Main biomarkers associated with age-related plasma zinc decrease and copper/zinc ratio in healthy elderly from ZincAge study

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R. Giacconi[1] L. Costarelli[1] F. Piacenza[1] A. Basso[1] L. Rink[2] E. Mariani[3] T. Fulop[4] G. Dedoussis[5] G. Herbein[6] M. Provinciali[7] J. Jajte[8] I. Lengyel[9] E. Mocchegiani[1] M. Malavolta[1]

Abstract

Purpose :Zinc (Zn) plays an essential role in many biologi-cal processes including immune response. Impaired Zn sta-tus promotes immune dysfunction, and it has been associ-ated with enhanced chronic inflammation during aging. It has been suggested that the measurement of circulating Zn by itself could not reflect the real Zn status of an individ-ual. It is therefore necessary to identify other determinants associated with plasma Zn to better understanding how physiopathological conditions during aging may affect the concentration of this metal.

Methods We have investigated the association between Zn levels and some biomarkers in 1090 healthy elderly from five European countries to increase the accuracy in the assessment of the Zn status. Stepwise multivariate linear regression models were used to analyze the influence of factors such as age, dietary intake, inflammatory mediators, laboratory parameters and polymorphisms previously associated with Zn homeostasis.

Results Plasma Zn decrement was most strongly predicted by age, while positive correlations were found with albumin, RANTES and Zn intake after adjustment for multiple confounders. HSP70 1267 AA genotype was an independent factor associated with Zn plasma concentrations. Cu/Zn ratio was positively associated with markers of systemic inflammation and age and negatively associated with albumin serum levels.

Conclusions Our findings show the most important independent determinants of plasma Zn concentration and Cu/Zn ratio variability in elderly population and suggest that the decline with age of Zn circulating levels is more dependent on physiopathological changes occurring with aging rather than to its nutritional intake.

Keywords Zinc plasma levels · Inflammation · Polymorphisms · Zinc homeostasis · Aging

R. Giacconi giacconi@inrca.it

[1] Translational Research Ctr. of Nutrition and Ageing, Scientific and Technological Pole, Italian National Institute of Health and Science on Aging (INRCA), Ancona, Italy

[2] Institute of Immunology, Medical Faculty, RWTH Aachen University, Aachen, Germany

[3] Laboratory of Immunereumatology and Tissue Regeneration/ RAMSES, Department of Medical and Surgical Sciences, Rizzoli Orthopedic Institute, University of Bologna, Bologna, Italy

[4] Department of Medicine, Faculty of Medicine, Research Center on Aging, University of Sherbrooke, Sherbrooke, Canada

[5] Department of Dietetics and Nutritional Science, Harokopio University of Athens, Athens, Greece

[6] Department Pathogens and Inflammation EA 4266, Université Bourgogne Franche-Comté, CHRU Besançon, Besançon, France

[7] Advanced Technology Center for Aging Research, Scientific and Technological Pole, Italian National Institute of Health and Science on Aging (INRCA), Ancona, Italy

[8] Department of Toxicology, Faculty of Pharmacy, Medical University, Lodz, Poland

[9] UCL Institute of Ophthalmology, University College London, 11-43 Bath Street, London, UK

Introduction

Zinc (Zn) is an essential micronutrient required for many bio-logical processes including growth and development, neuro-logical function and dysfunction, reproduction, cancer and diabetes. Impaired Zn status has also been associated with immune dysregulation, increased susceptibility to infections and chronic inflammation especially in the elderly [1, 2].

Several studies show a decline of Zn plasma levels with age [3–6] that may be attributed to a reduced dietary Zn intake, decreased intestinal absorption, alteration in Zn transporter proteins, inadequate mastication, drug inter-actions and dietary phytate intake [2, 7]. However, stud-ies comparing the effect of age in Zn absorption between young and elderly individuals are inconclusive [8]; there-fore, it has not been fully clarified what determines the decrease of Zn concentrations in the plasma. Phytic acid intake seems clearly associated with an impaired Zn bioa-vailability [9, 10]; however, similar phytate intake has been found between young and elderly populations, especially after adjusting for differences in energy intake [11].

Cellular Zn homeostasis is regulated by two main classes of proteins: Zn transporters and metallothioneins (MT). Zn transporters include two families: hZIP (pro-tein solute carrier family SLC39A) and hZnT (human Zn transporter, SLC30A) which play critical roles in cellular and physiological functions by modulating the Zn influx, efflux and compartmentalization across biological mem-branes [12]. MT exert a homeostatic buffering role, main-taining the free intracellular concentration of Zn ions at a low level and supplying Zn to various protein targets, such as Zn-dependent enzymes and Zn finger-dependent tran-scription factors [13]. MT and Zn transporters expression is closely related to Zn plasma concentrations [14, 15] and is influenced by genetic variations, for example in single-nucleotide polymorphisms [15–19].

