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# Trace Element Status (Se, Cu, Zn) in Healthy Portuguese Subjects of Lisbon Population

## *A Reference Study*

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## ABSTRACT

Serum levels of selenium, copper, and zinc were systematically determined in healthy subjects of the Lisbon population. The sample consisted of 183 blood donors of both genders who were divided into two age groups: 20–44 and 45–70 yr of age; relationships with gender, age, the lipid profile, and tobacco consumption were investigated. In the female group, the intake of oral contraceptives and pregnancy were considered for the youngest women, and hormonal replacement therapy (HRT) was taken into account for the oldest ones. Serum concentrations of these elements were in the same range as those found for populations of other European countries. Differences between genders were observed for the three elements studied, with serum selenium and zinc concentrations higher in men and copper levels higher in women. Age-dependent differences were found for selenium: The oldest subjects (regardless gender) presented the highest concentrations of

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selenium in serum as further demonstrated by the positive correlation with age. In both pregnant and contraceptive-using women, copper was greatly increased, confirming the influence of estrogen status and/or oral contraceptive intake on increased serum copper levels. However, in postmenopausal women, HRT did not significantly affect serum copper levels. Selenium, copper, and zinc status were not different between normolipidemic and hyperlipidemic subjects for the same gender and age range, but selenium levels tended to increase with hyperlipidemia when considering the whole group of subjects. With respect to the lifestyle, higher serum zinc levels were found in tobacco-consuming men. Albumin serum levels were similar for all considered subgroups, except for the pregnant women, for whom a decrease in this parameter was observed. The present study allowed one to obtain reference values for this healthy group of population, which will serve for a comparative study with groups having pathological conditions, such as cardiovascular disease.

**Index Entries:** Trace elements; selenium; copper; zinc; albumin; lipid profile; hormonal status; smoking habits.

## INTRODUCTION

Trace elements such as selenium (Se), copper (Cu), and zinc (Zn) are accepted as essential for optimal human health, because of their diverse well-documented metabolic functions. They have catalytic, structural, and regulatory roles and interact with macromolecules such as enzymes, hormones, and other biological molecules *(1).*

Selenium exerts its role in mammals mainly through selenoproteins with redox activities that contain selenocysteine in their primary structure *(2,3).* The selenocysteine-containing enzymes include the glutathione peroxidase family, two or three types iodothyronine deiodinase, and at least two thioredoxin redutases *(2).* In addition, new health-related functions have been identified for some other selenoproteins—for instance in the male reproductive system, where the phospholipid hydroperoxide glutathione peroxidase can exert both a protective activity (redox enzyme) and a structural role *(3,4).* Moreover, there is now considerable evidence that Se plays a key role in the modulation of the immune response and persuasive epidemiological studies demonstrated that it reduces cancer risk. However, experimental and epidemiological studies have not yet been conclusive for a protective effect of Se against other chronic pathological states, such as cardiovascular diseases *(3).*

Copper acts as a cofactor of about 30 Cu-dependent enzyme systems such as cytochrome-*c* oxidase, dopamine β-hydroxylase, thyroxinase, cytosolic and extracellular superoxide dismutases, and lysyl oxidase *(5,6).* In humans, the element is known as having a protective role against leukopenia, bone demineralization, arterial fragility, and demyelinization of nerve tissues, in relation to the activities of Cu-dependent enzymes *(6).* However, even if Cu is an essential element with diverse biological functions, includ-

ing antioxidant properties mostly the result of the Cu,Zn-SOD (superoxide dismutase) enzyme activities, it is also considered a pro-oxidant catalyst because of its transition metal condition, which is, in part, prevented in the extracellular compartment by its binding with ceruloplasmin *(7)*. The latter has antioxidant activity, not only because of its binding ability but also because it has free-radical-scavenging properties and functions as a ferroxidase enzyme *(7)*. Most of the changes observed in plasma Cu concentrations are associated with changes in ceruloplasmin that depend on factors such as age, gender, hormone therapy, pregnancy, or an inflammatory process *(8)*.

