products, seafood, nuts and cereals), are important sources of Zn (Sandstead, 2015); but there are no associations in the literature between food or food groups and toenail Zn (Gutiérrez-González et al., 2019). In our study, we only observed an inverse

Table 4

Association between toenail Zn and potential Zn sources by genetic background measured by tertiles of polygenic score ($PS_{Zn\ 1\text{-}out}$).

	Men									Women								
	All		$PS_{Zn1\text{-}out}$ T1		$PS_{Zn1\text{-}out}$ T2		$PS_{Zn1\text{-}out}$ T3		p-het	All		$PS_{Zn1\text{-}out}$ T1		$PS_{Zn1\text{-}out}$ T2		$PS_{Zn1\text{-}out}$ T3		p-het
	N	Gmean Ratio (CI 95 %)	N	Gmean Ratio (CI 95 %)	N	Gmean Ratio (CI 95 %)	N	Gmean Ratio (CI 95 %)		N	Gmean Ratio (CI 95 %)	N	Gmean Ratio (CI 95 %)	N	Gmean Ratio (CI 95 %)	N	Gmean Ratio (CI 95 %)	
Total Zn intake									0.68									0.13
T1: Men < 8.44; Women < 7.52	398	Ref	114	Ref	138	Ref	146	Ref		341	Ref	107	Ref	114	Ref	120	Ref	
T2: Men 8.44–10.62; Women 7.52–9.57	399	0.97 (0.92,1.02)	138	0.99 (0.90,1.10)	139	0.96 (0.84,1.09)	122	0.98 (0.89,1.09)		336	1.00 (0.97,1.04)	117	0.97 (0.88,1.07)	121	0.99 (0.91,1.08)	98	1.02 (0.94,1.11)	
T3: Men > 10.62; Women > 9.57	382	0.99 (0.94,1.05)	141	1.06 (0.96,1.17)	117	0.95 (0.83,1.09)	124	1.01 (0.91,1.11)		342	1.00 (0.96,1.04)	111	0.92 (0.83,1.01)	107	1.06 (0.97,1.16)	124	0.98 (0.90,1.06)	
p-trend		0.82		0.22		0.462		0.950			0.82		0.08		0.22		0.60	
Supplement intake									0.66									0.05
No	1091	Ref	365	Ref	368	Ref	358	Ref		887	Ref	295	Ref	293	Ref	299	Ref	
Yes, not specified	31	0.97 (0.85,1.10)	13	1.11 (0.89,1.39)	5	0.84 (0.52,1.36)	13	1.01 (0.80,1.26)		51	1.05 (0.98,1.12)	17	1.05 (0.88,1.25)	19	1.20 (1.03,1.40)	15	0.88 (0.75,1.04)	
Yes, containing zinc	33	1.00 (0.87,1.15)	10	0.99 (0.77,1.28)	9	0.85 (0.58,1.23)	14	1.18 (0.95,1.47)		61	1.01 (0.94,1.07)	18	0.90 (0.75,1.07)	23	1.05 (0.91,1.21)	20	1.10 (0.95,1.27)	
Tobacco									0.04									0.24
Never	374	Ref	119	Ref	137	Ref	118	Ref		637	Ref	217	Ref	203	Ref	217	Ref	
Former smoker	602	1.07 (1.02,1.13)	210	1.02 (0.93,1.12)	192	1.17 (1.05,1.32)	200	1.04 (0.95,1.15)		219	0.99 (0.95,1.03)	72	1.03 (0.92,1.14)	77	1.00 (0.91,1.10)	70	0.96 (0.88,1.04)	
Current smoker	287	1.09 (1.03,1.16)	85	0.95 (0.85,1.07)	105	1.11 (1.08,1.41)	97	1.08 (0.97,1.21)		226	1.03 (0.99,1.07)	66	1.07 (0.96,1.20)	83	0.96 (0.88,1.06)	77	1.06 (0.97,1.16)	
p-trend		0.01		0.48		0.01		0.15			0.290		0.22		0.454		0.31	
Zn soil									0.77									0.11
T1: Men < 4.21; Women < 4.14	409	Ref	134	Ref	130	Ref	145	Ref		294	Ref	92	Ref	105	Ref	97	Ref	
T2: Men 4.21–4.63; Women 4.14–4.58	423	1.03 (0.94,1.13)	135	0.89 (0.76,1.05)	157	1.06 (0.87,1.30)	131	1.07 (0.91,1.25)		477	1.04 (0.97,1.10)	161	1.11 (0.96,1.28)	156	0.97 (0.85,1.12)	160	1.03 (0.91,1.16)	
T3: Men > 4.63; Women > 4.58	426	1.33 (0.95,1.88)	147	1.04 (0.67,1.61)	143	1.69 (0.58,4.92)	136	1.33 (0.58,3.02)		312	0.99 (0.88,1.11)	102	1.17 (0.92,1.50)	103	0.86 (0.68,1.07)	107	0.96 (0.77,1.19)	
p-trend		0.31		0.34		0.47		0.36			0.72		0.13		0.23		0.94	
Any Zn-emitting industry within 3 km									0.92									0.91
No	758	Ref	244	Ref	260	Ref	254	Ref		688	Ref	223	Ref	228	Ref	237	Ref	
Yes	504	1.00 (0.94,1.05)	172	0.98 (0.88,1.17)	173	1.07 (0.94,1.21)	159	0.99 (0.89,1.10)		396	1.04 (1.00,1.09)	132	1.01 (0.92,1.11)	136	1.05 (0.96,1.15)	128	1.04 (0.95,1.13)	

