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Assessment of gene–nutrient interactions on inflammatory status of the elderly with the use of a zinc diet score — ZINCAGE study☆

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Abstract

Although zinc plays an important role in health status of the elderly, their dietary habits in relation to zinc intake are not well documented. The main objective of the current study was the assessment of dietary zinc intake in European old populations and the investigation of its impact on plasma zinc and inflammatory cytokines concentrations, in relation to genetic markers. Within the ZINCAGE study, 819 healthy old Europeans (≥60 years old) were recruited. Plasma zinc, interleukin-6 (IL-6) and interleukin-8 (IL-8) were measured. Genotype data were obtained for the −174G/C polymorphism in the IL-6 gene. Dietary data were collected with a food frequency questionnaire and were used to calculate a zinc diet score. Zinc score was validated using additional dietary data (24-h recalls), in a subsample of 105 subjects. Zinc score was different among most of the European centres ($P<.001$), while an age-dependent decline was documented ($P=4.4\times10^{-12}$). Plasma zinc concentrations were significantly correlated with the zinc score (standardized $\beta=0.144$, $P=8.8\times10^{-5}$). The minor allele frequency for the −174G/C polymorphism was f(C) 0.31. There was a significant interaction of zinc diet score and GG (−174G/C) genotype on higher plasma IL-6 levels ($\beta\pm$S.E.$=0.014\pm0.0$, $P=.008$). The main finding of our study was the detection of gene–nutrient and biochemical–nutrient interactions in a multiethnic cohort based on a common dietary assessment tool.

Keywords: Zinc diet score; Plasma zinc levels; Inflammation; Genes; Elderly; ZINCAGE study

1. Introduction

Ageing is a process that affects all physiological systems and increases susceptibility to disease and death. In particular, a dysregulation of the immune system with ageing is believed to contribute to morbidity and mortality, due to the greater incidence of infections and cancer, as well as to inflammatory phenomena involved in major age-related diseases, such as atherosclerosis [1].

Nutrition is a strong modulator of risk factors for chronic diseases. This is especially important for the elderly, as proper nutrition plays a crucial role in maintaining good health and functioning. Epidemiological studies suggest that nutrition influences longevity [2–5], cardiovascular events [6,7], neoplastic diseases [8,9] and cognitive function [10,11] in the elderly. Thus, dietary habits of the elderly, both in terms of dietary patterns and of single nutrient intake, need to be taken into account in order to clarify the relation between nutrition and health or disease in advanced age.

Zinc is an essential trace mineral for human health. It is a catalytic, structural and functional component of many proteins, enzymes, hormones and hormone receptors. Especially in elderly populations, zinc is of great importance due to its impact on immune functions [11,12], bone mass preservation [13,14], antioxidant defence [15], DNA repair [16] and cognitive function [17]. While the importance of zinc

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ion bioavailability in the elderly is well documented, there is limited data on the relationships between dietary zinc intake, zinc status and healthy ageing [18,19]. Providing information about dietary habits of the elderly and their possible impact on zinc and inflammatory status would be useful for the determination of specific needs and dietary guidelines in relation to zinc.

On the other hand, low-grade elevation of inflammatory mediators has been recognized as a risk factor for age-related inflammation, frailty, cardiovascular disease and all-cause mortality in the elderly [20,21]. Amongst the inflammatory markers, IL-6 and IL-8 have been positively associated with advancing age in several studies [21–24]. Nevertheless, genetic background also has an evident contribution to immunosenescence [25]. Among the potential genetic markers of inflammation, the −174G/C polymorphism in the promoter of the IL-6 gene has been shown to affect cytokine production, therefore modulating susceptibility to age-related diseases and mortality [26,27]. Several studies support that GG genotype is associated with elevated IL-6 levels, impaired innate immune response and high prevalence of inflammatory pathologies in the elderly [15,27,28].

Within the ZINCAGE project, biochemical, genetic and lifestyle factors for healthy ageing are studied in a European cohort [26]. For the purpose of the current study, healthy old subjects (≥60 years old) were recruited and dietary and genetic data, plasma zinc, IL-6 and IL-8 measurements were obtained. We designed and calculated a zinc score, representative of zinc dietary habits, in order to assess the differential dietary intake of zinc in European old populations and to investigate its impact on zinc and inflammatory markers concentrations, in relation to genetic background.