Zinc is a component of the catalytic site of numerous enzymes, with at least one in every enzyme class. In addition, 30% of cellular Zn is found within the nucleus of mammalian cells, and a large number of proteins playing a role in the regulation of gene expression have been either shown to or suspected of contain Zn *(9)*. It is presently accepted that Zn-dependent metabolic functions are present in every tissues. Organ systems known to be clinically affected by severe Zn deficiency include the epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems *(9,10)*. One of the most studied clinical features of Zn deficiency is the impairment of physical growth. Studies in developing countries are now under way to give more attention to pregnancy and the effects of maternal Zn status on both prenatal and postnatal development *(11,12)*.

Previous epidemiological studies have shown that essential element status depends on factors such as age, gender, and pathophysiological conditions *(8,9,13–15)*. In addition, geographical location seems to be an important factor in determining blood levels of some trace elements because the levels can be linked to the influences of geochemistry on soils, food, and water supply *(8,16–19)*. Finally, social and environmental conditions and lifestyle such as tobacco consumption are also factors influencing the trace element status *(16,19–23)*.

Some clinical prospective studies have suggested a relationship between these trace element levels and the serum lipid profile *(19,24,25)*. Moreover, it is believed that the impairment of this trace element status might be linked to the occurrence of degenerative diseases associated with vascular damage and atherogenesis *(24,26–29)*. Previous epidemiological studies on trace element status have been carried out in different European countries, mainly in the central and northern ones *(30,31)*. However, in the Iberian Peninsula and particularly in Portugal, studies on trace elements in humans focusing on their relationship with influencing factors remain scarce.

The present study is part of a larger project aiming at investigating blood parameters associated with antioxidant systems in humans from urban, fishing, and rural areas of Portugal. The objective is to evaluate serum Se, Cu, and Zn levels in healthy subjects of a representative sample of the Portuguese population of Lisbon and to assess the relationship of these elements with gender, age, lipid profile, pregnancy, and drug treatment with oral contraceptives or hormonal replacement therapy (HRT) in younger and older women, respectively. With reference to life habits, the

influence of tobacco consumption is also investigated. Furthermore, serum albumin levels were also quantified, as serum albumin is known as an indicator of general health condition, namely in the aging process and in hypercholesterolemic status in humans.

## SUBJECTS AND METHODS

### Subjects

The protocol of this study was approved by the Human Ethics Committee of the National Health Institute Dr. Ricardo Jorge. The population group consisted of 183 volunteers (136 women and 47 men) of Portuguese origin living in the city of Lisbon and between 20 and 70 yr of age. They belonged to the middle socioeconomic class with urban dietary habits. Informed consent was obtained from each donor before blood was drawn. All subjects were screened to determine eligibility, determined by completing a clinical report stating all information relevant to the study. Information regarding age, gender, drug intake (oral contraceptives, HRT, and others), smoking habits, and general clinical state was obtained. The original study group was much larger, but because the main point of this work was to establish reference values in a healthy reference population, subjects with pathological conditions were not included. The study group was divided into different subgroups according to gender and age, taking into account the hormonal status for women and the lipid profile and smoking habits for both sexes.

### Methods

#### Blood Collection

Blood collection took place at the National Health Institute Dr. Ricardo Jorge from November 1999 to July 2001. The venipuncture of the individuals was carried out in the morning after 12-h fasting. Peripheral blood samples were obtained by venipuncture and placed into S-Monovette® Z-Gel tubes. Serum was removed after centrifugation at 1500*g* for 10 min at 4°C and distributed in various aliquots for lipoprotein separation, lipid analysis, and trace element determinations. The aliquots for trace elements were frozen at –20°C until analysis.

#### Determination of Serum Se, Cu, and Zn Levels

Serum Se was determined by the direct electrothermal atomic absorption spectrometric procedure with a Zeeman background correction *(32).* Serum Cu and Zn were determined by the flame atomic absorption spectrometric procedure *(33).* The accuracy of the procedures was checked with standard reference materials (lyophilized human serum, Seronorm® from Nycomed) and participation in interlaboratory comparison trials.