Note: Ref: reference category. N: number of participants; Gmean: Geometric mean T: tertile; CI: confidence interval; $PS_{Zn1\text{-}out:}$ Cross validation (leave one out) of polygenic score for toenail Zn; p-het: Effect heterogeneity comparing geometric mean ratios of toenail Zn across tertiles of $PS_{Zn1\text{-}out}$ was assessed by Wald tests.

association of toenail Zn with the amount of fibre, nuts, vegetables and legumes and eggs intake in men. Some of these findings could be explained by the high content of phytate in some of these foods, which is known to interfere with Zn absorption (Sandstead, 2015). However, a positive association between fibre intake and toenail Zn levels has also been described (Gonzalez et al., 2008).

We found that tobacco consumption was positively associated in fully adjusted models with toenail Zn levels in men, but not in women. In our study, the percentage of smokers was higher among men, and also male smokers smoked a higher number of cigarettes per day than female smokers (Table 1). We also explored the relationship between toenail Zn and other variables of tobacco consumption like the number of cigarette/ day or pack-years (Supplementary Table 9), finding positive associations again only in men. This positive relationship between smoking and toenail Zn has been previously described in other studies (without stratification by sex) (Campos et al., 2007; Tang et al., 2021; Kilinc et al., 2020); although it has not been confirmed by other studies (Martin-Moreno et al., 2003; Park et al., 2016), Elevated Zn levels have also been found in other matrices such as serum in smokers compared to non-smokers (Badea et al., 2018). Although tobacco consumption is not identified as a common source of Zn in general population, non-negligible Zn concentrations have been measured in raw tobacco leaves and even higher in processed tobacco (Regassa and Chandravanshi, 2016). In light of all these findings, tobacco should be also considered as a possible source of exposure to Zn.

Our results showed that those women residing close (<3km) to one or more Zn-emitting industry had higher levels than those who did not. However, we did not observe associations with other sources of Zn exposure, such as Zn in soil. There are, however, some studies that found positive associations between Zn levels and place of residence (Nouri et al., 2008; Wilhelm et al., 1991; Were et al., 2009; Ndilila et al., 2014; Mohmand et al., 2015) or associations with Zn levels in air or dust (Marinho Reis et al., 2018; Ndilila et al., 2014; Raińska et al., 2005). These differences could be explained by the fact that our study uses controls randomly selected from the general population, while some of these studies are performed using participants living in industrial or mining areas that may be exposed to much higher Zn concentrations than the general population, and these high exposures may be better reflected by toenails.

As our study has shown, toenail Zn, in general, does not appear to be a good biomarker of exposure to most of the sources explored. This could be partly due to the characteristics of the matrix itself, but toenails have nevertheless been shown to be a good biomarker of exposure to other essential metals (e.g. selenium and dietary intake) (Gutiérrez-González et al., 2019). Moreover, this lack of association with some sources of exposure has been shown in matrices other than nails, such as plasma (Foster et al., 2011). Zinc is considered as a type 2 nutrient, similar to others such as potassium or magnesium, because it is necessary for multiple general metabolic functions (King, 2011). It is, therefore, an element subject to very effective homeostatic mechanisms, that protect the organism from exposure fluctuations (i.e. different amounts of intake through diet) (Virgili et al., 2019; Lowe et al., 2009) For example, when intake of Zn is low, there is a rapid decrease in excretion and an increase in zinc absorption, and zinc is also mobilized from intra- and extracellular pools (King et al., 2000). This may explain why individuals with different levels of exposure manage to keep adequate Zn circulating levels, which are subsequently deposited in the nail matrix (Jaramillo Ortiz et al., 2022; King, 2011).One of the novelties in our approach is the incorporation of genetic variability with regard to Zn metabolism into the assessment of toenail Zn determinants. We hypothesized that, given the tight control of plasmatic Zn levels in the organism, the metabolic regulation of this element could be a relevant factor determining toenail Zn. Genetic differences in the families of genes explored can result in changes in the affinity of the corresponding proteins to bind Zn (Giacconi et al., 2015; Suzuki and Koizumi, 2000). This indicates that the combination of some SNPs of Zn transporter genes could be an important determinant of toenail Zn. To the best of our knowledge, there are no other studies that have evaluated the association of SNPs of genes encoding Zn transporters with toenail Zn.

