## ORIGINAL CONTRIBUTION

# Dietary habits of Greek adults and serum total selenium concentration: the ATTICA study

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#### Abstract

Purpose The trace element selenium is an essential micronutrient for human health, and its low levels in serum are implicated in the pathogenesis of several chronic diseases. The determination of total serum selenium levels may contribute to the assessment of the health status of all populations. Since the serum selenium levels are highly affected by diet, we assessed its association with the dietary habits of Greek adults.

*Methods* Serum selenium levels were determined with inductively coupled plasma mass spectrometry in a cohort of 506 participants (men: 296, women: 210) aged 18–75 from the ATTICA study. Food consumption was evaluated with a validated food-frequency questionnaire.

Results Evaluation of the relationship between serum total selenium with major food groups and beverages by multi-adjusted analysis revealed that serum selenium was positively correlated with the consumption of red meat  $(2.37 \pm 0.91, p = 0.01)$  while the consumption of other selenium-containing foods (i.e., fish, cereals, dairy products, vegetables) did not demonstrate such a relationship. Moreover, principal component analysis revealed that the

adoption of a vegetarian type of diet is inversely correlated with total selenium ( $-3.94 \pm 2.28$ , p = 0.08).

Conclusions Among the dietary habits that were examined, red meat seems to be the major determinant of serum selenium in Greek adults.

**Keywords** Dietary habits · Serum selenium · Greeks · Red meat

#### Introduction

Selenium (Se) is an essential nutrient to human health [1-3]. Being part of several selenoproteins, such as thyroid hormone deiodinases, selenoprotein P and glutathione peroxidases, it is implicated in several antioxidants, hormonal and inflammatory systems of the human body [4]. The activity of these selenoproteins, and of others with as yet unidentified functions, depends on adequate Se supply from the diet. Several studies have shown that Se levels in serum reflect alimentary intake [5–7]. Dietary intake of Se is determined by its content in different foods, the bioavailability of its chemical forms and by the dietary patterns adopted by different populations [6, 8, 9]. The concentration of Se in soil is a major determinant of its transfer to the food chain, thus Se dietary intake shows a large geographical variation due to the differences of soil Se content in different countries [6, 10]. For example, the Se dietary intake can vary from 3 to 11 µg/ day in certain selenium-poor areas of China to 216 µg/day in seleniferous areas of United States. The dietary intake of Se reflects serum selenium levels (TSe), and a great variation of it has been observed in normal populations. Low levels have been found in the Balkan region (Serbia, Croatia, Bulgaria) while high TSe have been observed in the United States [11].

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According to the above, the estimation of Se content along the food chain is rather complicated. Red meat, poultry, eggs and fish are considered to be a rich source of Se although their Se content may be affected by several factors. Specifically, Se in animal tissue is totally dependent on dietary supply which in turn is dependent on soil's Se content. On the other hand, animals kept in captivity for all or part of their lives consume foods which may come from a great distance away and/or be mixed with other foods. Moreover, animals are generally supplemented with selenium either through concentrates or by gavages or injection; thus, an animal in a low-selenium area consuming a low-selenium diet may still have adequate selenium in the muscle. Finally, animals metabolize selenium depending on its chemical form. Animals consuming adequate amounts of selenomethionine (such as in wheat) may have higher selenium in the muscle than animals consuming high amounts of Se as a salt or as a methylated form [6]. Also, the Se content of foods can be considerably reduced by food processing [12]. Bioavailability of Se is an important criterion when judging the nutritional quality of food and determines not only the serum selenium levels but also the relative concentration of the different dietary Se species that are absorbed by the human body [5]. Selenium bioavailability is affected by its chemical form. Selenocysteine and selenomethionine are more bioavailable than inorganic forms of Se (selenate, selenite). Moreover, the food matrix (content of total protein, fat, heavy metals and dietary fibers) may modulate Se absorption. Moreover, sulfur status, especially sulfur acids, may be the primary matrix factor that modulates selenium absorption. The selenium found in animal foods, with high protein content, is readily bioavailable while the absorption of plant foods Se may be inhibited by the presence of dietary fibres [13].