### *Determination of Serum Lipids*

Separation of high-density lipoproteins (HDLs) was obtained by adding polyethylene glycol to the fresh samples in order to precipitate other lipoproteins *(34).* The direct quantitative determinations of cholesterol (C) and HDL cholesterol (HDL-C) in human serum were based on enzymatic in vitro assays using CHOD-PAP® and the HDL-C Plus® analysis kits from Roche, respectively. The concentration of low-density lipoprotein (LDL) cholesterol (LDL-C) was calculated by using the Friedewald formula *(35).* The quantitative determination of serum triglycerides (TGs) was based on an enzymatic in vitro test using the GPO-PAP® analysis kit from Roche.

### *Determination of Serum Albumin*

The quantitative determination of serum albumin was performed using a Randox kit (AB 362®) and expressed as grams per deciliter.

## *Statistics*

Before statistical analysis, the homoscedasticity of each sample was checked using Bartlett's test. In cases of significant heteroscedasticity, those data that deviated more than 3 to 1 in relation to the standard deviation were omitted. The standardized skewness and the standardized kurtosis were also tested in order to determine whether each sample could be adequately modeled by a normal distribution *(36).* For the comparison of means between samples, a *t*-test was performed assuming equal variances. For non-normally distributed samples, the Mann–Whitney *U*-test was used. Pearson or Spearman correlation coefficients were applied for normally and non-normally distributed samples in order to test possible causal relationships between the analyzed parameters and between each parameter and age. In particular, for the relationship between selenium and age, a simple linear model was chosen because of its explanatory power and because it does not differ significantly from any other model. These regression lines are graphically illustrated. In this context, analysis of variance (ANOVA) statistics for testing the statistical significance of the fitted relationship between selenium and age was used. To evaluate the degree of explanation of the fitted model, $r^2$ was calculated. The conventional, albeit arbitrary, $\alpha$ level was 0.05. The software used for statistical analysis was Statgraphics Plus for Windows 4.0® by Statistical Graphics Corp.

# RESULTS

## *Trace Element Concentrations According to Gender and Age*

The results of serum trace element levels in relation to gender and age are summarized in Table 1. Significant differences were observed for the three

Table 1
Serum Se, Cu, and Zn Concentrations in a Group of the Lisbon Population, According to Gender and Age (Range: 20–44 and 45–70 yr).

| | Women | | | Men | | |
|---|---|---|---|---|---|---|
| **Elements** | **Total** | **20 – 44** | **45 - 70** | **Total** | **20 - 44** | **45 - 70** |
| | **(n = 136)** | **(n = 75)** | **(n = 61)** | **(n = 47)** | **(n = 28)** | **(n = 19)** |
| **Se** | $81 \pm 14^{b,c,d,e}$ | $77 \pm 13^{c,f,g,h,i}$ | $85 \pm 14^{g}$ | $88 \pm 15^{b}$ | $88 \pm 17^{d,f,h}$ | $87 \pm 13^{e,i}$ |
| **(μg/L)** | (36 - 123) | (36 - 122) | (52 - 123) | (64 - 127) | (64 - 127) | (68 - 119) |
| **Cu** | $132 \pm 50^{k}$ | $140 \pm 60^{a,l}$ | $123 \pm 33^{m}$ | $103 \pm 38^{k}$ | $104 \pm 45^{l}$ | $100 \pm 28^{a,m}$ |
| **(μg/dL)** | (42 - 375) | (60 - 375) | (42 - 280) | (40 - 260) | (54 - 260) | (40 - 144) |
| **Zn** | $99 \pm 17^{j}$ | $98 \pm 17^{n}$ | $100 \pm 16$ | $105 \pm 18^{j}$ | $106 \pm 19^{n}$ | $104 \pm 17$ |
| **(μg/dL)** | (65 - 192) | (65 - 192) | (79 - 192) | (61 - 150) | (74 - 150) | (61 - 136) |

*Note:* Values represent the mean ± SD and ranges are given in parentheses. Statistical significance: $p$-value using the Student's $t$-test: $p<0.05^{a,b,c,d,e,f,g,h,i}$; $p$-value using Mann–Whitney $U$-test: $p<0.05^{j,k,l,m,n}$.

