

## Trace element status (Se, Cu, Zn) and serum lipid profile in Portuguese subjects of San Miguel Island from Azores'archipelago

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### Summary

Data on selenium, copper and zinc in serum of Portuguese inhabitants of the city of Ponta Delgada - Azores Archipelago are reported for the first time.

The subjects are of both sexes, aged 20 to 60 years, and non-abusers of alcohol, tobacco or drugs.

Serum concentrations of these elements are in the same range than those found for populations of Lisbon and of some other European countries. Differences between sexes are observed, with serum selenium and zinc levels being higher in males than in females, and the copper levels being higher in females as compared to males. These results can be explained by the hormonal status and/or oral contraceptive steroids intake, particularly for copper.

Concerning lipid profile, the majority of individuals have serum lipid parameters within the normal range. In addition, no difference in trace element levels between normo and hyperlipidemic individuals is observed and no conclusive results about the relationship of all evaluated parameters to alcohol, tobacco and drug consumption are observed, in agreement with data obtained in the population of Lisbon.

**Keywords:** Trace elements, selenium, copper, zinc, lipid profile, Azores.

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### Introduction

There is increasing evidence that free radical damage is implicated in the development of degenerative diseases such as

atherosclerosis and carcinoma (1, 2), in drug-induced toxicity (3) and in the normal ageing process (4). Selenium, copper and zinc are essential trace elements involved in cellular protection against the deleterious effects of reactive oxygen species. Selenium plays an important role as a cofactor of glutathione peroxidase enzymes (Se-GSH-Px), which destroy hydrogen peroxide and lipid hydroperoxides using glutathione as an electron donor (5 - 7). Recently it was observed that the element is a component of mammalian thioredoxin reductases that promote the NADPH-dependent reduction of thioredoxin, and have an important role in the regulation of the cellular redox balance (8 - 10). On the other hand selenium status seems to influence the metabolism of cholesterol and lipoproteins (11) and it might even be an anticarcinogenic agent (12). Copper and zinc are cofactors of superoxide dismutase (Cu/Zn-SOD), an antioxidant enzyme that detoxifies the superoxide radical and whose activity depends on copper availability (13). However, free copper can act as a prooxidant catalyst thus, its sequestration by plasma proteins, e.g. ceruloplasmin and albumin, is an important biological strategy of antioxidant defense (14). Apart from its role in antioxidant systems, especially as a stabiliser of Cu/Zn-SOD, zinc also participates in certain enzymatic systems required for DNA and RNA synthesis and it seems to influence the proliferative response of normal human lymphocytes (15).

Several epidemiological studies have shown that essential element levels depend on factors such as age, sex, physiological and pathological conditions (15,16). In general, geographical location also seems to be an important factor in determining blood levels of trace elements because it can be linked to the influences of geochemical characteristics, food and water sup-

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ply, social and environmental conditions, lifestyle and other factors (16-18). In addition, some clinical prospective studies have suggested correlations between the status of these trace elements and the serum lipid profile (3,11), as well as the composition in fatty acids of cellular membranes (19). Moreover, it is well known that the impairment of the status of these trace elements is linked to the occurrence of many diseases such as cardiovascular conditions (3, 11, 16, 20).

The main purpose of the present study was to evaluate the serum selenium, copper and zinc levels in Portuguese subjects living in the city of Ponta Delgada, S. Miguel Island, which is one of several volcanic islands belonging to the Azores Archipelago. The relationship between these trace elements and the age, sex, serum lipid profile (triglycerides, total cholesterol, HDL and LDL cholesterol) and life habits such as alcohol, tobacco and drug consumption were also considered.

## Subjects and Methods

### Subjects

The population group consisted of 102 volunteer Portuguese subjects (45 men and 57 women), born living in the city of Ponta Delgada - Azores. The age (20-60 years) and sex of the subjects, as well as drug, alcohol and tobacco consumption were registered. The number of individuals in each group is shown in the results. The donors belong to a middle - high socio-economic stratus with urban dietary habits. They were non-abusers of alcohol and drugs, had no recognizable chronic diseases as stated by their clinical reports. They were asked to begin fasting 12 h before blood sampling, which occurred at about the same time (8-10h) in the morning. The collection was carried out in the Atlantilab laboratories.