It appears that there is strict relationship between dys-regulation of Zn transporter expression, Zn homeostasis and inflammatory response, and this phenomenon may contribute to low Zn status and immune dysfunction dur-ing aging contributing to the development of age-related diseases [20–22]. Inflammatory conditions can be accom-panied by an increase in copper (Cu) serum concentration leading to an altered copper/zinc ratio (Cu/Zn) [23]. An increased Cu/Zn ratio has been suggested to represent an inflammatory–nutritional biomarker and a sensitive pre-dictor of disability and mortality in elderly subjects aged 70 years and above [24].

To date, there is no single reliable biomarker of Zn sta-tus, and, despite the known limitations, plasma Zn concen-tration remains the measurement used in most studies. It is therefore important to identify other determinants that are associated with plasma Zn levels both to reveal possible confounding factors and to use this information to develop a better understanding of how physiopathological processes (e.g., aging) affect plasma Zn concentration. The present study will analyze the main predictors of Zn plasma lev-els in a large cohort of healthy old people including dietary intake, inflammatory mediators, polymorphisms previously associated with Zn homeostasis and laboratory parameters.

Materials and methods

Subjects

The study included 1090 elderly healthy independent-living subjects enrolled in the ZincAge project (www.zincage.org). Subjects were recruited by announcements at general prac-titioners' surgeries from five European centers located in Italy (n =361), Greece (n =209), Poland (n 158), France (n =213) and Germany (n 149). According to the inclu-sion criteria of the ZincAge protocol [15], all subjects (602 females and 488 males; mean age = 74.6 8.7) were in good health without functional impairment. The participants of the study were free of steroids, diuretics, anticonvulsants, anti-depressive drugs, antibiotics, antimetabolites, nonster-oid anti-inflammatory drugs and micronutrient supplementa-tion. The health status was evaluated by a specific question-naire on health and morbidity planned for the ZincAge study and completed by the general practitioner of the participants after medical examination and review of the clinical records. Subjects were excluded if they had autoimmune, neurode-generative, cardiovascular, kidney or liver disease, diabe-tes, infections, cancer, chronic inflammatory bowel disease, acrodermatitis enteropathica, sickle cell anemia, chronic skin ulcerations or endocrine disorders. All participants gave informed consent for the genetic analysis and the ethics com-mittees from each country approved the protocol.

Laboratory determinations

Venous peripheral blood samples, collected after an over-night fast, underwent basal biochemical laboratory tests. The hematocrit and hemoglobin counts and erythrocyte sedimentation rate were performed by standard automated procedures (Sysmex XE-2100).

Blood concentrations of total cholesterol [limit of detec-tion (LoD) was 3 mg/dl; high values ≥ 240 mg/dl], HDL cholesterol, glucose (LoD was 2 mg/dl, normal values 70–110 mg/dl) and albumin (LoD was 0.2 g/dl, normal values 3.5–5.2 g/dl) were measured by an enzymatic col-orimetric test on modular automated clinical chemistry analyzers (Roche–Hitachi). Serum concentration of high-sensitive C-reactive protein (hs-CRP) was determined by amplified immunonephelometry assay (CardioPhase

hsCRP—Dade Behring Inc Deerfield, IL). Serum, plasma and buffy coats were separated, aliquoted and stored frozen at −80 °C in the Biological Bank of INRCA until analysis.

Assessment of dietary Zn intake and the Mediterranean diet score

A qualitative food frequency questionnaire, designed for the needs of ZincAge project (ZincAge project, 2004–2007, www.ZincAge.org), was used for the assessment of dietary Zn intake in healthy elderly subjects. The consumption of 53 food items was recorded and, based upon these data, a "zinc score" for each volunteer was determined. To provide a continuous variable, representative of Zn dietary habits, frequency, quantity estimation and Zn content of foods consumed were all considered for the "zinc score" calculation (zinc score = frequency 9 quantity 9 zinc content). A validation study of the "zinc score" has been previously reported [25]. A dietary pyramid has been developed to describe the Mediterranean dietary pattern. According to this dietary pattern, we calculated each participant's special diet score, which assessed adherence to the Mediterranean diet (range 0–55), as described elsewhere [26]. In brief, for the consumption of items presumed to be close to this pattern (non-refined cereals, fruit, vegetables, potatoes, legumes, fish and olive oil), it has been assigned a score of 0 for no consumption and scores 1–5 for rare to daily consumption. On the other hand, for the consumption of foods presumed to be away from this diet (red meat and red meat products, poultry and full-fat dairy products), it has been assigned the opposite scores (i.e., 0 when a participant reported daily consumption to 5 for rare or no consumption). For alcohol, the assigned score was 5 for consumption of >300 ml/day, a score of 0 for consumption of more than 700 ml/day, and scores of 1–4 for consumption of 300, 400, 500 and 600 ml/day (containing 12 g of ethanol per 100 ml).