studied elements, as serum Se and Zn levels were higher in men than in women and Cu levels were higher in women than in men. Statistically significant differences were found between age classes for Se. When comparing women of different age groups, the older women showed much higher Se levels than the younger ones. Moreover, men in the younger group had more elevated serum Se levels than women of the same group. However, no statistically significant differences were observed when older men were compared to women of the same age group (*see* Table 1). The mean serum Cu was higher in women than in men for both age groups considered (*see* Table 1). Concerning Zn, higher values were found in younger men when compared to the same age group of women. In the older groups and similarly for Se, the difference between genders was not significant (*see* Table 1).

As far as serum albumin levels are concerned, no significant differences were found either for gender (women = 4.11 ± 0.477 g/dL; men = 4.21 ± 0.342 g/dL) or age range (younger women = 4.04 ± 0.570 g/dL; older women = 4.19 ± 0.318 g/dL; younger men = 4.20 ± 0.335 g/dL; older men = 4.22 ± 0.358 g/dL).

## Trace Element Concentrations According to the Lipid Profile

In order to study the relationship between trace element levels and the lipid profile, serum lipid parameters were also determined (*see* Table 2). The subjects from each gender were divided into two groups, according to their levels of cholesterol and/or triglycerides in serum. The first group consisted of normolipidemic subjects with cholesterol below 200 mg/dL and/or triglycerides below 150 mg/dL, in accordance with the recommendations of the Expert Panel *(37).* The group of hyperlipidemic subjects had one or both parameters above these values. No significant differences were found for the three elements when comparing normolipidemic and hyperlipidemic individuals of the same gender (*see* Table 2). Also, the trace element levels did not differ with the lipid profile according to the age range for each sex. Differences were a consequence of those already reported in Table 1. However, the lipid profile occasionally modified the degree of data significance, which is illustrated in Table 2. Taking both genders together and using the nonparametric Mann–Whitney *U*-test, it was observed that all hyperlipidemic subjects showed higher serum Se values than normolipidemic individuals ($p < 0.05$).

The values found for serum albumin are identical when comparing normolipidemic and hyperlipidemic individuals (normolipidemic women = 4.16 ± 0.384 g/dL; hyperlipidemic women = 4.12 ± 0.324 g/dL; normolipidemic men = 4.21 ± 0.399 g/dL; hyperlipidemic men = 4.22 ± 0.284 g/dL).

## Trace Element Concentrations According to Hormonal Condition in Women

Serum trace element levels according to age range and hormonal status in women are presented in Fig. 1. In the younger women group, the

Table 2
Serum Se, Cu, and Zn Levels and Serum Lipid Concentrations (Cholesterol, HDL-Cholesterol, LDL-Cholesterol, and Triglycerides) in Normolipidemic and Hyperlipidemic Subjects from the Lisbon Population, According to Gender

| | Normolipidemic | | Hyperlipidemic | |
|---|---|---|---|---|
| **Parameter** | **Women** (n = 69) | **Men** (n = 25) | **Women** (n = 67) | **Men** (n = 22) |
| **Se (μg/L)** | 78 ± 14[a] (36 - 122) | 85 ± 16 (64 - 127) | 83 ± 14 (53 - 123) | 91 ± 15[a] (67 - 123) |
| **Cu (μg/dL)** | 131 ± 52[b] (42 - 375) | 94 ± 30[b,c] (40 - 154) | 133 ± 49[c] (60 - 300) | 112 ± 45 (76 - 260) |
| **Zn (μg/dL)** | 98 ± 12 (65 - 122) | 102 ± 20 (61 - 150) | 100 ± 20 (74 - 192) | 109 ± 15 (93 - 136) |
| **Cholesterol (mg/dL)** | 165 ± 21[d] (123 - 199) | 166 ± 24[e] (105 - 197) | 234 ± 28[d] (197 - 334) | 234 ± 22[e] (204 - 279) |
| **HDL - Chol. (mg/dL)** | 63 ± 14 (34 - 103) | 49 ± 13 (25 - 77) | 66 ± 19 (24 - 119) | 59 ± 21 (30 - 121) |
| **LDL - Chol. (mg/dL)** | 89 ± 18[f] (46 - 121) | 99 ± 20[g] (60 - 153) | 146 ± 28[f] (88 - 206) | 152 ± 23[g] (98 - 190) |
| **Triglycerides (mg/dL)** | 71 ± 28[h] (28 - 132) | 82 ± 32[i] (34 - 143) | 111 ± 61[h] (43 – 411) | 116 ± 59[i] (38 - 242) |