### Methods

Blood samples were collected in acid-washed tubes by venipuncture. In all cases a tourniquet was used and quickly re-

Table 1. Serum selenium, copper and zinc levels in the population of Ponta Delgada (S. Miguel)

Sex	Age (Mean±SD)	Serum concentrations (Mean±SD / Range)		
		Se (µg/L)	Cu (mg/L)	Zn (mg/L)
Women	42±12	88±15* (58-131)	1.38±0.40* (0.77-2.42)	1.02±1.60** (0.80-1.40)
Men	41±10	98±16* (65-137)	1.15±0.19* (0.67-1.72)	1.08±0.15** (0.60-1.40)

Values represent the mean±SD for women (n=57) and for men (n=45). Asterisks denote the significance of differences between means of men and women based on the t test (\*P<0.01; \*\*P<0.05). Ranges are in parentheses.

moved as soon as puncture was started. Serum was removed after centrifugation at 1800 g for 15 min at 4 °C and aliquots were kept frozen at -20 °C until trace element analysis. Serum selenium was determined by the direct electrothermal atomic absorption spectrometric procedure with Zeeman background correction (21). Serum copper and zinc were determined by the flame atomic absorption spectrometric procedure (22). The accuracy of the procedures was checked with standard reference materials (lyophilized human serum, Seronorm from Nycomed) and participation in interlaboratory comparison trials.

HDL lipoproteins were obtained by adding polyethylene glycol to fresh samples in order to precipitate other lipoproteins (23). Serum total cholesterol as well HDL cholesterol were determined enzymatically by the cholesterol CHOD-PAP method using a kit from Boehringer (Mannheim, Germany). LDL cholesterol was evaluated by the Friedewald formula. Serum triglycerides were also determined by an enzymatic procedure based on the triglyceride GPO-PAP method using a kit from the same manufacturer.

### Statistics

The normality of the distributions was evaluated by the Chi-square test. Group means comparisons were tested for significance by Student's t-test. A variance analysis (ANOVA) was performed in order to compare age, sex, alcohol and tobacco consumption with the evaluated parameters. In order to study the change with age the individuals were divided into four age groups (20 to 29, 30 to 39, 40 to 49 and 50 to 60 years) and a STATISTICA programme for Windows (release 5, Statsoft Inc. Tulsa, USA) was applied.

## Results

Serum trace element concentrations for the total sampled population are presented in Table 1. A significant difference

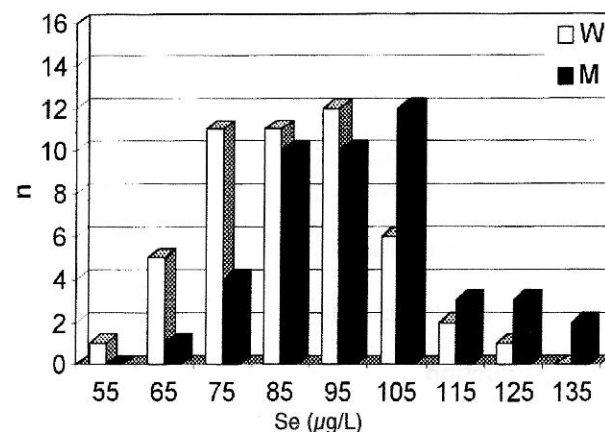


Figure 1. Frequency distribution of serum selenium concentration (µg/L) for the totality of male and female subjects aged 20 to 60 years. n=number of subjects