In our study, the PS_{Zn}, which summarized part of the individual variability in SNPs in genes encoding for proteins involved in Zn transportation and metabolism, such as MTs and ZnTs, was a relevant determinant of higher toenail Zn levels in both sexes. However, after applying a cross-validation method (leave one out), we cannot rule out that the observed effect could be due to overfitting. Despite our results, other studies carried out in biological matrices different from toenails have found that Zn may be influenced by genetic variability. Three SNPs from genes encoding Zn transporters [rs11126936 (*SLC30A3)*, rs233804 *(SLC39A8)*, and rs4872479 (*SLC39A14*)] have been positively associated with blood Zn concentration in Japanese population (Fujihara et al., 2018). Also, the SNP rs11126936 in *SLC30A3* Zn transporter was related with Zn serum concentrations (lower in carriers of C allele compared to T carriers) (da Rocha et al., 2014), and an association between the −5 A/G core promoter region SNP in the *MT2A* gene and Zn blood levels has been described (lower concentrations in carriers of G allele) (Kayaaltı et al., 2011). Another study found also an association between *SLC39A4* rs17855765 and high vagina tissue Zn levels in Hungarian women (Csikós et al., 2020). Also, this study found that some combinations of SNPs were associated both with lower or higher Zn vaginal tissue levels, and that a higher number of SNPs (6 or more) was associated with higher Zn vaginal tissue concentrations.

One of the strengths of our study is that we report toenail Zn in a population-based sample of controls, with a relatively large size and that toenail mass has been taken into account in the determination of toenail Zn levels, given that it can bias the results. In addition, we have explored its relationship to several sources of Zn exposure like dietary Zn, Zn supplements, tobacco and Zn in soil and proximity to industrial facilities releasing Zn. Finally, we have evaluated for the first time the association between toenail Zn and the genetic variability in Zn metabolism and transportation using an SNP-based PS_{Zn}, as well as assessed the possible interaction with the sources of exposure explored in this study, that, as a whole, represents a comprehensive review of Zn determinants.

Although our results are not conclusive, we cannot discard that some of the variability reflected by the biomarkers of exposure may be due to genetic variability. Therefore, given that the information provided by a biomarker may vary from person to person, in the current era of precision medicine we propose the inclusion of genetic variability into the picture when we are using biomarkers in order to get closer to a personalized approach to exposure measurement.

Our study is not exempt from limitations. Recall bias is frequent when information is self-reported using questionnaires. Estimates of Zn in air and soil could also be subject to an ecological fallacy. Also, although controls were randomly selected from general population, and recruited in different provinces, it is difficult to assess their possible representativeness of the Spanish population. Other circumstances like the presence of fungal infection should be considered, since it has been reported that Zn levels were lower in toenails of patients with onychomycosis compared to healthy subjects, although the prevalence of fungal infection is expected to be low (Kilinc et al., 2020). Another limitation is that we only explored the SNPs associated to Zn metabolism that were available in the microarray.

5. Conclusions

In summary, we have observed sex-related differences in the association with toenail Zn determinants like age, tobacco consumption, fibre intake or exposure to Zn from industries, while the association with other sources of exposure has not been confirmed. Also, genetic background should be considered when using this biomarker, as Zn levels may be the reflection of internal biological processes or genetic variability, and not only exposure to external sources. New studies including more SNPs, or genome wide association studies (GWAS) should be conducted to explore this possibility. Nowadays, we still need new data to understand the real meaning of Zn in toenails.

CRediT authorship contribution statement

Enrique Gutiérrez-González: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Pablo Fernández-Navarro:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Roberto Pastor-Barriuso:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Javier García-Pérez:** Investigation, Methodology, Writing – review & editing. **Gemma Castaño-Vinyals:** Writing – review & editing. **Vicente Martín-Sánchez:** Writing – review & editing. **Pilar Amiano:** Writing – review & editing. **Inés Gómez-Acebo:** Writing – review & editing. **Marcela Guevara:** Writing – review & editing. **Guillermo Fernández-Tardón:** Writing – review & editing. **Inmaculada Salcedo-Bellido:** Writing – review & editing. **Victor Moreno:** Writing – review & editing. **Marina Pinto-Carbó:** Writing – review & editing. **Juan Alguacil:** Writing – review & editing. **Rafael Marcos-Gragera:** Writing – review & editing. **Jesús Humberto Gómez-Gómez:** Writing – review & editing. **José Luis Gómez-Ariza:** Investigation, Methodology, Writing – review & editing. **Tamara García-Barrera:** Investigation, Methodology, Writing – review & editing. **Elena Varea-Jiménez:** Writing – review & editing. **Olivier Núñez:** Investigation, Methodology, Writing – review & editing. **Ana Espinosa:** Writing – review & editing. **Antonio J. Molina de la Torre:** Writing – review & editing. **Amaia Aizpurua-Atxega:** Writing – review & editing. **Jessica Alonso-Molero:** Writing – review & editing. **María Ederra-Sanz:** Writing – review & editing. **Thalia Belmonte:** Writing – review & editing. **Nuria Aragonés:** Writing – review & editing. **Manolis Kogevinas:** Funding acquisition, Writing – review & editing. **Marina Pollán:** Funding acquisition, Investigation, Methodology, Writing – review & editing. **Beatriz Pérez-Gómez:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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.