*Note:* Values represent the mean ± SD and ranges are given in parentheses. Statistical significance: *p*-value using the Student's *t*-test: $p<0.05^{a,b,c,d,e}$; *p*-value using Mann–Whitney *U*-test: $p<0.05^{f,g,h,i}$.

contraceptive users and pregnant women (classes 2 and 3) showed greatly increased serum Cu levels in comparison to those using no hormonal contraceptives (class 1). In addition, a nonsignificant decrease in serum Se and Zn levels was observed in pregnant women (class 3) in comparison to both groups of younger women (classes 1 and 2). In the older women group, no significant differences were found for the three elements studied between women under HRT (class 5) and those who were not (class 4). A slight but not significant increase in Cu levels was observed in women under HRT (class 5) as compared to nonmedicated postmenopausal women (class 4).

In relation to albumin levels, pregnant women showed decreased levels of this protein ($p < 0.05$). For the remaining groups and using the numerical order established, the values found were all very similar (class 1: 4.05 ± 0.667 g/dL; class 2: 4.16 ± 0.382 g/dL; class 3: 3.53 ± 0.250 g/dL; class 4: 4.18 ± 0.322 g/dL; class 5: 4.23 ± 0.312 g/dL).

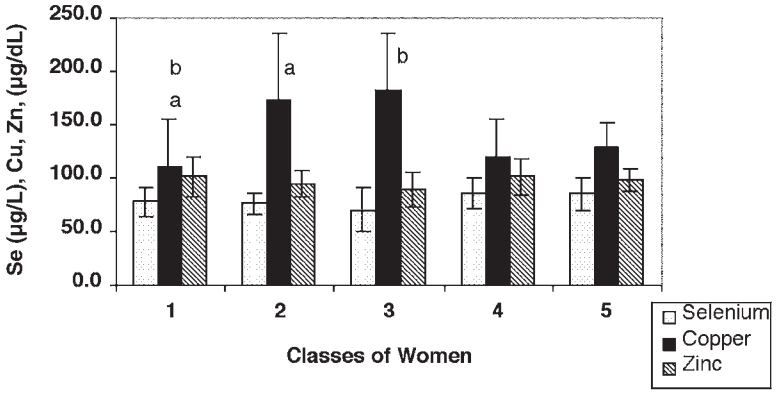


Fig. 1. Serum Se, Cu, and Zn levels in women classes, according to age and hormonal status. Class 1: noncontraceptive users of the younger group ($n$=41); class 2: contraceptive users of the younger group ($n$=27); class 3: younger pregnant women ($n$=7); class 4: postmenopausal women without HRT ($n$=44); class 5: postmenopausal women under HRT ($n$=17). $p$-Value using the Student's $t$-test: $p<0.05^{a,b}$.