Previous studies have focused mainly on the intake of Se that was estimated with the help of food composition tables which have incorporated Se content of foods. However, only few studies have attempted to correlate TSe levels and the dietary habits of the participants [14, 15]. Concerning the limited available data on this issue and the absence of data for the Greek population, aim of our study was to assess the effect of dietary habits of apparently healthy Greeks on the levels of TSe in a subpopulation of the ATTICA study.

# Materials and methods

Study population

The "ATTICA" epidemiological study has been carried out in the province of Attica (including 78% urban and 22% rural areas), Greece [16]. For the purposes of this

work, a random sub-sample from the ATTICA Study's database, consisted of 296 males ( $40 \pm 11$  years) and 210 females ( $38 \pm 12$  years), was selected, and the serum total Se was determined. The study was approved by the Medical Research Ethics Committee of the supervising Institution and was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and all participants gave their informed written consent in order to participate in the study.

## Dietary assessment

The evaluation of the nutritional habits was based on a validated food-frequency questionnaire (i.e., the EPIC-Greek questionnaire [17]) that was kindly provided from the Nutrition Unit of Athens Medical School. All participants were asked to report the daily or weekly average intake of several food groups and choices that they had consumed (during the last year). Then, the frequency of consumption was quantified approximately in terms of the number of times per month a food or a drink was consumed. Thus, daily consumption was multiplied by 30 and weekly consumption was multiplied by 4.3, and a value of 0 was assigned to food groups rarely or never consumed. Portions of foods were specified (e.g., small, normal and large) and in some cases photos assisted the participant to decide the exact portion of food consumed. Mixed foods were taken into account and assigned into the respective food groups. In particular, study participants were instructed by trained dieticians to complete the questionnaire that included 156 foods and beverages commonly consumed in Greece. In this work, the interest was focused on the consumption of food groups, like cereals, such as bread (white or dark) or hardtack (hard and dry type of cracker), pasta, rice and other non-refined cereals, fruits (grapes, avocado, bananas, watermelon, apples, pears, oranges, grapefruit, strawberries, peaches, apricots, etc.), greens (spinach, iceberg, lettuce etc.), vegetables (broccoli, cabbage etc.), legumes (beans, peas etc.), potatoes, small (silverside, anchovy, etc.) or big (swordfish, gilthead etc.) fish, various types of meat (beef, pork, lamb, poultry) and its products (bacon, hot-dogs etc.), dairy products (low, i.e., 0%, 2% or total fat) like cheese, yogurt, milk, alcoholic beverages (like wine, beer, whiskey, ouzo), as well as drinking of coffee, tea and decaffeinated coffee. All participants were asked about their usual frequency of consumption of coffee (instant coffee, «Greek» type-boiled, filtered or «cappuccino», decaffeinated coffee) and tea in cups/day over the last year. Alcohol consumption was measured in wine glasses (100 mL) and quantified by ethanol intake (g/drink). One wine glass (100 mL) was equal to 12% ethanol concentration, a bottle of beer (330 mL) was equal to 5% ethanol concentration, a glass of



martini/vermouth (45 mL) was equal to 16% ethanol concentration, a glass of liquor (30 mL) was equal to 35% ethanol concentration and a glass of ouzo (30 mL) was equal of 40% ethanol concentration. Finally, a glass of other alcohol beverages (45 mL), such as whiskey, vodkagin, as well as brandy-cognac was equal to 40% ethanol concentration.

Based on the Mediterranean diet pyramid, a diet score has been developed to describe a traditional dietary pattern [18, 19] in order to evaluate overall dietary habits of the participants [20]. In brief, the Mediterranean pattern consists of daily consumption of non-refined cereals and products, fruits and vegetables, olive oil (as the main added lipid) and non-fat or low-fat dairy products; weekly consumption of fish, poultry, potatoes, olives, pulses and nuts and rarely eggs and sweets and monthly consumption of red meat and products. It is also characterized by moderate consumption of wine (one to two wineglasses a day). Thus, according to the previous dietary pattern and the reported monthly frequency consumption of the above food groups, a special diet score for each participant was calculated assessing adherence to the Mediterranean diet (range 0-55). In particular, for the consumption of groups presumed to be close to this pattern (i.e., those suggested on daily basis or more than 4 servings per week), a score of 0 was assigned when a participant reported no consumption, 1 when reported consumption of 1–4 times a month, 2 for 5-8 times, 3 for 9-12 times, 4 for 13-18 times and 5 for more than 18 times. On the other hand, for the consumption of foods presumed to be away from this diet (like meat and meat products), the opposite scores were assigned (i.e., 0 when a participant reported almost daily consumption to 5 for rare or no consumption). Especially, for alcohol intake, a score of 5 was assigned for consumption of less than three wineglasses per day, 0 for consumption of more than seven and 4-1 for consumption of three, four to five, six and seven. Higher values of this diet score indicate greater adherence to the Mediterranean diet [21].

