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Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention

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Ability in Atherosclerosis and Cancer Prevention
 Stress in Humans
 Oxidant Activity
 Oxidative DNA Damage and Repair
 Use of a Commercially Available
 Use of Farnesol and
 Vitamin E and Carotenoids
 and Cancers
 in the Human Organism
 Different Forms of Carotenoids
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SELENIUM STATUS AND CARDIOVASCULAR RISK FACTORS IN POPULATIONS FROM DIFFERENT PORTUGUESE REGIONS

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1 INTRODUCTION

Selenium as a cofactor of glutathione peroxidase, which prevents lipid peroxidation in mammals ¹, takes part in the direct protection of endothelial cells against reactive oxygen species that have been implicated in atherogenesis ²⁻⁴; moreover, it is involved in the biosynthesis of arachidonic acid derivatives in platelets ^{5,6} and in the regulation of lipoprotein cholesterol metabolism in human beings and in animal models ⁷⁻¹⁰. These aspects are relevant enough to conclude that low selenium status may be related to atherosclerosis and, consequently, to the occurrence of cardiovascular diseases ¹¹.

Clinical studies showed a decrease in plasma selenium of patients with congestive cardiomyopathy and/or myocardial infarction ^{12,13}. A significant inverse correlation between plasma selenium and severity of coronary atherosclerosis was also reported in man ¹⁴. However, prospective epidemiological studies on the relationship between selenium and cardiovascular disease are rather controversial ^{15,16}.

The aim of this work was to compare the selenium status by determining serum levels of this element in inhabitants of two urban and one rural portuguese regions. The relationship between serum selenium levels and generally accepted cardiovascular risk factors was also an objective. In this context, serum selenium and serum lipid parameters (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) were evaluated. Age and sex as well as alcohol and tobacco consumption were also considered.

2 SUBJECTS AND METHODS

2.1. Subjects

The studied groups consisted of 101 (39 women and 62 men), 98 (50 women and 48 men) and 35 (19 women and 16 men) volunteer portuguese subjects, aged 20 to 60 years and living in Lisbon-Mainland (urban region), Ponta Delgada - Azores Archipelago (urban region) and Salvaterra de Magos - Mainland (rural region), respectively.

The donors were non-alcoholic persons and they did not abuse drugs. Age and sex as well as the date of sampling were registered. The existence of chronic diseases and a history of any cardiovascular condition or stroke were also considered. The subjects were asked to begin to fast 12 h before blood sampling, which occurred in the morning and was carried

2. Methods

2.2.1. Analytical procedures. Blood was collected in polyethylene tubes by venipuncture. Serum was removed after centrifugation without addition of anticoagulants and an aliquot kept frozen at -20°C until analysed for selenium.

HDL proteins were obtained by adding polyethylene glycol to fresh samples to precipitate other lipoproteins. Their cholesterol content as well as the serum total cholesterol were determined enzymatically by the cholesterol CHOD-PAP method (Boehringer, Mannheim, FRG). Serum triglycerides were determined enzymatically by the triglycerides GPO-PAP method (same manufacturer). LDL cholesterol concentration was calculated using the Friedewald formula.

Serum selenium was quantified by a direct electrothermal atomic absorption spectrometric procedure with Zeeman background correction¹⁷. Accuracy of the procedures was checked with standard reference material.

2.2.2. Statistics. Normality of the distribution was evaluated by the Kolmogorov-Smirnov test. Distribution was studied by drawing frequency polygons after division into class intervals.

Individual mean comparisons were tested for significance by the Student's t-test or by the Mann-Whitney test.

The correlations of serum selenium with age and lipid parameters were analysed by linear regression or correlation coefficient. For the discrete variables, smoking and alcohol consumptions associations with other parameters were made by the Student's t-test.

3 RESULTS AND DISCUSSION

3.1. Results

3.1.1. Serum selenium concentrations - Intrapopulation and interpopulation differences. A significant difference ($p < 0.01$) of average serum selenium concentrations was found between the two sexes in the Azores population. A less important variation ($p < 0.05$) was also observed in the population of Lisbon (Table 1).

Women and men of the rural population from Salvaterra de Magos exhibited significant differences ($p < 0.01$) in the average serum selenium concentration when compared with both the urban populations (Lisbon and Azores). An exception was found for the women of the Azores, which had a serum selenium concentration similar to the women of Salvaterra de Magos (Table 1).

Table 1. Serum selenium concentration ($\mu\text{g l}^{-1}$) of populations from Lisbon, Ponta Delgada - Azores and Salvaterra de Magos.