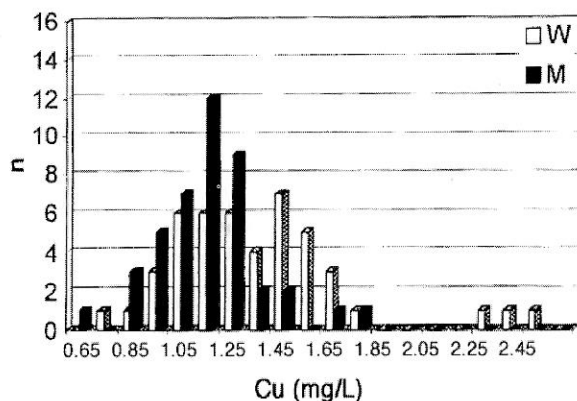


Figure 2. Frequency distribution of serum copper concentration (mg/L) for the totality of male and female subjects aged 20 to 60 years. n=number of subjects

( $P < 0.01$ ) in both selenium and copper levels was observed between women and men. Selenium was about 17 % lower and copper about 12 % higher in women than in men. In addition, men seemed to have somewhat but significantly higher serum zinc concentration than women ( $P < 0.05$ ). The frequency distribution for the three elements, taking women and men separately, could be considered normal, as can be seen in the Figures 1-3.

Taking into consideration the age of the subjects (Table 2), an increase in serum selenium levels with age was observed (ANOVA:  $F = 3.58$ ,  $P < 0.02$ ), when both sexes were considered together; however when the two sexes were considered separately, no difference could be observed, either for men (ANOVA:  $F = 2.01$ ,  $P < 0.13$ ) or for women (ANOVA:  $F = 2.79$ ,  $P < 0.051$ ).

In the same way zinc levels appeared to change across the age range for all the subjects (ANOVA:  $F = 2.74$ ,  $P < 0.05$ ), but no relationship was between the element and age when the two sexes were considered separately (Table 2). Copper did not show any change with age across the age range (ANOVA:  $F = 0.31$ ,  $P < 0.82$ ).

In order to study the relationship between the levels of selenium, copper and zinc and the lipid profile, serum lipid parameters were also determined (Table 3). The subjects from each sex were divided up into two groups according to their levels of total cholesterol and/or triglycerides in serum. The first group consisted of normolipidemic subjects with total cholesterol be-

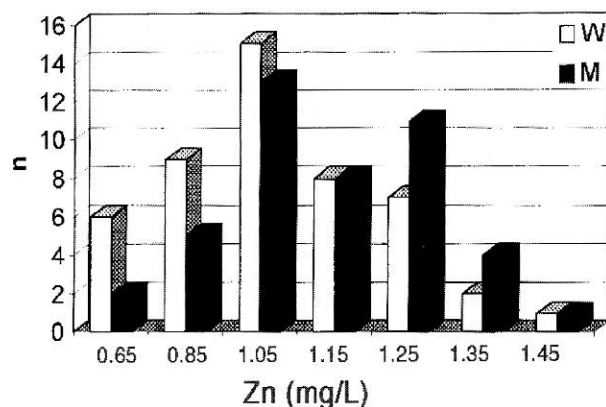


Figure 3. Frequency distribution of serum zinc concentration (mg/L) for the totality of male and female subjects aged 20 to 60 years. n=number of subjects

low 250mg/dL and/or triglycerides below 115mg/dL for women and below 155mg/dL for men. The group of hyperlipidemic subjects had one or both parameters above those reference values.

For hyperlipidemic women there were eleven with triglyceride contents and five with cholesterol levels above normal values. Only one of them had cholesterol and triglyceride contents  $> 300$  mg/dL. In addition, the levels of HDL and LDL cholesterol were within the normal range as related to total cholesterol for normo- and hyperlipidemic groups.

For men, there were eleven men with total cholesterol levels and nineteen with triglyceride levels higher than normal values. Among these only two men had cholesterol above 300 mg/dL and four had triglycerides above 300mg/dL. As it was observed for women, the levels of HDL and LDL cholesterol were within the normal range as related to the total cholesterol for both groups (Table 3).

Serum levels of selenium, copper and zinc were not different between normo and hyperlipidemic individuals for both sexes (Table 3). In addition, no significant correlation was observed between the level of each trace element and these lipid parameters.