Data availability

The authors do not have permission to share data.

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Ethics

The Ethics Committee of all participating centres approved the study protocol.

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Appendix A. Supplementary data

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References

Agnew, U.M., Slesinger, T.L., 2022. Zinc Toxicity. In: *StatPearls*. StatPearls Publishing; 2022. Accessed March 30, 2022. http://www.ncbi.nlm.nih.gov/books/NBK554548/.

Amaral, A.F.S., Porta, M., Silverman, D.T., Milne, R.L., Kogevinas, M., Rothman, N., Cantor, K.P., Jackson, B.P., Pumarega, J.A., López, T., Carrato, A., Guarner, L., Real, F.X., Malats, N., 2012. Pancreatic cancer risk and levels of trace elements. Gut 61 (11), 1583–1588. https://doi.org/10.1136/gutjnl-2011-301086.

Badea, M., Luzardo, O.P., González-Antuña, A., Zumbado, M., Rogozea, L., Floroian, L., Alexandrescu, D., Moga, M., Gaman, L., Radoi, M., Boada, L.D., Henríquez-Hernández, L.A., 2018. Body burden of toxic metals and rare earth elements in non-smokers, cigarette smokers and electronic cigarette users. Environ. Res. 166, 269–275. https://doi.org/10.1016/j.envres.2018.06.007.

Bales, C.W., Freeland-Graves, J.H., Askey, S., et al., 1990. Zinc, magnesium, copper, and protein concentrations in human saliva: age- and sex-related differences. Am. J. Clin. Nutr. 51 (3), 462–469. https://doi.org/10.1093/ajcn/51.3.462.

Bel-Serrat, S., Stammers, A.-L., Warthon-Medina, M., Moran, V.H., Iglesia-Altaba, I., Hermoso, M., Moreno, L.A., Lowe, N.M., 2014. Factors that affect zinc bioavailability and losses in adult and elderly populations. Nutr. Rev. 72 (5), 334–352. https://doi.org/10.1111/nure.12105.

Berglund, M., Lindberg, A.-L., Rahman, M., Yunus, M., Grandér, M., Lönnerdal, B.o., Vahter, M., 2011. Gender and age differences in mixed metal exposure and urinary excretion. Environ. Res. 111 (8), 1271–1279. https://doi.org/10.1016/j.envres.2011.09.002.

Berglund, M., Larsson, K., Grandér, M., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Castaño, A., Esteban, M., Angerer, J., Koch, H.M., Schindler, B.K., Schoeters, G., Smolders, R., Exley, K., Sepai, O., Blumen, L., Horvat, M., Knudsen, L.E., Mørck, T.A., Joas, A., Joas, R., Biot, P., Aerts, D., De Cremer, K., Van Overmeire, I., Katsonouri, A., Hadjipanayis, A., Cerna, M., Krskova, A., Nielsen, J.K.S., Jensen, J.F., Rudnai, P., Kozepesy, S., Griffin, C., Nesbitt, I., Gutleb, A.C., Fischer, M.E., Ligocka, D., Jakubowski, M., Reis, M.F., Namorado, S., Lupsa, I.-R., Gurzau, A.E., Halzlova, K., Jajcaj, M., Mazej, D., Tratnik, J.S., Lopez, A., Cañas, A., Lehmann, A., Crettaz, P., Hond, E.D., Govarts, E., 2015. Exposure determinants of cadmium in European mothers and their children. Environ. Res. 141, 69–76. https://doi.org/10.1016/j.envres.2014.09.042.

Bergomi, M., Vinceti, M., Nacci, G., Pietrini, V., Brätter, P., Alber, D., Ferrari, A., Vescovi, L., Guidetti, D., Sola, P., Malagu, S., Aramini, C., Vivoli, G., 2002. Environmental exposure to trace elements and risk of amyotrophic lateral sclerosis: a population-based case-control study. Environ. Res. 89 (2), 116–123.

Brockman, J.D., Guthrie, J.M., Morris, J.S., Davis, J., Madsen, R., Robertson, J.D., 2009. Analysis of the toenail as a biomonitor of supranutritional intake of Zn, Cu, and Mg. J. Radioanal. Nucl. Chem. 279 (2), 405–410. https://doi.org/10.1007/s10967-007-7279-3.

Campos, F.I., Koriyama, C., Akiba, S., Carrasquilla, G., Serra, M., Carrascal, E., Yamamoto, M., Nakano, A., 2007. Toenail zinc level and gastric cancer risk in Cali, Colombia. J. Cancer Res. Clin. Oncol. 134 (2), 169–178. https://doi.org/10.1007/s00432-007-0266-1.

Canada H. Sixth report on human biomonitoring of environmental chemicals in Canada. Published December 14, 2021. Accessed May 4, 2022. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/environmental-contaminants/sixth-report-human-biomonitoring.html.