Genotyping

Genomic DNA was isolated from whole blood using a commercially available kit (Wizard® Genomic DNA Purification Kit, Promega Corporation) according to the manufacture's instruction. Genotyping was performed for the following single-nucleotide polymorphisms:

1267 A/G (rs1061581) HSP70-2; −308 G/A (rs1800629) TNF-α; −174 G/C (rs 1800795).
IL-6; Gln/Arg/Leu variation Zip-2 (rs2234632); +647 A/C (rs11640851) and +1245 A/G (rs8052394) MT1A; −209 A/G (rs1610216) MT2A.
All polymorphisms were performed following the methods reported in our previous studies [17, 19, 27–29].

Zinc and copper plasma measurements

The plasma zinc (Zn) and copper (Cu) concentrations were determined by a Thermo XII Series ICP-MS (Thermo Electron Corporation, Waltham, MA, USA) by adapting methods used for the measurement of trace elements in human plasma with slight modifications [24]. Plasma samples were diluted 1:10, with a diluent containing 0.1 % triton and 0.15 % HNO3, to ensure that trace elements are maintained in solution and to favor the washout of these elements between samples. External calibration solutions containing Zn and Cu (blank to 2000 ppb) were prepared by serial dilution of a parent multi-element solution (1000 ppm for Zn and Cu) (VHG Labs, Manchester, USA), using the same diluent used for the samples. Rhodium (Rh) at 200 ng/ml was used as internal standard. Data were acquired for 66Zn, 65Cu.

Quality of the analysis was assured by the assessment of "quality standard samples" (SERONORM™ TRACE ELEMENT SERUM, Sero AS, Billingstad, Norway). Zn and Cu levels of the quality standard samples were within 10 % of the certified levels, as previously reported [24]. Limits of detection estimated with the post-column calibration were 0.5 ppb for 65Cu and 5 ppb for 66Zn. The instrument was operated with a Peltier cooled impact bead spray chamber, single-piece quartz torch (1.5 mm i.d. injector) together with Xi interface cones and a Cetac-ASX 100 autosampler (CETAC Technologies, Omaha, NE). A Burgener Trace nebulizer was used as this device does not block during aspiration of clinical samples. The instrument was operated in standard mode (non-CCT), using 1400 WRF power, 1.10 l/min nebulizer gas flow, 0.70 l/min auxiliary gas flow, 13.0 l/min cool gas flow, 70 ms dwell time, 30 s sample uptake and 35 s wash time (2 repeats per sample).

Multiple immunoassay for chemokine and cytokine plasma assessment

Fourfold-diluted plasma samples were assayed in duplicate using commercially available multiplex bead-based immunoassay kits. IL-6, IL-8, TNF-α, MCP-1, MIP-1α and RANTES concentrations were simultaneously evaluated using multiplex reagent kits and the Bio-Plex Protein Array System (Bio-Rad Laboratories, Hercules, CA, USA) as previously described [29]. Standard curves used for each soluble factor ranged for IL-6, from 39,525 to 2.4 pg/ml; for IL-8 from 31,592 to 1.9 pg/ml; for MCP-1 from 22,802 to 1.4 pg/ml; for MIP-1α from 15,460 to 0.9 pg/ml; for RANTES from 22,134 to 1.4 pg/ml; and for TNF- α from 126,859 to 7.7 pg/ml.

Values presenting a coefficient of variation beyond 10 % were discarded before the final data analysis. Data were analyzed using the Bio-Plex Manager software version 3.0 (Bio-Rad Laboratories, USA) and expressed as pg or ng/ml.