Table 4 shows that life habits, such as tobacco and alcohol do not appear to affect the levels of these trace elements, except for zinc levels, where a significant decrease ( $P < 0.05$ ) was observed in smoker women when compared with non-smokers. Concerning contraceptive intake the most striking change is in

Table 2. Serum selenium, copper and zinc levels for each age group in a population from Ponta Delgada (S. Miguel)

Age groups (years)	Se ( $\mu$ g/L)			Cu (mg/L)			Zn (mg/L)		
	W+M	W	M	W+M	W	M	W+M	W	M
20-29	87	79	95	1.28	1.56	1.10	1.01	0.97	1.09
30-39	86	84	88	1.28	1.40	1.16	0.98	0.91	1.04
40-49	95	89	100	1.20	1.30	1.12	1.09	1.05	1.10
50-60	99	96	103	1.29	1.43	1.19	1.07	1.05	1.11

Values represent the mean of serum selenium, copper and zinc concentrations for each age group, considering the whole population (W+M), women (W) and men (M)

Table 3. Serum selenium, copper and zinc levels and serum lipid concentrations (cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) in normolipidemic and hyperlipidemic subjects in a population from Ponta Delgada (S. Miguel Island)

Parameter	Normolipidemic		Hyperlipidemic	
	Women (n=46)	Men (n=26)	Women (n=11)	Men (n=19)
Selenium ( $\mu\text{g/L}$ )	86 $\pm$ 15 (58-131)	98 $\pm$ 16 (65-132)	93 $\pm$ 13 (71-112)	98 $\pm$ 16 (77-137)
Copper (mg/L)	1.36 $\pm$ 0.35 (0.77-2.30)	1.15 $\pm$ 0.21 (0.67-0.61)	1.44 $\pm$ 0.40 (0.91-2.42)	1.14 $\pm$ 0.18 (0.82 $\pm$ 1.72)
Zinc (mg/L)	1.01 $\pm$ 0.17 (0.80-1.40)	1.11 $\pm$ 0.13 (0.90-1.40)	1.01 $\pm$ 0.12 (0.80-1.10)	1.05 $\pm$ 0.17 (0.60-1.20)
Cholesterol (mg/dL)	184 $\pm$ 29 (129-246)	190 $\pm$ 35 (145-246)	230 $\pm$ 43* (261-309)	235 $\pm$ 51* (258-326)
HDL cholest. (mg/dL)	58 $\pm$ 11 (32-80)	41 $\pm$ 12 (27-74)	43 $\pm$ 5* (38-64)	37 $\pm$ 9 (38-64)
LDL cholest. (mg/dL)	117 $\pm$ 31 (68-173)	131 $\pm$ 33 (70-181)	146 $\pm$ 47** (124-208)	147 $\pm$ 50 (70-243)
Triglycerides (mg/dL)	77 $\pm$ 30 (40-113)	106 $\pm$ 34 (46-145)	194 $\pm$ 77* (117-396)	257 $\pm$ 100* (159-589)

Values represent the mean $\pm$ SD of parameters in normo- and hyperlipidemic subjects. Asterisks denote the significance of the t test of differences between means of normolipidemic and hyperlipidemic groups for each sex (\* $P < 0.01$ ; \*\* $P < 0.05$ ). Ranges are in parentheses

the copper level, which is higher for those women taking oral contraceptives ( $P < 0.02$ ).

The relationship of all the evaluated parameters with alcohol, drug and tobacco consumption was established by variance analysis, considering both sexes individually, but no conclusive results were obtained.

## Discussion

This study reports for the first time data on selenium, copper and zinc in the serum of Portuguese inhabitants of the city of Ponta Delgada - Azores Archipelago. Serum selenium concentrations are in the same range as those found for the population of Lisbon, and a similar significant difference between selenium levels in women and men has already been observed (24) for that population on the mainland ( $87 \pm 20$  mg/L for women and  $100 \pm 11$  mg/L for men). However, a difference based on sex has not been generally observed in populations of other European countries (18). Hormonal status, for example, probably can explain the difference and even different food habits between the two sexes can contribute to the discrepancies reported in the literature (16, 18).