Castaño-Vinyals, G., Aragonés, N., Pérez-Gómez, B., Martín, V., Llorca, J., Moreno, V., Altzibar, J.M., Ardanaz, E., de Sanjosé, S., Jiménez-Moleón, J.J., Tardón, A., Alguacil, J., Peiró, R., Marcos-Gragera, R., Navarro, C., Pollán, M., Kogevinas, M., 2015. Population-based multicase-control study in common tumors in Spain (MCC-Spain): rationale and study design. Gac. Sanit. 29 (4), 308–315. https://doi.org/10.1016/j.gaceta.2014.12.003.

Cervantes, M., Sierra, A., García-Barrera, T., et al., 2015. Toenail metal concentrations: interlaboratory reproducibility study. Poster presented at the II Iberoamerican Public Health and Epidemiology Congress Santiago de Compostela, Spain. Published online September 2.

Chuang, H.-C., Juan, H.-T., Chang, C.-N., Yan, Y.-H., Yuan, T.-H., Wang, J.-S., Chen, H.-C., Hwang, Y.-H., Lee, C.-H., Cheng, T.-J., 2014. Cardiopulmonary toxicity of pulmonary exposure to occupationally relevant zinc oxide nanoparticles. Nanotoxicology. 8 (6), 593–604. https://doi.org/10.3109/17435390.2013.809809.

Coelho, P., Costa, S., Costa, C., Silva, S., Walter, A., Ranville, J., Pastorinho, M.R., Harrington, C., Taylor, A., Dall'Armi, V., Zoffoli, R., Candeias, C., da Silva, E.F., Bonassi, S., Laffon, B., Teixeira, J.P., 2014. Biomonitoring of several toxic metal (loid)s in different biological matrices from environmentally and occupationally exposed populations from Panasqueira mine area, Portugal. Environ. Geochem. Health 36 (2), 255–269. https://doi.org/10.1007/s10653-013-9562-7.

Csikós, A., Takacs, P., Miklós, I., 2020. Comparison of novel single nucleotide polymorphisms of zinc transporters with zinc concentration in the human blood and vaginal tissues. Biometals 33 (6), 323–337. https://doi.org/10.1007/s10534-020-00249-x.

da Rocha, T.J., Korb, C., Schuch, J.B., Bamberg, D.P., de Andrade, F.M., Fiegenbaum, M., 2014. SLC30A3 and SEP15 gene polymorphisms influence the serum concentrations of zinc and selenium in mature adults. Nutr. Res. 34 (9), 742–748. https://doi.org/10.1016/j.nutres.2014.08.009.

Durrleman, S., Simon, R., 1989. Flexible regression models with cubic splines. Stat. Med. 8 (5), 551–561. https://doi.org/10.1002/sim.4780080504.

Efron, B., Tibshirani, R.J., 1994. An Introduction to the Bootstrap. CRC Press.

Esteban, M., Castaño, A., 2009. Non-invasive matrices in human biomonitoring: a review. Environ. Int. 35 (2), 438–449. https://doi.org/10.1016/j.envint.2008.09.003.

Farag, M.A., Hamouda, S., Gomaa, S., Agboluaje, A.A., Hariri, M.L.M., Yousof, S.M., 2021. Dietary micronutrients from zygote to senility: updated review of minerals' role and orchestration in human nutrition throughout life cycle with sex differences. Nutrients 13 (11), 3740. https://doi.org/10.3390/nu13113740.

Farrán, A., Zamora, R., Cervera, P., 2003. Tablas de Composición de Alimentos Del Cesnid. https://www.sennutricion.org/es/2013/05/13/tablas-de-composicin-de-alimentos-del-cesnid.

Foster, M., Hancock, D., Petocz, P., Samman, S., 2011. Zinc transporter genes are coordinately expressed in men and women independently of dietary or plasma zinc. J. Nutr. 141 (6), 1195–1201. https://doi.org/10.3945/jn.111.140053.

Fourth National Report on Human Exposure to Environmental Chemicals, 2021. National Center for Environmental Health. doi:10.15620/cdc:105345.

Fujihara, J., Yasuda, T., Kimura-Kataoka, K., Takinami, Y., Nagao, M., Takeshita, H., 2018. Association of SNPs in genes encoding zinc transporters on blood zinc levels in humans. Leg Med. (Tokyo). 30, 28–33. https://doi.org/10.1016/j.legalmed.2017.10.009.

García-Pérez, J., Gómez-Barroso, D., Tamayo-Uria, I., Ramis, R., 2019. Methodological approaches to the study of cancer risk in the vicinity of pollution sources: the experience of a population-based case-control study of childhood cancer. Int. J. Health Geogr. 18 (1), 12. https://doi.org/10.1186/s12942-019-0176-x.

Garland, M., Morris, J.S., Rosner, B.A., et al., 1993. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. Cancer Epidemiol Biomarkers Prev. 2 (5), 493–497.

Garland, M., Morris, J.S., Colditz, G.A., Stampfer, M.J., Spate, V.L., Baskett, C.K., Rosner, B., Speizer, F.E., Willett, W.C., Hunter, D.J., 1996. Toenail trace element levels and breast cancer: A prospective study. Am. J. Epidemiol. 144 (7), 653–660.