Lifestyle, anthropometric, clinical, and biochemical parameters

Information about lifestyle, anthropometric parameters was collected using a standardized questionnaire developed for the study through face-to-face interviews. Current smokers were defined as those who smoked at least one cigarette per day; former smokers were defined as those who had stopped smoking for at least 1 year and the rest of the participants were defined as non-smokers. Occasional smokers (fewer than seven cigarettes per week) were recorded and combined with current smokers due to their small sample size.

Height was measured, to the nearest 0.5 cm, without shoes, back square against the wall tape, eyes looking

straight ahead (visual axis is horizontal when the top of external auditory meat us is level with the inferior margin of bony orbit), with a right-angles triangle resting of the scalp and against the wall. Weight was measured with a lever balance, to the nearest 100 g, without shoes, in light undergarments. Body mass index was measured as weight (in kilograms) divided by standing height (in meter squared). Details regarding the aims and the design of the ATTICA study have already presented elsewhere [22].

Blood samples were collected from the antecubital vein between 8 and 10 AM, after 12 h of fasting and avoidance of alcohol. The biochemical evaluation was carried out in the same laboratory that followed the criteria of the World Health Organization Reference Laboratories. Arterial blood pressure was measured at the end of the physical examination, with the individual in a sitting position and after a rest of at least 30 min. Blood pressure measurements were taken by a cardiologist who was unaware of the answers to dietary questionnaires. Three measurements were made, at the right arm, with the arm relaxed and well supported by a table and at an angle of 458 from the trunk (ELKA aneroid manometric sphygmometer, Von Schlieben Co., Munich, West Germany). The systolic blood pressure was determined by the first perception of (a tapping) sound. The diastolic blood pressure was determined by phase V when the repetitive sounds became completely muffled (disappeared). Changes in loudness were not taken into account. Patients whose average blood pressures were equal to or greater than 140/90 mmHg or who were receiving antihypertensive medication were classified as hypertensive, as is common practice in epidemiological studies.

Interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF  $\alpha$ ) were measured in all participants using a high sensitivity enzyme linked immunoassay (R&D Systems Europe Ltd, Abingdon, United Kingdom). High sensitivity C-reactive protein (hsCRP) was assayed by particle-enhanced immunonephelometry (N Latex, Date-Behring Marburg GmbH, Marburg, Germany). Serum total cholesterol, HDL cholesterol and triglycerides were measured by a chromatographic enzymatic method in a Technicon automatic analyzer RA-1000 (Dade Behring, Marburg, Germany). LDL cholesterol calculated using the Friedewald formulae: {total cholesterol} — {HDL cholesterol} — 1/5 (triglycerides). Blood glucose levels were measured immediately after collection with a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA).

Selenium was determined in serum using inductively coupled plasma mass spectrometry (ICP-MS) by a previously published method [23] with some minor modifications. For the purpose of this study, an X Series (Thermo Scientific) ICP-MS was used. Three Se isotopes were monitored for Se, i.e., <sup>77</sup>Se, <sup>78</sup>Se and <sup>82</sup>Se, as well as <sup>115</sup>In

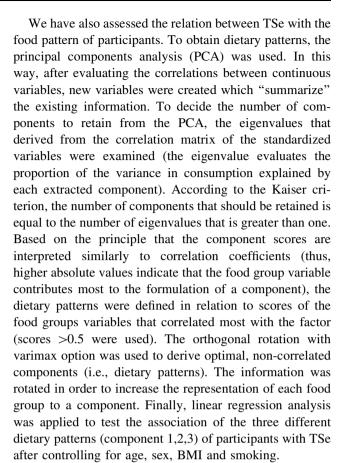


for the internal standard. Because of the potential for spectroscopic interferences, the isotope  $^{77}$ Se was used for quantitative purposes. The aqueous diluent for the total Se analysis consisted of Triton X-100 0.5%, nitric acid 1%, butanol 6%. All solutions were made to also contain 5 ng per mL of the internal standard. All serum samples were diluted 1:10 and vortexed prior to being analyzed. Three replicates were analyzed for each sample. The method limit of detection was determined to be 0.1 µg Se/L. Linear calibration curves (r=0.999) were observed for Se concentrations ranging from 1 to 20 µg Se/L that were used to analyze the 1:10 diluted serum samples.