Serum copper concentrations are similar to those found in

other European countries (16). In addition, these levels seem to be higher in women than in men and similar observations for different populations have been reported by many investigators (16, 25). In this study, women were aged from 20 to 60 years and 70 % of them were between 20 and 45. Once more, the hormonal status, mainly the use of oral contraceptives, can be associated with the increased concentration in serum copper. Serum zinc concentrations seem to be slightly higher in men than in women, and the range values for both sexes are similar to the values observed elsewhere (16). An identical sex-dependent difference in zinc levels has been reported for other populations, but the opposite has also been observed, and many studies have found no significant differences between the sexes (16).

An altered plasma lipid profile in humans, mainly high cholesterol levels and alterations of its distribution among lipoproteins, is considered to be a risk factor for the development of atherosclerosis and, subsequently, the occurrence of cardiovascular diseases. In the present study when examining the levels of selenium, copper and zinc in serum, no difference in serum trace element levels based on the lipid profile can be observed. Concerning selenium, this finding is in accordance with the results obtained in the population of Lisbon for normolipidemic subjects (24). In fact, nearly all the subjects had a lipidic profile within normal values, mainly the serum levels of cholesterol

Table 4. Serum selenium, copper and zinc levels in subjects from the population of Ponta Delgada (S. Miguel Island) based on different life habits. Women taking oral contraceptives are also shown

Subjects	Sex	Number	Serum concentrations (Mean $\pm$ SD)		
			Se ( $\mu\text{g/L}$ )	Cu (mg/L)	Zn (mg/L)
Non smokers	W	49	87 $\pm$ 16	1.40 $\pm$ 0.40	1.03 $\pm$ 0.16
Non smokers	M	27	101 $\pm$ 17	1.10 $\pm$ 0.20	1.11 $\pm$ 0.16
Smokers	W	8	90 $\pm$ 7	1.40 $\pm$ 0.40	0.90 $\pm$ 0.13**
Smokers	M	18	96 $\pm$ 15	1.20 $\pm$ 0.20	1.07 $\pm$ 0.12
Non- drinkers	W	50	86 $\pm$ 15	1.40 $\pm$ 0.40	1.02 $\pm$ 0.16
Non- drinkers	M	19	98 $\pm$ 15	1.20 $\pm$ 0.20	1.11 $\pm$ 0.18
VDrinkers	W	7	93 $\pm$ 14	1.40 $\pm$ 0.10	1.00 $\pm$ 0.10
VDrinkers	M	26	100 $\pm$ 17	1.10 $\pm$ 0.20	1.08 $\pm$ 0.12
Non oralcontraceptives	W	13	88 $\pm$ 15	1.30 $\pm$ 0.30	1.01 $\pm$ 0.16
Oral contraceptives	W	44	88 $\pm$ 16	1.70 $\pm$ 0.50*	1.06 $\pm$ 0.16

Values represent the mean $\pm$ SD of serum selenium, copper and zinc for each case. v Alcohol drinker, non abuser. Asterisks denote the significance of the t test of differences between means of consumers and non-consumers for each sex (\* $P < 0.02$ ; \*\* $P < 0.05$ )

(total cholesterol, HDL cholesterol and LDL cholesterol) and the hyperlipidemic individuals had lipid levels somewhat above reference values. This is the result for a sample that is composed of healthy people. However results appearing in the literature on the relationship between these trace elements and the serum lipid profile are contradictory (3,10,16, 20).

Finally, the fact there are no significant variations in the serum concentrations of the elements studied with the consumption of alcohol, tobacco and/or drugs by some individuals in the sample, can be due to the fact that they do not consume excessive quantities. Thus, low consumption has no effect on the status of the studied elements.

The present results have encouraged the carrying out of a further investigation of these antioxidant trace elements comparing this urban population and others of S. Miguel (rural and fishing ones, for instance) with different dietary habits, taking into account the considered cardiovascular risk factors and other parameters that may be indicators of oxidative stress. The results of this on-going study will be reported at a later time.

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