2. Materials and methods

2.1. Subjects and study design

The study was carried out in a sample of 819 (272 from Italy, 163 from Greece, 137 from Germany, 128 from France and 119 from Poland) healthy noninstitutionalized men and women older than 60 years. Moreover, the participants of the study had to be free of medication such as steroids, diuretics, anticonvulsants, antidepressive drugs, antibiotics, antimetabolites, non-steroidal anti-inflammatory drugs and micronutrient supplementation. Subjects were excluded if they had autoimmune, neurodegenerative, cardiovascular, kidney or liver disease, diabetes, infections, cancer, chronic inflammatory bowel disease or acrodermatitis enteropathica, sickle cell anaemia, chronic skin ulcerations and endocrine disorders. Medical history was recorded by the family doctor or through a medical examination when possible. Ethical approval was obtained at all of the centers of recruitment and all subjects signed an informed consent form. The study was part of the ZINCAGE study [26].

2.2. Anthropometric and biochemical measurements

The anthropometrical measurements included weight and height, and were obtained using standardized techniques and equipment. Body mass index (BMI) was calculated as weight (kg)/height (m) squared. Blood samples were collected after 12 h of fasting, using trace element-free vacutainers. Plasma zinc levels were analyzed by induction coupled plasma mass spectrometry (ICP-MS) according to the manufacturer's instructions (Thermo Electron Corporation, Waltham, MA, USA). Plasma concentrations of IL-6 and IL-8 were evaluated using commercially available multiplex bead-based sandwich immunoassay kits (Bio-Rad Laboratories, Hercules, CA, USA), as described previously [23].

2.3. Genotyping

Genomic DNA from peripheral blood was extracted with phenol/chloroform using standard protocols. IL-6 −174G/C (rs1800795) polymorphism was screened, as described elsewhere [27].

2.4. Assessment of dietary zinc intake

A qualitative food frequency questionnaire, designed for the needs of ZINCAGE, was used for the assessment of dietary zinc intake. The food frequency questionnaire included 53 food items, representative of the major food groups (red meat and poultry, fish and seafood, dairy products, refined and nonrefined grains, raw and cooked vegetables, fruits, eggs, sweets, beverages, and oil and added fat). The consumption of all food items was recorded in terms of frequency (never or less than once a month, occasionally, sometimes, daily consumption) and quantity (no, small, medium, abundant consumption). Based upon these data, we determined a "zinc score" for each volunteer. To provide a continuous variable, representative of zinc dietary habits, frequency, quantity estimation and zinc content of foods consumed were all considered for the zinc score calculation.

Individual food consumption was calculated as frequency (1 for never or less than once a month, 2 for occasionally, 3 for sometimes and 4 for consumption everyday) multiplied by quantity (0 for no consumption, 1 for small, 2 for medium and 3 for abundant). Taking into account the large variations of zinc content in foods, even within food groups, all food items were considered separately. European National and USDA food composition tables were used to define zinc content for all 53 food items included in the food frequency questionnaire. Zinc content of all food items used for the zinc diet score calculation is presented in Table 1. Consumption of each food was then multiplied to the correspondent zinc content. A zinc score was calculated for each old volunteer as the sum of all estimated zinc intakes derived from all listed food items. The general formula used for zinc score calculation was: Zinc score=Frequency×Quantity×Zinc content. The calculation of the zinc score provided with a continuous variable as a comparative estimate of dietary zinc intakes among recruited old subjects.

A validation study of the developed zinc score was conducted in a subsample of 111 old subjects (51 males and 60 females), because of the high cost of complete dietary assessment in all subjects of the ZINCAGE study. Three 24-h recalls were collected from each old participant of the validation study. Dietary data were assessed using the Nutritionist Pro software (Axxya Systems) and published national food composition tables, for the estimation of mean daily dietary zinc intake.