Quality control of the analytical data was achieved by analyzing the Certified Reference Material BCR®-637 (European Commission, Joint Research Centre, Institute for Reference Materials and Measurements), which is a human serum material certified for its Se content, with every batch of forty to fifty samples. The concentration of Se in the BCR®-637 reference material determined in this study was  $82 \pm 6 \mu g$  Se/L (n = 12). This is in good agreement with the value of  $81 \pm 7 \mu g$  Se/L which has been certified for this reference material. In our study, the reported standard deviation of  $\pm 6$  for n = 12 for BCR 637 reflects the method's inter batch precision. Whereas, intra batch relative standard deviations of 2-3% were typically observed. For all samples analyzed, %RSD values for Se concentrations were between 2 and 5%.

## Statistical analysis

Continuous variables (age, BMI, systolic-diastolic pressure, glucose, total cholesterol, LDL-C, HDL-C, triglycerides, CRP, TSe) are presented as mean values  $\pm$  standard deviation, while categorical variables (current smokers) are presented as relative frequencies (%). Associations between current smokers and sex of the participants were tested by the calculation of chi-squared test, while associations between sex of the participants and age, BMI, systolic-diastolic pressure, glucose, total cholesterol, LDL-C, HDL-C, triglycerides, CRP and TSe were tested by Student's t-test. Moreover, associations between TSe and groups of coffee consumption (no consumption,  $\leq 1, >1-2$ , 2-3, >3 cups/day), tea consumption (no consumption, <1, >1 cups/day) as well as wine consumption ( $<1, \ge 1$  drinks/ day) were tested by analysis of variance. Correlations between TSe and food groups intake and ethanol consumption were tested using the Spearman rho correlation coefficient. Finally, linear regression analysis was applied to test the association of red meat/vegetables consumption as well as ethanol consumption with TSe after controlling for age, sex, BMI, smoking and adherence to Mediterranean diet (Med.Diet Score).



Selenium in serum was normally distributed. Food groups consumption as well as ethanol, tea, coffee and wine consumption had skew distributions. Normality was graphically assessed through Q–Q plots, and Levene's test was used to evaluate homoscedacity. All reported *p*-values were based on two-sided tests and compared to a significance level of 5%. SPSS 16 (SPSS Inc., Chicago, Illinois, USA) software was used for statistical analysis. Statistical power analysis revealed that the enrolled participants are adequate to achieve power >0.80 at 0.05 probability level for evaluating standardized differences of Se concentrations equal to 0.5 units for meat consumption.

# Results

Anthropometric, biochemical and lifestyle parameters of participants

Table 1 summarizes the main anthropometric, biochemical and lifestyle characteristics of the population along with TSe levels. The mean concentration of TSe is 91.9  $\pm$  33.7 µg/L (n=506). No significant difference of Se levels was observed between genders (p=0.28). As we have previously reported, the dietary patterns of males and females do not differ significantly, partly explaining the