Table 1 Baseline characteristics of elderly subjects

	All subjects $N = 1090$	Females	Males	p value
Age (years)	74.6 ± 8.7	74.3 ± 9.0	73.8 ± 8.2	0.41
BMI	25.8 ± 3.9	26.0 ± 5.1	25.3 ± 3.4	0.71
WBC ($10^3/\mu$l)	6.32 ± 2.68	6.32 ± 2.63	6.46 ± 2.75	0.10
Lymphocytes (%)	31.3 ± 8.3	32.2 ± 8.2	30.1 ± 8.3	0.001
Neutrophils (%)	58.0 ± 8.8	57.6 ± 8.8	57.6 ± 8.7	0.13
Erythrocytes ($10^6/\mu$l)	4.6 ± 0.5	4.5 ± 0.4	4.8 ± 0.5	0.001
Hemoglobin (g/dl)	14.0 ± 1.4	13.5 ± 1.3	14.6 ± 1.3	0.001
CRP (pg/ml)	0.45 ± 1.20	0.41 ± 0.81	0.54 ± 1.47	0.060
Albumin (g/dl)	4.20 ± 0.41	4.14 ± 0.44	4.21 ± 0.40	0.01
Glycemia (mg/dl)	94.3 ± 14.9	93.1 ± 15.2	95.5 ± 15.0	0.01
TG (mg/dl)	119.8 ± 62.0	123.5 ± 62.0	118.5 ± 64.7	0.23
TC (mg/dl)	220.2 ± 42.2	227.5 ± 41.1	211.8 ± 40.2	0.001
HDL-C (mg/dl)	59.1 ± 14.8	62.4 ± 15.6	55.4 ± 13.8	0.001
Zinc score	156 ± 94	147 ± 93	168 ± 95	0.001
Mediterranean diet scores	28.9 ± 4.1	28.6 ± 4.2	29.4 ± 4.1	0.003
Zinc plasma levels (μM)	12.1 ± 2.3	12.1 ± 2.2	12.2 ± 2.3	0.36
Cu/Zn ratio	1.54 ± 0.43	1.63 ± 0.45	1.4 ± 0.43	0.001

Data are mean ± SD

Comparisons between males and females were performed by ANCOVA adjusting for age and country

WBC white blood cells, *CRP* C-reactive protein, *TG* triglycerides, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *Cu/Zn* copper/zinc ratio

Statistical analysis

An automatic linear modeling was carried out to explore the main predictors of Zn plasma levels. The variables inserted were: age, gender, Zn score, Mediterranean score, center, cytokines and chemokines, C-reactive protein, albumin and LDL cholesterol serum levels, BMI and genetic determinants [MT1A +647 (Asp/Thr), MT1A 1245 (Lys/Arg), MT2A −209 A/G, TNF-α −308 A/G, IL-6 174 G/C, ZIP2 Gln/Arg/Leu (rs2234632), HSP70 1267 A/G SNPs].

A stepwise model selection procedure was used to build a multiple linear regression model for determining effect sizes and significance of associations between Zn plasma levels SNPs, Zn score, Mediterranean score, inflammatory mediators and biochemical parameters. Log transformation of the variables was performed if they were not normally distributed as assessed by the Kolmogorov–Smirnov test. Data analyses were performed with SPSS Statistics version 20.0.0 (IBM, IL, USA).

Results

Baseline characteristics of healthy elderly population

Baseline characteristics of the ZincAge sample are shown in Table 1. No differences between gender were observed for age, WBC, BMI, percentage of neutrophils, Zn plasma concentrations, CRP and triglyceride serum levels. Females displayed higher percentage of lymphocytes, total and HDL cholesterol, Cu/Zn ratio and lower values of hemoglobin, erythrocytes, albumin and fasting glucose than males ($p < 0.01$). Zn score and Mediterranean diet scores were higher in males as compared to females (Table 1; $p < 0.01$). Genotypic frequencies of MT1A +647 (Asp/Thr), MT1A +1245 (Lys/Arg), MT2A 209 A/G, TNF-α −308 A/G, IL-6 174 G/C, ZIP2 Gln/Arg/Leu (rs2234632), and HSP70 1267 A/G SNPs ZIP2 Gln/Arg/Leu in the ZincAge population were consistent with the Hardy–Weinberg equilibrium ($p > 0.05$). No significant differences in the genotype or allele frequencies distribution were observed in relation to gender (Supplementary Materials, Table 1S–7S).

Automatic linear modeling and multivariate linear regression analyses for variables independently associated with Zn plasma levels and Cu/Zn ratio

Figure 1 shows a negative correlation of age with plasma Zn (A) and zinc score (B) in elderly males and females. According to the automatic linear modeling, we have categorized the top 7 important predictors of Zn plasma level variations as follows: center of recruitment, albumin serum levels, age, RANTES plasma levels, Zn score, Mediterranean score and HSP70 SNP (Fig. 2). To confirm the