2.5. Statistical methods and analysis

Statistical analysis was performed with SPSS edition 13.0. Continuous variables are presented as mean values±S.D. The normal distribution of the investigated variables was assessed through the Kolmogorov–Smirnov criterion. Due to skewed distribution, log-transformed values of zinc score, plasma IL-6, IL-8 and zinc levels were used. Correlations were evaluated by calculation of Pearson r coefficient. Student's t test, analyses of variance (ANOVA) and analyses of covariance (ANCOVA) were used to compare the variables among subgroups of the sample. Because of multiple comparisons, Tukey's correction was used to control for overall type I error. The distribution of the investigated polymorphism was compared with the expected frequency through the Hardy–Weinberg equilibrium (HWE), using Pearson's chi-squared test. Multiple linear regression and univariate analysis of variance models were applied, after taking into account the effect of potential cofounders. All reported P values are based on two-sided tests and were compared with a significance level of 5%.

Table 1
Zinc content of all food items used for the zinc diet score calculation

Food item (serving)	Zn content/ serving (mg)	Food item (serving)	Zn content/ serving (mg)
Oysters (3 oz)	74.00	Whole-wheat bread (1 slice)	0.50
Calf (3 oz)	5.50	Egg (1 item)	0.50
Liver (3 oz)	4.50	Tomatoes cooked (0.5 cup)	0.45
Shellfish (3 oz)	4.00	Potatoes cooked (1 cup)	0.40
Lamb (3 oz)	4.00	Other cooked vegetables (1 cup)	0.40
Horse (3 oz)	3.00	Fish[a] (3 oz)	0.40
Canned meat (3 oz)	3.00	Ice cream (0.5 cup)	0.40
Offal (3 oz)	3.00	Wheat bread (1 slice)	0.20
Turkey (3 oz)	2.80	Raw vegetables (1 cup)	0.20
Pork (3 oz)	2.50	Vegetable soup (1 cup)	0.20
Other meat (3 oz)	2.50	Fruit juice (1 cup)	0.20
Yoghurt (1 cup)	2.00	Cake/snack (1 piece)	0.13
Chicken (3 oz)	1.70	Fruits (1 medium)	0.10
Legumes cooked (0.5 cup)	1.30	Canned fruits (0.5 cup)	0.10
Peas cooked (1 cup)	1.10	Dried fruits (1 medium)	0.10
Canned fish (3 oz)	1.00	Chocolate (0.5 bar)	0.10
Milk[b] (1 cup)	1.00	Cola-type beverage (1 cup)	0.05
Hard matured cheese (1 oz)	0.90	Cookies (1 item)	0.02
Pizza (1 slice)	0.80	Tea (1 cup)	0.02
Cheese[c] (1 oz)	0.80	Butter, lard (1 tablespoon)	0.001
Pasta/rice (1 cup)	0.70	Sugar/honey (1 teaspoon)	0.00
Cold meat/salami (2 slices)	0.65	Oil[d] (1 teaspoon)	0.00

[a] Includes codfish/bluefish, frozen fish and other fresh fish.
[b] Includes full-cream, semi-skimmed and skimmed milk.
[c] Includes fresh and soft cheese.
[d] Includes extravirgin olive oil, olive oil, seed oil and margarine.

3. Results

A total of 819 subjects (128 from France, 137 from Germany, 163 from Greece, 272 from Italy and 119 from Poland) entered the study. Demographic, biochemical and diet characteristics of the recruited subjects are shown in Table 2.

In the subsample of 105 healthy older subjects, who enrolled in the validation study, the assessed mean daily dietary zinc intake was 8.2±3.5 mg/day and the calculated mean zinc score was 126.9±54.6. Zinc score was significantly associated with daily dietary zinc intake (mg/day) (standardized β=0.260, P=.007, after controlling for age, sex and country of origin). This finding is illustrated in Fig. 1A. Plasma zinc values showed significant positive correlations with dietary zinc score in all elderly (standardized β=0.144, $P=8.8\times10^{-5}$, after controlling for age, sex, country of origin and BMI), as illustrated in Fig. 1B.