Table 1 Anthropometric, lifestyle and biochemical parameters of the participants

Characteristics of the participants	Total sample $(n = 506)$	Male $(n = 296)$	Female $(n = 210)$	p value (by gender)
Age (year)	39 ± 11	40 ± 11	38 ± 12	0.03*
BMI (kg/m <sup>2</sup> )	$26 \pm 5$	$27 \pm 5$	$24 \pm 4$	<0.001*
Current Smokers (n, %)	231 (45%)	154 (52%)	77 (37%)	0.001
Diastolic Blood pressure (mmHg)	$78 \pm 13$	$81 \pm 13$	$75 \pm 12$	<0.001*
Systolic Blood pressure (mmHg)	$120 \pm 17$	$124 \pm 16$	$115 \pm 16$	
Glucose (mmol/L)	$4.8 \pm 1.1$	$4.9 \pm 1.2$	$4.7 \pm 1.0$	0.04*
Total Cholesterol (mmol/L)	$4.20 \pm 0.92$	$4.23 \pm 0.95$	$4.55 \pm 0.85$	<0.001*
LDL-C (mmol/L)	$3.00 \pm 0.88$	$3.12 \pm 0.90$	$2.78 \pm 0.75$	<0.001*
HDL-C (mmol/L)	$1.20 \pm 0.34$	$1.09 \pm 0.29$	$1.34 \pm 0.34$	<0.001*
Triglycerides (mmol/L)	$1.4 \pm 1.0$	$1.61 \pm 1.10$	$1.10 \pm 0.55$	<0.001*
IL-6 (pg/mL)	$1.38 \pm 0.33$	$1.44 \pm 0.34$	$1.31 \pm 0.32$	<0.001*
TNF- $\alpha$ (pg/mL)	$6.05 \pm 2.56$	$7.37 \pm 2.34$	$4.74 \pm 2.79$	<0.001*
C-RP (mg/L)	$1.80 \pm 0.17$	$1.85 \pm 0.22$	$1.69 \pm 0.12$	0.43
TSe (µg/L)	$91.9 \pm 33.7$	$90.5 \pm 35.0$	$93.9 \pm 31.6$	0.28

Data are presented as mean  $\pm$  SD or relative frequencies. Means are significantly different at p < 0.05 based on Student's t-test

almost same mean concentrations of serum Se of the two groups [24].

#### Consumption of foods groups and TSe

The Spearman correlation analysis between TSe and the consumption of food groups showed that the consumption of red meat, as well as poultry, was positively correlated with TSe, while vegetables' consumption was inversely correlated with TSe (Table 2). However, when splitting the total sample by gender, the above correlations remain significant only in males with the exception of poultry. Multiple regression analysis revealed that the significant association between TSe and consumption of poultry (b = 5.39, SE = 3.60, p = 0.13), vegetables (b = -0.30, p = 0.13)SE = 0.20, p = 0.13) disappeared after adjusting for age, sex, BMI, smoking and adherence to Mediterranean diet (Med.Diet Score). On the other hand, the association between TSe and the consumption of red meat remained significant even after adjusting for the same confounders (Table 3). Specifically, for every servings of red meat consumed per week, a 2.37 µg/L increase in TSe was observed (Table 3).

Moreover, we evaluate the relation of TSe with dietary patterns of the participants with the technique of PCA. In the PCA analysis, three components of dietary patterns were extracted that explained 53% of the total variation in intake. Specifically, component 1 displays a vegetarian food pattern which loaded heavily on vegetables, cereals, legumes, fruits and fish, component 2 is a food pattern mainly characterized by the intake of dairy products and eggs while component 3 describes a diet

rich in red meat, poultry and potatoes (Table 4). These components were then entered in the multiple adjusted regression models, in order to evaluate the effects of dietary habits (i.e., vegetables consumption or red meat consumption) on TSe of the participants. Component 1 has a negative significant effect on TSe whereas component 3 has a positive significant effect on TSe of participants (Table 5). The inclusion of age in the regression models lowers the statistical significance of the above correlations which, however, remain marginally significant. Component 2 has no significant effect on TSe of participants (Table 5).

### Beverages or alcoholic beverages consumption and TSe

Tea consumption revealed no significant association with TSe in all participants (p = 0.44). On the other hand, coffee consumption has a marginal positive association with TSe (p = 0.05) in total sample; however, this association was masked after the inclusion of age in multiple regression analysis model (p = 0.96). No association between decaffeinated coffee consumption with TSe was observed (p = 0.88). Concerning alcoholic beverages, we observed that total ethanol consumption is inversely correlated with TSe in total sample (rho = -0.18, p = 0.02). However, this correlation remains significant for males (rho = -0.18, p = 0.02), whereas no significant correlation appears for females (rho = -0.04, p = 0.65). The association between TSe and ethanol consumption was not significant after adjusting for age and other potential confounders (p = 0.15). Finally, wine consumption was not associated with TSe in all participants (p = 0.38).