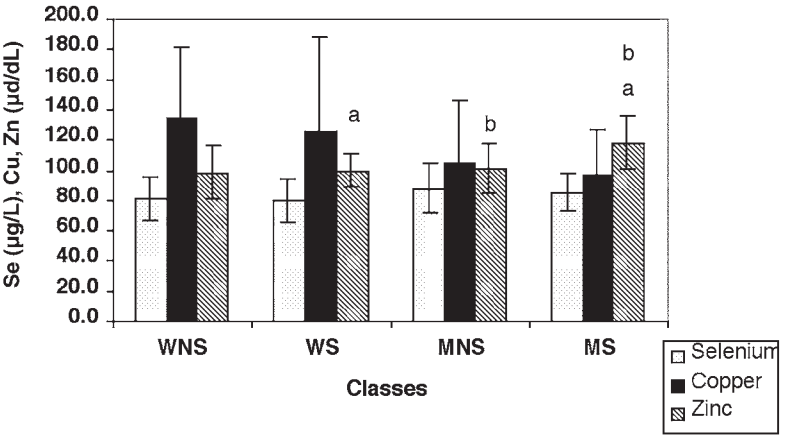


Fig. 2. Serum Se, Cu, and Zn levels, according to gender and tobacco consumption. WNS=women nonsmoking ($n$=110); WS=women smoking ($n$=26); MNS=men nonsmoking ($n$=35); MS=men smoking ($n$=12). $p$-Value using the Student's $t$-test: $p<0.05^{a,b}$.

## *Trace Element Concentrations According to Smoking Habits*

Results according to gender and smoking habits are illustrated in Fig. 2. Because significant differences regarding tobacco consumption were generally not found between age classes for both genders, the results were presented without taking into account the age range. Selenium was not

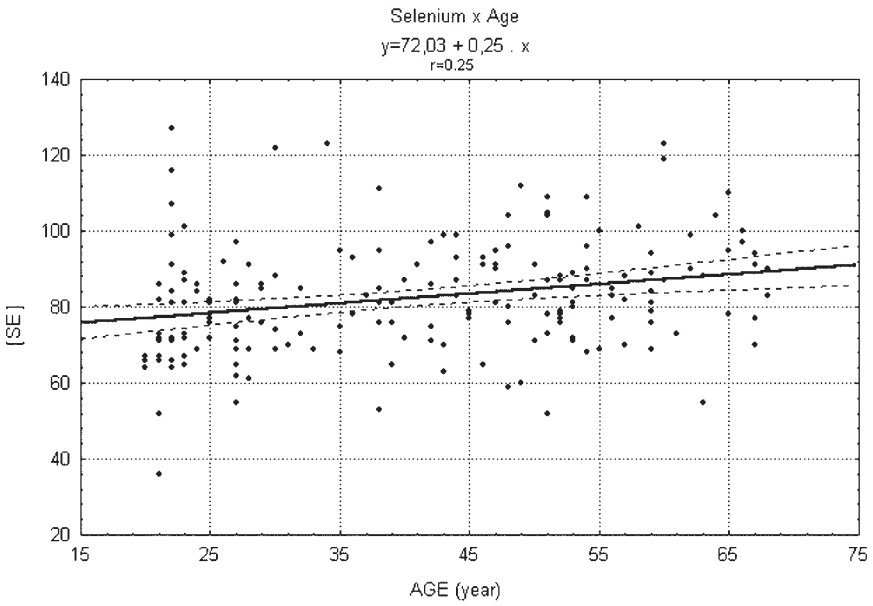


Fig. 3. Relationship between age and selenium in a sample from the Lisbon population.

significantly modified as a consequence of tobacco use. Mean Cu levels for nonsmoking women and men were slightly more elevated than in smoking women and men, but the difference did not reach statistical significance. As far as zinc is concerned, male smokers had higher levels compared to nonsmokers.

Apparently, smoking habits did not affect the protein levels of albumin in serum, as no statistical differences between nonsmoker and smoker groups (nonsmoking women = 4.15 ± 0.354 g/dL; smoking women = 4.11 ± 0.371 g/dL; nonsmoking men = 4.21 ± 0.352 g/dL; smoking men = 4.25 ± 0.333 g/dL) were observed.

## Correlations Between Parameters

Considering all subjects, positive correlations were found by Pearsons' correlation coefficient for the following variables: age and Se ($r = 0.25$; $p < 0.05$), Cu and Zn ($r = 0.15$; $p < 0.05$), and triglycerides and Cu ($r = 0.21$; $p < 0.05$). Figure 3 gives the regression line between age and selenium. Other associations between serum lipid parameters and age were found for the whole group of subjects through Spearman's correlation coefficient: cholesterol and age ($r = 0.40$; $p < 0.05$), triglycerides and age ($r = 0.21$; $p < 0.05$), and Se and cholesterol ($r = 0.25$; $p < 0.05$). In normolipidemic subjects, an association between cholesterol and age was only observed ($r = 0.24$; $p < 0.05$). However, in hyperlipidemic subjects, significant correlations between

cholesterol and age ($r = 0.27$; $p < 0.05$), Se and age ($r = 0.23$; $p < 0.05$), and Se and cholesterol ($r = 0.22$; $p < 0.05$) were also observed.