Ghayour-Mobarhan, M., Taylor, A., New, S.A., Lamb, D.J., Ferns, G.A.A., 2005. Determinants of serum copper, zinc and selenium in healthy subjects. Ann. Clin. Biochem. 42 (Pt 5), 364–375. https://doi.org/10.1258/0004563054889990.

Giacconi, R., Costarelli, L., Malavolta, M., Cardelli, M., Galeazzi, R., Piacenza, F., Gasparini, N., Basso, A., Mariani, E., Fulop, T., Rink, L., Dedoussis, G., Herbein, G., Jajte, J., Provinciali, M., Busco, F., Mocchegiani, E., 2015. Effect of ZIP2 Gln/Arg/Leu (rs2234632) polymorphism on zinc homeostasis and inflammatory response following zinc supplementation. BioFactors 41 (6), 414–423. https://doi.org/10.1002/biof.1247.

Gonzalez, A., Peters, U., Lampe, J.W., Satia, J.A., White, E., 2008. Correlates of toenail zinc in a free-living U.S. population. Ann. Epidemiol. 18 (1), 74–77. https://doi.org/10.1016/j.annepidem.2007.07.100.

Goulle, J.P., Saussereau, E., Mahieu, L., Bouige, D., Groenwont, S., Guerbet, M., Lacroix, C., 2009. Application of inductively coupled plasma mass spectrometry multielement analysis in fingernail and toenail as a biomarker of metal exposure. J. Anal. Toxicol. 33 (2), 92–98.

Graham, N.M., Sorensen, D., Odaka, N., et al., 1991. Relationship of serum copper and zinc levels to HIV-1 seropositivity and progression to AIDS. J. Acquir. Immune Defic. Syndr. 4 (10), 976–980.

Gutiérrez-González, E., García-Esquinas, E., de Larrea-Baz, N.F., Salcedo-Bellido, I., Navas-Acien, A., Lope, V., Gómez-Ariza, J.L., Pastor, R., Pollán, M., Pérez-Gómez, B., 2019. Toenails as biomarker of exposure to essential trace metals: A review. Environ. Res. 179, 108787. https://doi.org/10.1016/j.envres.2019.108787.

Harris, D.C., 2020. Quantitative chemical analysis. Macmillan Learning.

Hashemian, M., Poustchi, H., Pourshams, A., Khoshnia, M., Brockman, J.D., Hekmatdoost, A., Abnet, C.C., Malekzadeh, R., 2016. The nail as a biomonitor of trace element status in golestan cohort study. Middle East J. Dig. Dis. 8 (1), 19–23. https://doi.org/10.15171/mejdd.2016.02.

Hopps, H.C., 1977. The biologic bases for using hair and nail for analyses of trace elements. Sci. Total Environ. 7 (1), 71–89.

Institute of Medicine (US) Panel on. *Zinc*. National Academies Press (US); 2001. Accessed April 27, 2022. https://www.ncbi.nlm.nih.gov/books/NBK222317/.

Jaramillo Ortiz, S., Howsam, M., van Aken, E.H., Delanghe, J.R., Boulanger, E., Tessier, F.J., 2022. Biomarkers of disease in human nails: a comprehensive review. Crit. Rev. Clin. Lab. Sci. 59 (2), 125–141.

Kambe, T., Tsuji, T., Hashimoto, A., Itsumura, N., 2015. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. Physiol. Rev. 95 (3), 749–784. https://doi.org/10.1152/physrev.00035.2014.

Kayaaltı, Z., Aliyev, V., Söylemezoğlu, T., 2011. The potential effect of metallothionein 2A–5A/G single nucleotide polymorphism on blood cadmium, lead, zinc and copper levels. Toxicol. Appl. Pharmacol. 256 (1), 1–7. https://doi.org/10.1016/j.taap.2011.06.023.

Kilinc, E., Buturak, B., Alkan, F.A., 2020. Level of trace elements in serum and toenail samples of patients with onychocryptosis (ingrown toenail) and onychomycosis. J. Trace Elem. Med. Biol. 61, 126509. https://doi.org/10.1016/j.jtemb.2020.126509.

Kimura, T., Kambe, T., 2016. The functions of metallothionein and ZIP and ZnT transporters: an overview and perspective. Int. J. Mol. Sci. 17 (3), 336. https://doi.org/10.3390/ijms17030336.

King, J.C., 2011. Zinc: an essential but elusive nutrient, 679S-84S Am. J. Clin. Nutr. 94 (2). https://doi.org/10.3945/ajcn.110.005744.

King, J.C., Shames, D.M., Woodhouse, L.R., 2000. Zinc homeostasis in humans, 1360S-6S J. Nutr. 130 (5S Suppl). https://doi.org/10.1093/jn/130.5.1360S.

King, J.C., Brown, K.H., Gibson, R.S., et al., 2016. Biomarkers of nutrition for development (BOND)—zinc review. J. Nutr. 146 (4), 858S–885S. https://doi.org/10.3945/jn.115.220079.

Locutura-Rupérez, J., *Atlas Geoquímico de España*. Vol cesnid.; 2012. Accessed January 15, 2022. https://info.igme.es/catalogo/resource.aspx?portal=1&catalog=3&ctt=1&lang=spa&dlang=eng&llt=dropdown&master=infoigme&shdt=false&shfo=false&resource=8309.