**Table 2** Correlation of selenium status in serum ( $\mu$ g/L) with consumption of certain food groups (servings/week)

Food groups	Serum total Se	Serum total Se (µg/L)			
(servings/week)	Total sample	Males	Females		
Fish	$rho^a = -0.08$	rho = -0.16	rho = 0.04		
	p = 0.19	p = 0.05	p = 0.64		
Nuts	rho = 0.05	rho = 0.06	rho = 0.06		
	p = 0.38	p = 0.41	p = 0.50		
Legumes	rho = -0.05	rho = -0.13	rho = 0.08		
	p = 0.38	p = 0.11	p = 0.39		
Dairy products	rho = 0.07	rho = 0.07	rho = 0.04		
	p = 0.23	p = 0.34	p = 0.66		
Fruits	rho = 0.01	rho = 0.07	rho = 0.03		
	p = 0.86	p = 0.92	p = 0.75		
Vegetables	rho = -0.14	rho = -0.19	rho = -0.04		
	p = 0.02*	p = 0.01*	p = 0.64		
Potatoes	rho = 0.02	rho = -0.02	rho = 0.08		
	p = 0.72	p = 0.79	p = 0.42		
Cereals	rho = -0.02	rho = -0.09	rho = -0.07		
	p = 0.73	p = 0.24	p = 0.43		
Sweets	rho = 0.08	rho = 0.07	rho = 0.10		
	p = 0.18	p = 0.38	p = 0.28		
Red meat	rho = 0.13	rho = 0.18	rho = 0.13		
	p = 0.03*	p = 0.02*	p = 0.19		
Eggs	rho = 0.03	rho = 0.08	rho = 0.05		
	p = 0.62	p = 0.32	p = 0.63		
Poultry	rho = 0.13	rho = 0.09	rho = 0.21		
	p = 0.03*	p = 0.24	p = 0.03*		

<sup>&</sup>lt;sup>a</sup> rho Spearman correlation coefficient

**Table 3** Results from multiple linear regression analysis that evaluate the association between selenium status in serum (dependent variable) with red meat consumption (independent variable)

	$b^{\mathrm{a}}$	SE <sup>b</sup>	p
Age (per 1 year)	-0.67	0.22	0.002
Male versus female gender	0.75	5.21	0.87
Body mass index (per 1 kg/m <sup>2</sup> )	-0.40	0.55	0.46
Smoking (yes/no)	-8.16	4.55	0.07
Med.diet score (0-55 units)	-0.04	0.38	0.90
Red meat consumption (servings/week)	2.37	0.91	0.01

 $<sup>^{</sup>a}$  b = b-coefficient

# Discussion

Several epidemiological studies have suggested that a low Se status is associated with an increased risk for a number of diseases [2–5]. Moreover, numerous studies have shown that TSe reflects alimentary intake [1, 6]. Therefore, the

**Table 4** Score coefficients derived from principal components analysis regarding food groups consumed by study participants

Food groups	Component <sup>a</sup>			
	1	2	3	
Fish	0.780	-0.083	0.020	
Nuts	0.442	0.258	0.240	
Legumes	0.699	0.116	0.098	
Dairy products	0.212	0.732	0.107	
Fruits	0.505	0.505	-0.067	
Vegetables	0.767	0.172	-0.003	
Potatoes	0.122	0.212	0.694	
Cereals	0.617	0.280	0.353	
Sweets	0.406	0.462	0.379	
Red meat	0.046	0.193	0.769	
Eggs	-0.004	0.760	0.076	
Poultry	0.046	-0.157	0.654	

Score coefficients are similar to the correlation coefficients. Higher absolute values indicate that the food variable is correlated with the respective component. Numbers in bold indicate loadings greater than 0.5

**Table 5** Multiple linear regression model with selenium status in serum as dependent variable and PCA components scores as independent variables

	$b^{\mathrm{a}}$	SE <sup>b</sup>	p
Component 1			_
Fish, vegetables, legumes, cereals, fruits	-3.94	2.28	0.08
Component 2			
Dairy products and eggs	-0.41	0.51	0.86
Component 3			
Red meat or white meat and potatoes	3.51	0.15	0.13