Amongst the overall healthy old populations examined, those in France had the highest zinc scores, followed by the Italian, German, Greek and, lastly, the Polish (Fig. 2). Multiple comparisons showed significant differences in mean zinc score for all counties (P<.001, adjusted for age and gender), except between Germany and Italy, occurring with the same zinc score values (P=.408, adjusted for age and sex). The same order of magnitude was observed when only females were considered, with all multiple comparisons statistically significant (P<.027, adjusted for age), except between German and Greece female populations (P=.767, adjusted for age). Among healthy male populations, the French had the highest zinc score, followed by the German and the Italian, who had the same zinc scores (P=.111, adjusted for age). Polish and Greek male populations presented with the lowest zinc scores and were not different from each other (P=.287, adjusted for age). Mean zinc score values were significantly different between males and females for Italy and Poland (Table 2). Italian and Polish old males had higher mean zinc scores compared to their female counterparts (P=.012 and $P=3.3\times10^{-4}$, respectively, adjusted for age and BMI).

There was a significant decline in zinc score with age in the majority of the healthy old volunteers. A significant negative correlation between zinc score and age was observed in the Italian, Polish, German and Greek old population ($P=5.3\times10^{-12}$, $P=9.4\times10^{-7}$, P=.044, P=.004, respectively, adjusted for gender).

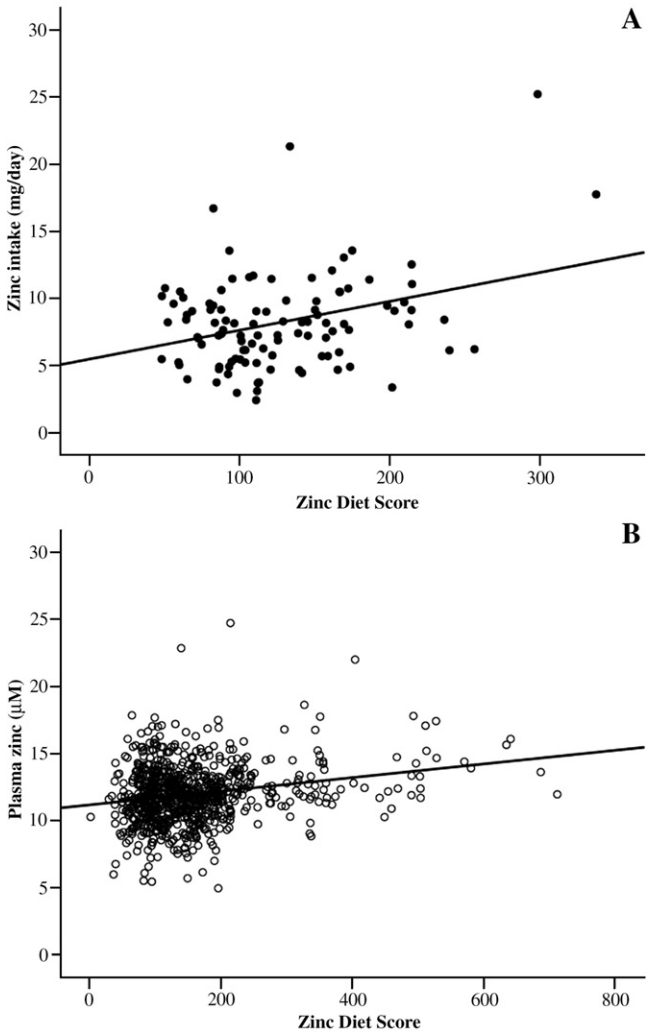

Fig. 1. Positive correlation between zinc diet score and dietary zinc intake (A) in healthy old subjects (n=105, standardized β=0.260, P=.007, after controlling for age, sex and country of origin), and plasma zinc values (B) in all old subjects (N=819, standardized β=0.144, $P=8.8\times10^{-5}$, after controlling for age, sex, country of origin and BMI).

Table 2
Characteristics of the ZINCAGE study subjects, stratified by country of origin and gender