Lowe, N.M., Fekete, K., Decsi, T., 2009. Methods of assessment of zinc status in humans: a systematic review, 2040S-51S Am. J. Clin. Nutr. 89 (6). https://doi.org/10.3945/ajcn.2009.27230G.

Marinho Reis, A.P., Cave, M., Sousa, A.J., Wragg, J., Rangel, M.J., Oliveira, A.R., Patinha, C., Rocha, F., Orsiere, T., Noack, Y., 2018. Lead and zinc concentrations in household dust and toenails of the residents (Estarreja, Portugal): a source-pathway-fate model. Environ. Sci. Process Impacts. 20 (9), 1210–1224.

Martin-moreno, J.M., Boyle, P., Gorgojo, L., Maisonneuve, P., Fernandez-rodriguez, J.C., Salvini, S., Willett, W.C., 1993. Development and validation of a food frequency questionnaire in Spain. Int. J. Epidemiol. 22 (3), 512–519.

Martin-Moreno, J.M., Gorgojo, L., Riemersma, R.A., Gomez-Aracen, J., Kark, J.D., Guillen, J., Jimenez, J., Ringstad, J.J., Fernandez-Crehuet, J., Bode, P., Kok, F.J., 2003. Myocardial infarction risk in relation to zinc concentration in toenails. Br. J. Nutr. 89 (5), 673–678. https://doi.org/10.1079/BJN2003825.

Matthews, N.H., Koh, M., Li, W.Q., et al., 2019. A prospective study of toenail trace element levels and risk of skin cancer. Cancer Epidemiol. Biomarkers Prev. 28 (9), 1534–1543. https://doi.org/10.1158/1055-9965.EPI-19-0214.

Milunsky, A., Morris, J.S., Jick, H., Rothman, K.J., Ulcickas, M., Jick, S.S., Shoukimas, P., Willett, W., 1992. Maternal zinc and fetal neural tube defects. Teratology. 46 (4), 341–348. https://doi.org/10.1002/tera.1420460405.

Mohmand, J., Eqani, S.A.M.A.S., Fasola, M., Alamdar, A., Mustafa, I., Ali, N., Liu, L., Peng, S., Shen, H., 2015. Human exposure to toxic metals via contaminated dust: Bio-accumulation trends and their potential risk estimation. Chemosphere 132, 142–151. https://doi.org/10.1016/j.chemosphere.2015.03.004.

Ndilila, W., Callan, A.C., McGregor, L.A., Kalin, R.M., Hinwood, A.L., 2014. Environmental and toenail metals concentrations in copper mining and non mining communities in Zambia. Int. J. Hyg. Environ. Health 217 (1), 62–69. https://doi.org/10.1016/j.ijheh.2013.03.011.

Nouri, M., Chalian, H., Bahman, A., et al., 2008. Nail molybdenum and zinc contents in populations with low and moderate incidence of esophageal cancer. Arch. Iran Med. 11 (4), 392–396. https://doi.org/10.08114/AIM.0010.

Núñez, O., Fernández-Navarro, P., Martín-Méndez, I., Bel-Lan, A., Locutura Rupérez, J.F., López-Abente, G., 2017. Association between heavy metal and metalloid levels in topsoil and cancer mortality in Spain. Environ. Sci. Pollut. Res. 24 (8), 7413–7421. https://doi.org/10.1007/s11356-017-8418-6.

O'Rorke, M.A., Cantwell, M.M., Abnet, C.C., Brockman, A.J.D., Murray, L.J., 2012. FINBAR Study Group. Toenail trace element status and risk of Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. Int. J. Cancer 131 (8), 1882–1891. https://doi.org/10.1002/ijc.27434.

Park, J.S., Xun, P., Li, J., Morris, S.J., Jacobs, D.R., Liu, K., He, K.a., 2016. Longitudinal association between toenail zinc levels and the incidence of diabetes among American young adults: The CARDIA Trace Element Study. Sci. Rep. 6 (1) https://doi.org/10.1038/srep23155.

Pinheiro, J.C., Bates, D.M. (Eds.), 2000. Extending the Basic Linear Mixed-Effects Model. In: Mixed-Effects Models in S and S-PLUS. Statistics and Computing. Springer, pp. 201–270. https://doi.org/10.1007/0-387-22747-4_5.

Plum, L.M., Rink, L., Haase, H., 2010. The essential toxin: impact of zinc on human health. Int. J. Environ. Res. Public Health 7 (4), 1342–1365. https://doi.org/10.3390/ijerph7041342.

Poddalgoda, D., Macey, K., Hancock, S., 2019. Derivation of biomonitoring equivalents (BE values) for zinc. Regul. Toxicol. Pharm. 106, 178–186. https://doi.org/10.1016/j.yrtph.2019.04.018.

PRTR España | Registro Estatal de Emisiones y Fuentes Contaminantes (PRTR-España). Accessed April 29, 2022. https://en.prtr-es.es/.