The multiple linear regression model was adjusted for age, sex, BMI and smoking

determination of TSe in all populations may contribute to the assessment of their nutritional and health status. As far as we know, there are limited studies determining nutritional selenium status in population-based Greek cohorts [25]. Moreover, no studies have previously attempted to correlate the dietary habits of Greek people with TSe. Under this perspective, the main purpose of this work was to assess whether the consumption of certain food groups or the adoption of certain dietary patterns may affect the TSe in a sub-sample of the ATTICA study.



b SE standard error

<sup>&</sup>lt;sup>a</sup> Description of the components: **component 1** = a vegetarian food pattern, **component 2** = a pattern that is mainly characterized by the intake of dairy products and eggs and **component 3** = a pattern describes a diet rich in red meat, poultry and potatoes

 $<sup>^{</sup>a}$  b = b-coefficient

b SE = standard error

As we have previously described. TSe between males and females did not differ despite the worse biological and anthropometric indices of males. The similar TSe between males and females is in accordance with the similar dietary habits of both genders [24]. According to our findings, among all food groups, only the consumption of red meat positively correlated with TSe, irrespective of age, sex, smoking, BMI as well as overall dietary habits of the participants (Med. Diet Score). The consumption of other foods (i.e., fishes, cereals, dairy products) had not demonstrated such a relationship. On the other hand, the consumption of vegetables inversely correlated with the TSe, though this relationship disappeared after adjustment for age. This inverse correlation can be attributed to the fact that a diet rich in vegetables usually corresponds to a diet low in meat consumption. Most of the aforementioned correlations remained significant only for males that are a more homogeneous group than females, whose menstrual cycle affects TSe [26]. The positive correlation between red meat consumption and TSe observed in our study is in accordance with the results of similar studies in French [14] and Polish [15] healthy populations. However, the French study has also demonstrated a positive correlation between fish consumption and TSe. Our study failed to demonstrate this probably because the dietary habits of modern Greeks are characterized by the consumption of large amounts of meat and much lower amounts of fish [27]. To which extent the consumption of foods influences the TSe values of participants also depends on the bioavailability of Se. Selenium of red meat is highly bioavailable [14, 15] while fish Se has a rather limited bioavailability [15, 28]. Moreover, the bioavailability of vegetable Se is negatively influenced by the presence of dietary fibers (especially pectins) and phytate in them [13]. On the other hand, red meat is a rich source of readily bioavailable Se whose consumption is able to drive the synthesis of selenoproteins, to some extent, as well as selenium-containing proteins [29, 30]. Last but not least, it should be mentioned that foods consumed in an urban region such as Athens comes from different part of the world. This contributes to the great variation of selenium content in foods which may influence the reflection of dietary selenium intake in serum selenium status. The above observations are confirmed by the results of the PCA analysis, which reveals that the adoption of vegetarian type of diet (component 1) is inversely correlated with TSe whereas a diet rich in red meat and poultry (component 3) is positively correlated with TSe.

Ethanol was inversely correlated with TSe, however, this association was lost after the inclusion of age and other lifestyle parameters in multiple regression analysis. Concerning the effect of alcoholic beverages on TSe, previous studies provided contradictory results [14, 15, 31–33].

However, it should be mentioned that the majority of our population was light drinkers; therefore, an effect of alcohol on liver dysfunction [33]—which lowers TSe—should be excluded. Moreover, in our study, an unadjusted marginal positive correlation between TSe and coffee consumption was observed in contrast to the only available study concerning this relationship [34]. However, this positive unadjusted association was masked when age was taken into account.

Despite the strengths reported above, this work has also some limitations. First of all, it should be mentioned that we have no data available for dietary supplements intake that may serve as source of selenium and others antioxidants. Moreover, dietary assessment was performed once and therefore, may be prone to seasonal bias; however, the FFQ used was repeatable and valid, while the sampling lasted throughout the year. Finally, this is a cross-sectional study that cannot claim for causal relationships but only generate hypotheses for further clinical research.

#### Conclusion

In conclusion, this is the largest study, so far, concerning the association between TSe and dietary habits in apparently healthy Greeks. The main outcome of the present study is the fact that red meat consumption seems to be a significant determinant of TSe while the adoption of vegetarian type of diet is inversely correlated with TSe.

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