	France	Germany	Greece	Italy	Poland
n					
Male	62	96	60	107	41
Female	66	41	103	165	78
Age (years)					
Male	68.3±5.9[a]	69.7±4.8	73.3±6.2	77.4±9.2	72.4±6.1
Female	68.6±6.0	70.2±4.9	71.0±6.3[b]	79.7±10.0	71.0±7.3
BMI (kg/m^2)					
Male	26.0±2.8	25.3±2.5	28.2±3.5	25.4±3.3	25.6±3.1
Female	23.5±3.2[b]	23.9±3.3[b]	30.1±3.4[c]	24.4±4.0[b]	27.5±3.6[b]
IL-6 (pg/ml)					
Male	22.1±7.6	19.0±11.2	12.8±4.6	19.8±8.9	22.8±9.2
Female	21.8±6.4	18.8±11.0	13.6±4.6	19.7±9.1	22.1±8.6
IL-8 (pg/ml)					
Male	9.7±5.2	8.4±4.4	8.2±3.6	8.5±4.2	14.6±5.5
Female	10.5±6.3	8.3±4.3	8.4±4.2	9.9±4.3[d]	14.1±5.4
Plasma zinc (μM)					
Male	13.3±4.0	12.4±1.6	11.2±1.7	11.4±2.7	12.5±2.4
Female	13.1±1.9	12.1±1.5	11.4±1.6	11.4±2.2	12.5±2.3
Zinc diet score					
Male	296.8±136.8	168.9±96.9	109.9±25.8	166.9±54.0	104.8±35.4
Female	251.8±130.4	140.0±94.0	104.3±25.5	146.4±45.5[b]	87.1±26.3[b]

[a]Mean±S.D., all such values. [b]Significant difference between genders, [c]after age adjustment, [d]after age BMI adjustments.

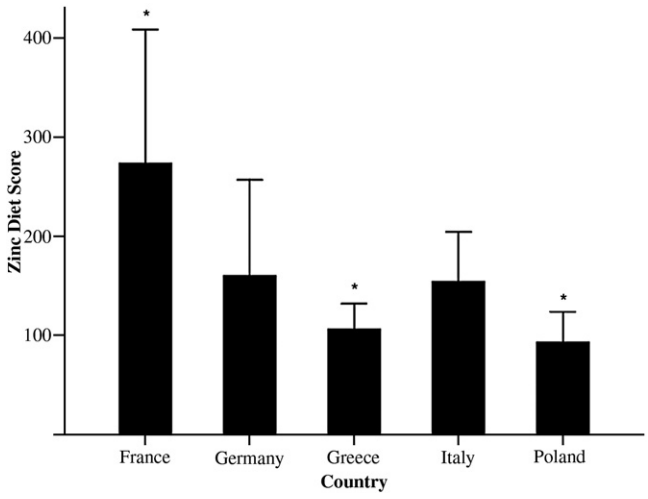

Fig. 2. Zinc score mean values in healthy old subjects across the European countries (P<.001, ANCOVA test adjusted for age and gender). *Significantly different from all other countries (P<.001, ANCOVA test adjusted for age and gender).

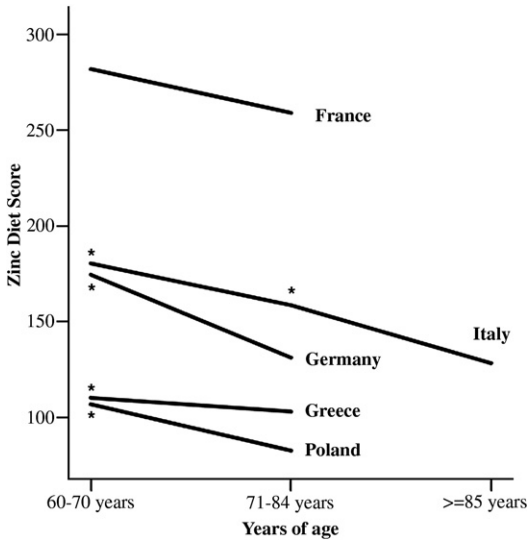

Fig. 3. Age dependent decline in zinc score in healthy old and very old subjects. *Significant different from all other age groups ($P<.001$, ANCOVA test adjusted for gender).

When all healthy old subjects were divided into two age groups (60–70 and 71–84 years of age), there was a significant decline in zinc score throughout these age groups ($P=4.4\times10^{-12}$, adjusted for gender and country of origin). A decline in mean zinc score values was significant in subjects aged 71–84 years compared to elders between 60 and 70 years in Germany, Greece and Poland ($P=.026$, $P=.042$ and $P=2.1\times10^{-7}$ accordingly, adjusted for gender). In contrast, no significant decline in zinc score between the age groups was observed in the French ($P=.361$ adjusted for gender). A decline in zinc score was also significant across all Italian age groups, including the oldest ones ($P=6.4\times10^{-10}$) (Fig. 3).