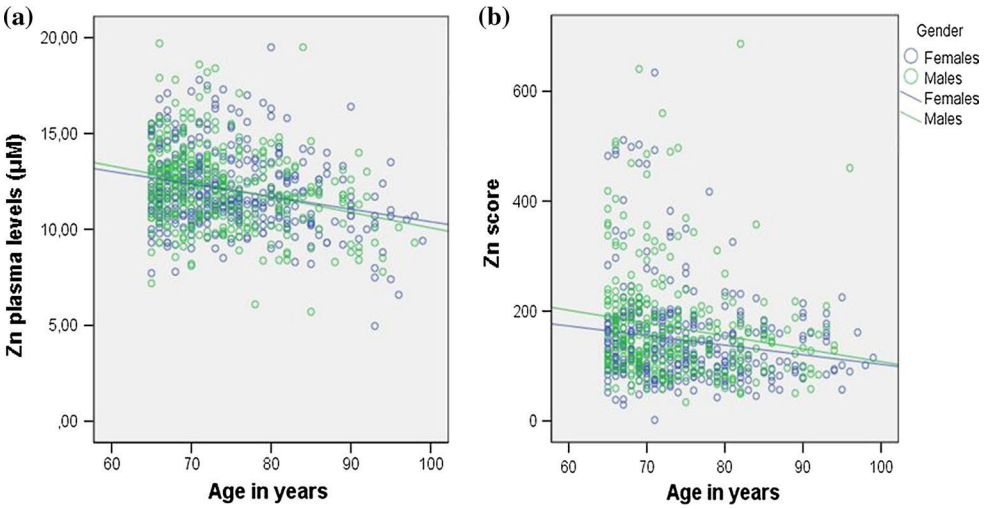

Fig. 1 Scatter plot illustrating the relationship between Zn plasma levels (**a**) and Zn score (**b**) with age $N = 1090$; females: $r = -0.312$ (**a**); $r = -0.19$ (**b**); males: $r = -0.332$ (**a**); $r = -0.312$ (**b**)

independent contributions of the variables to the Zn plasma concentrations, a multiple linear regression model was built (Table 2). The variability in the plasma Zn concentrations was best explained by age, albumin serum concentration, RANTES plasma levels, Zn score and HSP70+1267 A/G SNP.

The adjusted coefficient of determination (R^2) was 0.169 (Model 5, Table 2). Genetic Hsp70 determinant explained approximately 10 % of the total variability.

As expected from the previous literature [3], there was a negative association between plasma Zn concentration and age (Table 2; Fig. 1). In contrast, there was a positive association between Zn score, albumin serum levels and RANTES plasma concentrations (Table 2, Model 5). A significant association was found with 1267 A/G HSP70 SNP; indeed, AA variant showed lower Zn plasma levels than AG + GG ones (Fig. 3). Only a trend was observed for the recruitment center (data not shown, p 0.100). The positive association between Mediterranean score and Zn plasma levels in the automatic linear modeling has not been confirmed with the multiple linear modeling (data not shown, p 0.27).

Following, we evaluated the main determinants of Cu/Zn ratio changes that has been suggested an important inflammatory–nutritional biomarker and a sensitive predictor of disability and mortality in elderly subjects [24]. The multiple linear regression model showed that the Cu/Zn is associated with erythrocyte sedimentation rate (ESR), albumin levels, gender, age and CRP serum concentration. A positive correlation was observed with age, ESR and CRP, while a negative association was found with albumin levels. The adjusted coefficient of determination (R^2) was 0.239 (Model 5, Table 3). As previously reported [24], females under 85 years of age showed higher values of Cu/Zn ratio than males (Fig. 4; $p < 0.05$).

Discussion

Zn plays an important role in the regulation of the immune response, particularly T cell-mediated function [30]. Zn deficiency can impair immunity, induce inflammatory response [31] and increase susceptibility to infectious diseases, a major cause of mortality in the elderly [1, 32]. Plasma Zn concentrations are under tight homeostatic control and may not reflect changes in Zn intakes and status [23, 33]. Still, circulating Zn levels remains the most widely used method to determine Zn status, despite the known limitations.

For the first time in this study, we have analyzed the influence of multiple determinants involved in immune–inflammatory response and Zn homeostasis on Zn plasma concentrations in a large population of elderly subjects to better understanding how physiopathological states during aging affect circulating Zn levels. Age was the most important predictor of Zn plasma level changes followed by albumin and RANTES levels, Zn dietary intake (Zn score) and HSP70 +1267 A/G variants. Moreover, the Cu/Zn ratio appears to be strongly associated with systemic inflammatory factors. Herein, the plasma Zn concentrations decreased with age similarly to what observed in previous reports [3, 4, 6]. The negative correlation between plasma Zn and age remained significant even after adjustment for confounders. Consistent with previous studies [34, 35], we found a positive association between plasma Zn and its main serum carrier, albumin. An important factor that may have a dramatic impact on albumin and Zn during aging is inflammation [36]. Pro-inflammatory cytokines can also upregulate the Zn importer Zip14 in the liver that contributes to the serum hypozincemia during the inflammatory response [37]. Reduced intracellular Zn in immune cells has been also