## DISCUSSION

In the present study, serum Se, Cu, and Zn levels were systematically determined and for the first time in healthy subjects from Lisbon.

### *Trace Element Status in Relation to Gender, Age, and Hormonal Condition*

Serum Se concentrations were in the same range as found for the populations of other European countries *(18),* including Portuguese islands *(17,22,38).* Serum Se levels in men were lower than those found in fishing populations from the Azores archipelago (110 ± 25 µg/L) *(19)* and from Madeira Island (104 ± 21 µg/L) *(38),* but both genders had Se levels similar to those observed in rural populations of the Azores archipelago for men (88 ± 22 µg/L) and women (80 ± 17 µg/L) *(19).* Remarkably, the Se concentrations presently assessed were lower than those previously found for both genders in the Lisbon population (96 ± 15 µg/L for men and 89 ± 14 µg/L for women) *(25).* It therefore seems that a reduced intake of the element might have occurred for the last few years in this urban region. The positive correlation between Se and age presently reported was also observed in other Portuguese groups from Ponta Delgada of the Azores archipelago *(22)* and from Madeira Island (unpublished data). A similar tendency has been observed by other authors in several countries, even though the groups under study were often poorly defined *(16).* As far as gender is concerned, the serum Se levels were higher in men than in women, mainly in the youngest age groups. A similar significant gender-related difference has already been observed in an earlier study on the Lisbon population *(25)* and on fishing and urban populations from S. Miguel Island *(17,22).* Furthermore, a tendency for decreased serum Se levels was observed in pregnant women of this female sample, in accordance with literature data *(39,40).* These findings suggest that the hormonal status partially explains gender differences in serum Se concentrations. However, differences in Se status as a result of gender has not been generally described in populations from other European countries *(16).*

Serum Cu concentrations were generally in the same range as those found for other Portuguese populations from the Azores archipelago *(17,19,22)* and also for other European countries *(13).* Cu serum levels were here not influenced by age, but clearly gender-related with the women having the highest Cu values, such as already shown in previous studies *(13,41).* More recently, Pavão et al. *(17)* and Viegas-Crespo et al. *(22)* found a similar tendency when comparing men and women in several populations (fishing, rural, and urban ones) of the Azores archipelago. This difference is thought

to be related to estrogen status because it was less pronounced when both pregnant women and contraceptive users were excluded from this sample. Several studies have actually inferred a relationship between serum Cu levels and oral contraception and/or pregnancy, suggesting that the estrogen component of the oral contraceptives is responsible for increased Cu and ceruloplasmin levels in plasma *(13,42,43).* Estrogens induce ceruloplasmin synthesis in the liver, which leads to an increase in serum Cu levels *(13,43,44).* The marked elevated Cu status during pregnancy is probably necessary for adequate fetal development, as Cu-dependent enzymes play relevant roles in the organization of connective tissue and bone mineralization (see the Introduction). However, in postmenopausal women, HRT did not modify serum Cu concentrations. This is not in accordance with the results reported by other authors, who found increased Cu levels in serum in such women *(8,15).* The discrepancy could be related to the large diversity of HRT related to the type, dose, and process (transdermal or oral) of hormonal administration *(45).*

Serum Zn concentrations were slightly higher in men than in women and the range values for both sexes were similar to those found for other populations *(13,17,22).* Similar gender dependencies of Zn levels were reported for other populations, but the opposite or even no gender influence were also observed *(13).* Concerning hormonal status in women, no differences could be observed between pregnant and control younger women, suggesting an adequate intake of the element. In Western countries and mainly in developing countries, decreased Zn status during pregnancy has been reported *(46,47).* A weak correlation between Cu and Zn was also observed in the whole subjects, which might be related to serum Cu/Zn-SOD activity *(48).*