The minor allele frequency of the −174G/C polymorphism in the IL-6 gene was 0.31 for the C allele. Genotype frequency was in HWE. Demographic, biochemical and diet characteristics of the subjects, according to their genotypes, are presented in Table 3. Old subjects carrying the C allele for the −174G/C polymorphism in the IL-6 gene presented with significant higher plasma IL-8 levels ($P=.019$) compared to GG old subjects (Table 3).

In order to evaluate the impact of the −174G/C polymorphism and zinc diet score on plasma IL-6 and IL-8 levels, two models of univariate analysis of variance were applied (Table 4). Plasma IL-6 levels were negatively associated with the presence of the GG genotype ($\beta\pm$S.E.$=-2.371\pm01.2$, $P=.006$), while the impact of the zinc diet score was only borderline significant (Table 4). However, the −174G/C genotype–zinc diet score interaction was significant, as GG genotype was associated with higher plasma IL-6 levels compared to GC/CC genotypes, with increasing zinc diet score ($\beta\pm$S.E.$=0.014\pm0.0$, $P=.008$) (Table 4).

Plasma IL-8 levels were positively associated with advancing age ($\beta\pm$S.E.$=0.042\pm0.0$, $P=.013$), while the impact of either the −174G/C or the zinc diet score was statistically significant (Table 4).

Table 3
Differences in biochemical and dietary factors of the old subjects, stratified by the −174G/C IL-6 genotypes

	IL-6 −174G/C		
	GG	GC/CC	*P*
n	405	414	
Male/female (%)	46.3/53.7	43.0/57.0	.354
Age (year)	73.6±8.5[a]	72.6±8.2	.093
BMI (kg/m^2)	26.2±4.2	25.9±3.6	.236
IL-6 (pg/ml)	19.0±9.0	19.2±8.8	.520
IL-8 (pg/ml)	9.3±4.7	10.3±5.4	*.019*
Plasma zinc (μM)	11.9±2.2	12.1±2.5	.144
Zinc diet score	159.4±94.2	152.6±94.0	.065

[a] Mean±S.D., all such values. Values in italics represent statistically significant *P* value.

Table 4
Results from univariate analysis of variance models, which evaluated the association among plasma IL-6 and IL-8 levels, −176G/C polymorphism of the IL-6 gene and zinc diet score in the ZINCAGE cohort

Covariates	Plasma IL-6 (pg/ml)		Plasma IL-8 (pg/ml)	
	β coefficient±S.E.	*P*	β coefficient±S.E.	*P*
Age (year)	−0.075±0.1	.107	0.042±0.0	*.013*
BMI (kg/m^2)	−0.146±0.1	.104	0.027±0.1	.733
Female vs. male	0.229±0.6	*.034*	0.820±0.4	*.029*
Nonsmokers vs. current smokers	0.564±1.1	.981	1.327±0.7	*.023*
Country of origin	−0.915±0.2	*.001*	−0.210±0.1	.261
GG vs. GC/CC genotypes	−2.371±1.2	*.006*	−1.751±0.7	.488
Zinc diet score	0.007±0.0	.067	−0.003±0.0	.293
GG genotype×Zinc diet score vs. GC/CC genotypes×Zinc diet score	0.014±0.0	*.008*	0.004±0.0	.668

4. Discussion

In order to assess the dietary zinc intake in old Europeans, we designed a zinc score based upon data collected by a qualitative food frequency questionnaire. To provide a continuous variable, representative of zinc-related dietary habits, frequency, quantity estimation and zinc content of foods consumed were all considered for the zinc score calculation. Delineating associations between nutrition and health status is quite challenging in elderly populations, since dietary behaviour is largely modified by age-related difficulties in mastication, gastrointestinal function and psychosocial factors [28]. Furthermore, basic difficulties in nutritional assessment of the elderly and the complex influence of dietary habits on health and disease justify the use of “diet” scores. Various diet scores are used in nutritional epidemiology, providing an overall view of dietary habits. Mediterranean diet score [5,29], dietary pattern analysis [30], healthy eating index scores [31] and nutrient density scores [32] have all been developed and used in large-scale elderly cohorts, in order to reveal risk factors of morbidity. Furthermore, dietary scores have been successfully applied on single nutrients, like folate, providing a valid estimate of dietary intake in the elderly [33].