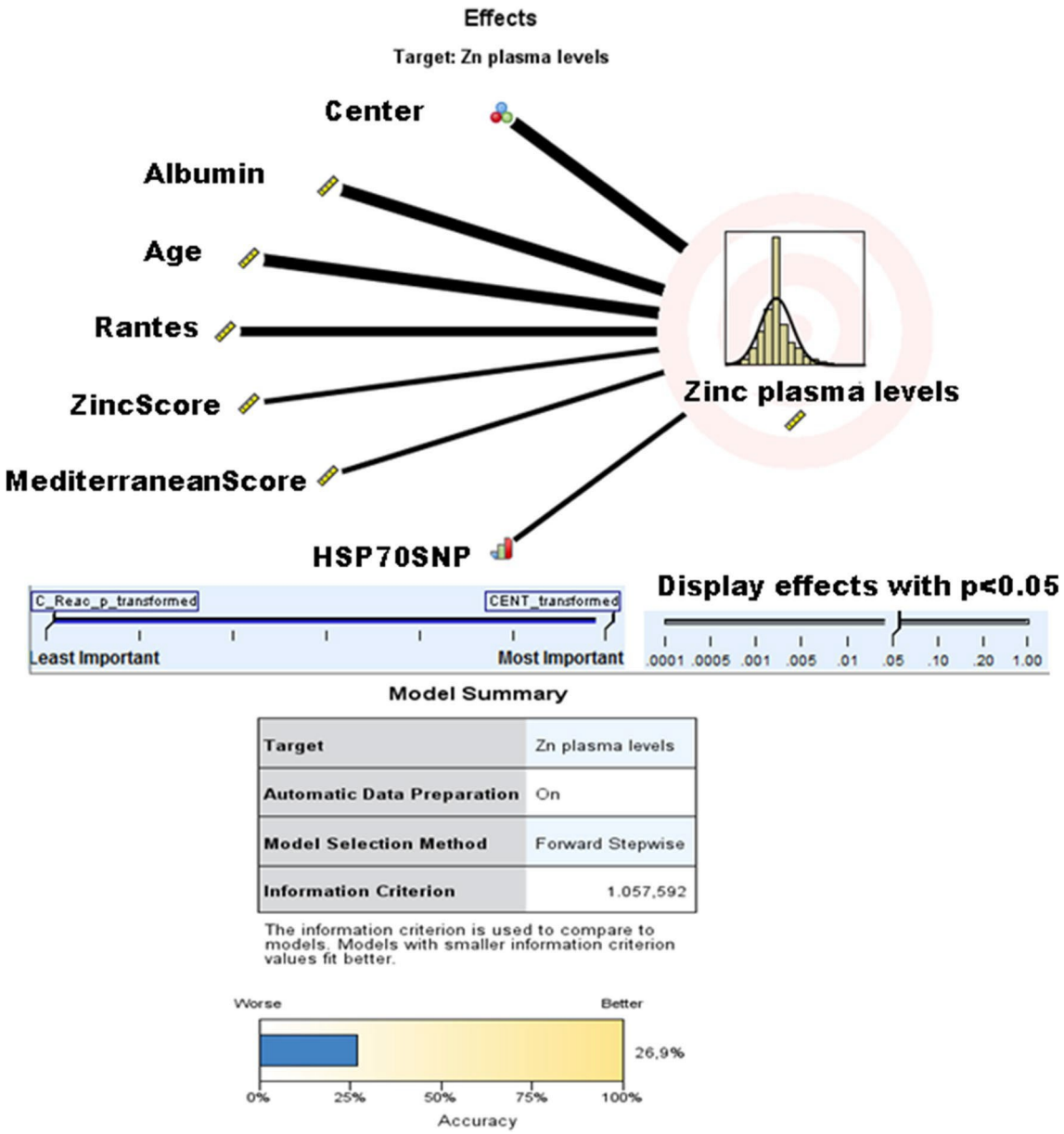

Fig. 2 Coefficients (SPSS automatic linear modeling) for Zn plasma levels. This *chart* displays the intercept first and then sorts effects *from top to bottom* in decreasing predictor importance. Within effect containing factors, coefficients are sorted by ascending order of data values. The width of *connecting lines* in the diagram reflects the coefficient significance, with greater line width corresponding to more significant coefficients (smaller *p* values)

linked to increased production of inflammatory mediators in aging [21, 29]. Surprisingly, we have not found a significant association with the main inflammatory factors, but a positive correlation with RANTES plasma levels. RANTES, also known as chemokine ligand 5 (CCL5), modulates leukocyte migration and plays a role in T cell activation, thus enhancing T cell proliferation and cytotoxicity [38], as well as promoting a prompt and efficacious inflammatory response and host defense against infection [39]. Contradictory results exist on circulating RANTES changes with aging [40, 41]. LPS-induced RANTES production was shown to be impaired in peritoneal leukocytes from old mice, but preserved in long-lived animals [42]. In our cohort, the circulating levels of RANTES are unaffected by age even after correction for the main covariates (Zn score, BMI, center) (Fig. 5S Supplementary Material), but our population consisted entirely of elderly subjects.