Raińska, E., Biziuk, M., Sarbu, C., Szczepaniak, K., Frontasyeva, M.V., Culicov, O., Bode, P., Astel, A., 2005. Assessment of phosphatic fertilizer production impact on occupational staff based on NAA of hair, nails, and inhald particles. J. Environ. Sci. Health A Tox. Hazard Subst. Environ. Eng. 40 (12), 2137–2152. https://doi.org/10.1080/10934520500234635.

Rakovic, M., Foltýnová, V., Pilecká, N., Glagolicová, A., Kucera, J., 1997. Assessment of metals and metalloids in skin derivatives of volunteers from capital city of Prague. Czech Republic. *Sb Lek*. 98 (2), 107–114.

Regassa, G., Chandravanshi, B.S., 2016. Levels of heavy metals in the raw and processed Ethiopian tobacco leaves. Springerplus. 5, 232. https://doi.org/10.1186/s40064-016-1770-z.

Roohani, N., Hurrell, R., Kelishadi, R., Schulin, R., 2013. Zinc and its importance for human health: An integrative review. J. Res. Med. Sci. 18 (2), 144–157. Accessed March 20, 2022. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3724376/.

Sandstead, H.H., 2015. In: Handbook on the Toxicology of Metals. Elsevier, pp. 1369–1385. https://doi.org/10.1016/B978-0-444-59453-2.00061-5.

Simon-Hettich, B., Wibbertmann, A., Wagner, D., et al., 2001. Zinc. World Health Organization. Accessed March 20, 2022. https://apps.who.int/iris/handle/10665/42337.

Skoog, D.A., Holler, F.J., Crouch, S.R., 2017. Principles of Instrumental Analysis. Cengage Learning.

Sukumar, A., 2006. Human nails as a biomarker of element exposure. In: Reviews of Environmental Contamination and Toxicology. Reviews of Environmental Contamination and Toxicology. Springer, New York, NY, pp. 141–177. https://doi.org/10.1007/0-387-30638-2_5.

Sureda, A., Bibiloni, M.D.M., Julibert, A., Aparicio-Ugarriza, R., Palacios-Le Blé, G., Pons, A., Gonzalez-Gross, M., Tur, J.A., Hu, Y.i., 2017. Trace element contents in toenails are related to regular physical activity in older adults. PLoS ONE 12 (10), e0185318. https://doi.org/10.1371/journal.pone.0185318.

Suzuki, K., Koizumi, S., 2000. Individual metal responsive elements of the human metallothionein-IIA gene independently mediate responses to various heavy metal signals. Ind. Health 38 (1), 87–90. https://doi.org/10.2486/indhealth.38.87.

Tang, W., Xun, P., Chen, C., Lu, L., Sood, A., Shikany, J.M., Kahe, K.a., 2021. Association between toenail zinc concentrations and incidence of asthma among American young adults: The CARDIA study. J. Trace Elem. Med. Biol. 64, 126683. https://doi.org/10.1016/j.jtemb.2020.126683.

Tubek, S., 2006. Gender differences in selected zinc metabolism parameters in patients with mild primary arterial hypertension. Biol. Trace Elem. Res. 114 (1–3), 55–63. https://doi.org/10.1385/BTER:114:1:55.

Virgili, F., Ambra, R., McCormack, J., Simpson, E.E.A., Ciarapica, D., Barnaba, L., Azzini, E., Polito, A., 2019. Genetic polymorphisms and zinc status: implications for supplementation in metabolic diseases. Curr. Pharm. Des. 24 (35), 4131–4143. https://doi.org/10.2174/1381612824666181016155903.

Wastney, M.E., Ahmed, S., Henkin, R.I., 1992. Changes in regulation of human zinc metabolism with age. Am. J. Physiol. 263 (5 Pt 2), R1162–R1168. https://doi.org/10.1152/ajpregu.1992.263.5.R1162.

Were, F.H., Njue, W.M., Murungi, J., Wanjau, R., 2009. Comparison of some essential and heavy metals in the toenails and fingernails of school-age children in Kenya. Bull. Chem. Soc. Ethiop. 23 (1), 117–122.

WHO, 1996. Trace Elements in Human Nutrition and Health. Published online.

Wilhelm, M., Hafner, D., Lombeck, I., Ohnosorge, F., 1991. Monitoring of cadmium, copper, lead and zinc status in young children using toenails: comparison with scalp hair. Sci. Total Environ. 103 (2-3), 199–207.

Willett, W.C., Howe, G.R., Kushi, L.H., 1997. Adjustment for total energy intake in epidemiologic studies, 1220S-1228S; discussion 1229S-1231S Am. J. Clin. Nutr. 65 (4 Suppl). https://doi.org/10.1093/ajcn/65.4.1220S.

World Health Organization, Agency IAE, Nations F and AO of the U. *Trace elements in human nutrition and health*. World Health Organization; 1996. Accessed May 4, 2022. https://apps.who.int/iris/handle/10665/37931.

Zinc | Toxicological Profile | ATSDR. Accessed June 29, 2022. https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=302&tid=54.