In the present study, we found a positive association between plasma zinc levels and zinc score for the overall old cohort (Fig. 1B). Other studies, investigating relations between dietary zinc intake and plasma zinc levels, reported contradictory results [13,18,34–36]. These discrepancies could be attributed to the lack of their employment of a zinc-specific dietary assessment tool, as well as to the variety of factors influencing zinc bioavailability. It is well documented that dietary sources and other components of the diet [37,38], as well as physiological factors, medications [39] and genetic background [40], are strong modulators of zinc bioavailability and plasma zinc concentrations. In particular, a recent study has shown a significant effect of food phytate content on zinc absorption and bioavailability [41]. However, it was not feasible to consider dietary factors implicated in zinc absorption in our study design.

Our analysis revealed a significant gene–nutrient interaction concerning the −174G/C IL-6 polymorphism and IL-6 plasma levels. In particular, GG old subjects occurred with higher plasma IL-6 levels compared to C allele carriers, with increasing zinc diet score. This finding is in accordance with previously published data from a zinc supplementation trial within the ZINCAGE study [42]; nevertheless, it is contradictory to previously published data on zinc-regulated

gene expression in cultured peripheral blood mononuclear cells with a 50 μM zinc dosage [27]. This last effect may be attributed to the pharmacological effect of large doses of zinc on pro-inflammatory cytokines production [43]. However, when supplementing with a physiological dose of zinc (10 mg/day), an increment in IL-6 levels of GG old individuals was observed [44]. This finding supports the hypothesis that zinc may have a role in stabilizing inflammation and modulating the immune response, especially in GG subjects, with subsequent prompt immune response against external noxiousness [44]. On the other hand, experiments in lymphocytes from aged donors, stimulated with LPS, have shown an increment of IL-6 levels after zinc addition (15 μM) *in vitro* [45]. These data suggest the importance of zinc as an immunomodulatory agent, while underlining that increased dietary zinc intake in GG elderly could be beneficial against external noxiousness, with subsequent good healthy status. Such a consideration could be important, taking into account that the GG genotype is considered detrimental for longevity [40].

Additionally, we found significant country-dependent differences in zinc scores across Europe, with the French having the highest zinc intake, then the Italian, followed by the German, while the Greek and Polish old subjects had the lowest zinc intake. To our knowledge, there are no published data to compare the dietary zinc intake of the elderly among different European countries, applying a common nutritional assessment tool. However, differential dietary habits across Europe are well documented [31] and could account for this country-dependent difference in zinc score. Interestingly, we have also documented several differential consumptions in food groups, within the ZINCAGE cohort [46]. Higher consumption of oysters in France, as well as fish and red meat in France, Italy and Germany, compared to Greece and Poland, could explain the large differences in zinc score [46].

Furthermore, a decline in zinc score with age was recorded for the majority of the investigated European old populations, except for the French (Fig. 3). Results from other studies are contradictory, as some investigators demonstrate a decline in dietary zinc intake with advancing age [47], while others do not observe any age-related change [18]. Unfortunately, the difficulty in recruiting very old subjects from European countries other than Italy is inhibitory for further comparisons among several age groups. However, it is well documented that the elderly are at nutritional risk as a result of multiple physiological, social, psychological and economic factors, which adversely affect dietary choices and eating patterns [48]. On the other hand, previously published results from the ZINCAGE study indicate a progressive age-related increase of plasma IL-6, IL-8, MCP-1 and TNF-alpha concentrations in concomitance with low circulating levels of zinc [23].

To sum up, we used a zinc diet score to assess gene–nutrient interactions on the inflammatory status of the elderly. With the application of a zinc score, different zinc intake on a gender-, age- and country-dependent manner is revealed. A dietary assessment tool, like zinc diet score, would be useful for the evaluation of gene–nutrient and biochemical–nutrient interactions. Additional statistical analysis, also including more genetic, biochemical and lifestyle data along with the zinc score, would provide a substantial database for gender- and country-specific dietary guidelines for the elderly.

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