Table 2 Multivariate linear regression analyses for variables independently associated with Zn plasma levels

Model	Predictors	Standardized beta coefficients	Adjusted R^2	p value
1	Age	−0.298	0.087	0.0001
2	Age	−0.225	0.123	0.0001
	Albumin	0.208		0.0001
3	Age	−0.201	0.153	0.0001
	Albumin	0.201		0.0001
	RANTES	0.183		0.0001
4	Age	−0.181	0.162	0.0001
	Albumin	0.202		0.0001
	RANTES	0.183		0.0001
	Zn score	0.103		0.025
5	Age	−0.177	0.169	0.0001
	Albumin	0.204		0.0001
	RANTES	0.184		0.0001
	Zn score	0.104		0.023
	HSP70 +1267 A/G SNP[a]	0.098		0.028

[a] Reference genotype was B+ (AG and GG)

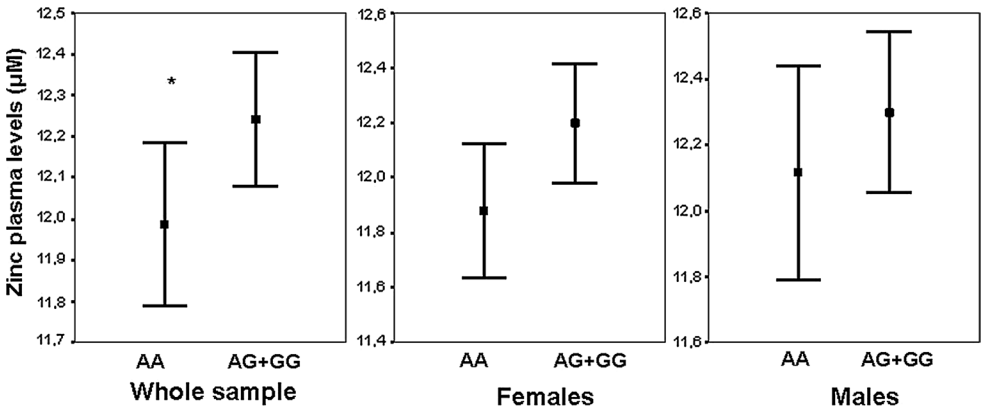

Fig. 3 Zn plasma levels according to HSP70 1267 A/G SNP in the total population and in elderly subjects subdivided by gender. Error bars represent 95 % CIs for the mean values. The genotype effect was determined by ANCOVA correcting for age, gender, Zn score, center, CRP, albumin levels and BMI *$p < 0.05$ as compared to AG and GG carriers

The positive association between RANTES and Zn may be explained by the influence of this cation on Zn finger transcription factor activity, including RFLAT-1, that represents a strong transactivator for RANTES in T cells [43, 44].

We confirmed the relationship previously observed [29] between HSP70 +1267 A/G SNP and circulating Zn levels. In this regression model, HSP70 polymorphism is found as the most important predictor among the SNPs included in the analysis, although its relevance in comparison with other non-genetic predictors is relatively low. Several investigations report that Zn may induce HSP70 production [45–47]. However, there is not a clear evidence on the link among HSP70+1267 A/G SNP, Hsp70 levels and Zn. Some studies report a reduced HSP70 protein or mRNA levels in 1267 HSP70 GG genotype, as compared to AG and AA genotypes, [48, 49] that may be in line with the reduced Zn levels in GG carriers herein found. Anyhow, more research should be performed to better clarify the relationship between HSP70 and Zn status and their role on the susceptibility to age-related diseases. Our linear regression model with a stepwise approach didn't show significant association between the 174 G/C IL-6 polymorphism and plasma Zn, but in this manuscript we have not considered the whole set of indexes for Zn status as previously reported [16]. In any case, an association between IL-6 SNP and Zn plasma concentration was found using ANCOVA correcting for center, age, gender, BMI and Zn score (Fig. 6S, Supplementary Material).