### Trace Element Status in Relation to the Lipid Profile

Hyperlipidemia is considered a risk factor for the development of vascular diseases and atherosclerosis. In the present study, the serum levels of Se, Cu, and Zn were not significantly related to the serum lipid profile for the same gender and age range. An impairment in the status of these trace elements has been observed in strong dyslipidemic subjects from other populations *(19,25,49).* In the present study, the sample was composed of healthy people, and individuals from the hyperlipidemic group had lipid levels within the borderline range *(37).* However, when considering the whole group of subjects, increased serum Se levels were observed in hyperlipidemic individuals. This finding could be partly the result of the binding of Se to lipoproteins, mainly the LDLs, which are claimed to contain a significant amount of Se *(50).* The simultaneous determination of other indicators of Se status in serum, such as selenoprotein P and glutathione peroxidase activity *(51),* could contribute to clarify this important point, as data appearing in the literature on the relationship between Se status and the serum lipid profile are generally inconclusive *(22,49,52).*

The tendency for elevated Cu levels in hyperlipidemic individuals and the correlation between Cu levels and triglycerides are also in accordance with data obtained for rural and fishing populations of the Azores archipelago (S. Miguel Island) in normolipidemic and hyperlipidemic individuals (unpublished data). Similar associations between high serum Cu levels and an unfavorable lipid profile were observed in Spanish children and adolescents *(28).* However, this phenomenon remains poorly understood. Salonen et al. *(24)* and Klipstein-Grobusch et al. *(53)* suggested that the increased serum Cu level (and also ceruloplasmin) is a risk factor for cardiovascular disease operating through oxidative modification of LDLs. Therefore, further investigations should be carried out to better characterize the relationship between serum Cu and hyperlipidemia.

### *Trace Element Levels in Relation to Tobacco Consumption*

Elevated serum Zn levels in smoking men but not in women are partially in accordance with Dubick and Keen *(54)* and Faruque et al. *(21),* but not with other authors who did not observe any influence of tobacco consumption *(55,56).* However, Dubick and Keen *(54)* reported decreased Cu and Se levels in serum from smokers, contrary to the present results. These findings suggest that smoking habits might cause an imbalance in trace element status, which is explained by several mechanisms. Increased Zn levels could be the result of the presence of cadmium in tobacco, which interacts with Zn metabolism *(57).* In another context, cigaret smoke induces a chronic inflammatory process that induces apoptosis of alveolar macrophages in vitro *(58).* Zn itself can modulate death processes in precursors of lymphocytes and there is substantial evidence that Zn supplementation reduces the development of several pathologies by preventing the dismantling of the immune system *(59).* Further studies should be carried out in order to clarify the role of zinc in smokers.

### *Serum Albumin Levels and Considered Subgroups*

In regard to serum albumin determinations, no variations between genders and age range were observed. Albumin is considered to be a very stable parameter, and for this reason, reduced levels of this protein are thought to be related with increased mortality risk *(60,61).* Moreover, a direct protective effect of this molecule has been suggested; current evidence that serum albumin may represent the major circulating antioxidant known to be exposed to continuous oxidative stress *(62–64).* The levels found for pregnancy are in accordance with values reported in the literature *(65,66),* since, especially in the third trimester of gestation in parallel with increased lipids retention, lower levels of this protein are usually observed. Concerning the lipid profile, no differences were found for albumin, although it is thought to be true that elevated total plasma homocysteine levels and decreased plasma albumin levels were associated with

hyperlipidemia, a risk factor for coronary heart disease *(61).* Finally, Pignatelli et al. *(66),* in a study conducted with lung cancer patients and current smokers, verified that, the major human plasma protein was neither nitrated nor oxidized, which certainly corroborates the identical values found for serum albumin between smoker and nonsmoker individuals.

In conclusion, the present study obtained reference values for trace element status and serum albumin levels in the Lisbon population. The simultaneous evaluation of the trace element status in other Portuguese regions and the relationship with some of the most relevant biological parameters contributed to a better knowledge of health condition of the Portuguese population. This will allow the possibility of a comparative study on the knowledge and characterization of pathological conditions, such as cardiovascular disease.

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