Cu/Zn ratio is an important predictor of disability and mortality [24]. This ratio is mostly associated with inflammatory mediators rather than nutritional factors [24, 50]. Herein, the multivariate linear regression analysis shows a positive association between Cu/Zn ratio with markers of systemic inflammation (ESR and CRP) and age, while

a negative association has been found with albumin serum levels, confirming our previous results [24]. On the other hand, inflammation determines a reduced albumin synthesis [51].

The increment of Cu/Zn ratio with aging may depend on several factors such as (1) a decrease in serum Zn due to a diminished requirement of Zn–albumin for proliferation and growth, (2) a reduced nutritional intake, (3) a dissociation of Zn from the serum albumin pool to other tissue and compartments to sustain stress response, and (4) an increment of Cu-ceruloplasmin (the major carrier of copper in serum) to counteract oxidative stress occurring with aging and age-related diseases [23].

Cu/Zn ratio is higher in females than males, and this is consistent with several previous reports [24, 52, 53]. The difference of Cu/Zn ratio in relation to gender may be in part explained by an increased Zn dietary intake in males than females, as observed in our cohort and by other authors [52], but this is likely to play a minor role compared to the gender differences reported for copper and ceruloplasmin [54]. Moreover, it has been shown that postmenopausal therapy is associated with increased concentrations of serum copper [53].

This study presents some limitations, such as the lack of data on medication consumption and dietary phytate intake that might affect Zn plasma concentrations. Moreover, in our model we have not considered the expression of Zn transporters that are the most important regulators of Zn status, although we have included the Gln/Arg/Leu Zip-2 SNP previously related to Zn transporter gene expression [19].

In conclusion, our findings show that the most important independent determinants of plasma Zn concentration are age, albumin and RANTES circulating levels, Zn dietary intake and HSP70 + 1267 A/G variants. Moreover, the main predictors of Cu/Zn ratio variability were represented by systemic inflammatory factors (ESR and CRP), albumin levels, gender and age. These results confirm that circulating Zn represents a weak indicator of Zn deficiency in elderly, as the decline with age of this trace element in the plasma is more dependent on physiopathological changes occurring with aging rather than to its nutritional intake.

Table 3 Multivariate linear regression analyses for variables independently associated with copper/zinc ratio

Model	Predictors	Standardized beta coefficients	Adjusted R^2	p value
1	ESR	0.418	0.172	0.0001
2	ESR	0.409	0.223	0.0001
	Albumin	−0.230		0.0001
3	ESR	0.351	0.254	0.0001
	Albumin	−0.204		0.0001
	Gender	0.194		0.0001
4	ESR	0.322	0.276	0.0001
	Albumin	−0.137		0.006
	Gender	0.218		0.0001
	Age	0.169		0.001
5	ESR	0.284	0.286	0.0001
	Albumin	−0.118		0.021
	Gender	0.239		0.0001
	Age	0.177		0.004
	CRP	0.115		0.018

Gender was categorized as follows: 0 = males and 1 = females

ESR erythrocyte sedimentation rate, *CRP* C-reactive protein

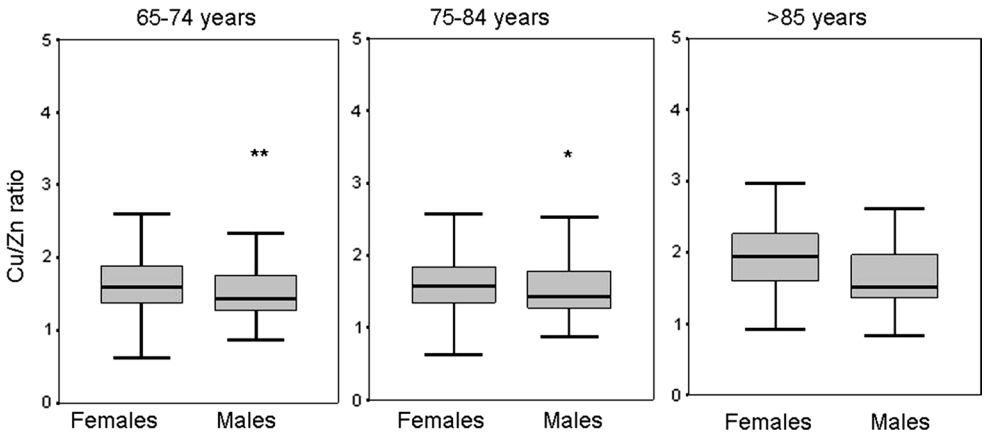

Fig. 4 Plasma Cu/Zn ratio in males and females at different age classes (65–74; 75–84; >85 years). The *box plots* display the median, interquartile range, the 5th and 95th percentile. $^{**}p < 0.001$; $^{*}p < 0.05$

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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