Sex	Lisbon	P. Delgada - Azores	Salvaterra Magos
W	94.4 ± 16.7	88.1 ± 14.5	84.2 ± 14.4
M	99.0 ± 20.5	97.9 ± 16.2	84.5 ± 16.5

Values represent the mean ± S.D..

W - women (n = 39, Lisbon; n = 50, P. Delgada - Azores; n = 19, Salvaterra);

M - men (n = 62, Lisbon; n = 48, P. Delgada - Azores; n = 16, Salvaterra).

For the azorean population, the results revealed an increase ($p < 0.02$) of serum selenium levels with age, when considering all samples irrespective of sex (Figure 1).

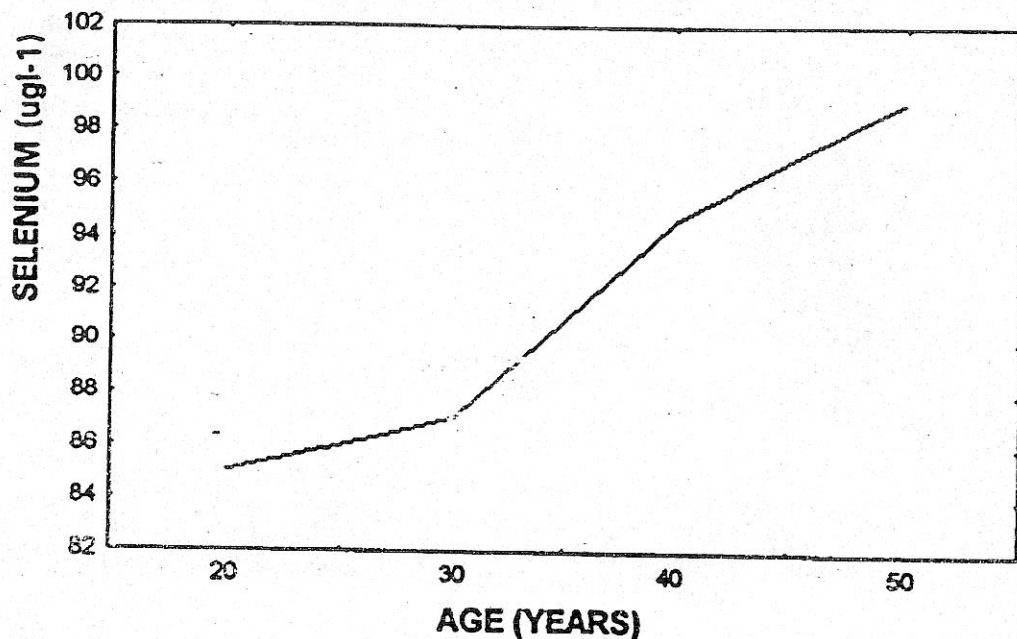


Figure 1. Change of serum selenium levels with age of subjects from the Azorean population.

3.1.2. Association between the serum selenium concentration and the serum lipid parameters. In order to study the relationship between the lipid parameters and serum selenium levels, the subjects were divided according to respective total cholesterol and triglycerides contents. The normolipidaemic group consisted of healthy subjects having serum total cholesterol and triglycerides concentrations $< 200 \text{ mg dl}^{-1}$; the hiperlipidaemic group consisted of subjects with impairment of lipid status, having the serum total cholesterol and /or triglycerides concentrations $> 200 \text{ mg dl}^{-1}$. The HDL cholesterol and the HDL cholesterol/total cholesterol ratio is within the normal range, for the normolipidaemic group, but this ratio is lower ($p < 0.01$) for the hiperlipidaemic individuals when compared with normal values for the three populations (Tables 2,3 and 4).

Mean serum selenium was not significantly different for the two groups of normolipidaemic and hiperlipidaemic subjects in the two populations from Azores and Salvaterra de Magos (Tables 3 and 4). However, a weak increase ($p < 0.05$) in that parameter was observed, for the hiperlipidaemic group of Lisbon, which exhibited also the highest values in cholesterol and triglycerides concentrations (Tables 2,3 and 4).

Table 2. Lipid parameters and selenium concentration in serum of the subjects from Lisbon population.

Parameter	Sex	Normolipidaemic	Hiperlipidaemic
Cholesterol (mg d l ⁻¹)	W	181.1±27.6	271.2±54.2*
	M	193.6±22.7	298.3±100.6*
HDL cholest. (mg d l ⁻¹)	W	56.1± 9.5	46.0±12.7**
	M	46.3±13.5	39.6±10.1**
HDL cholest./ cholesterol,%	W	31.4±6.0	17.0±5.8*
	M	23.8±7.0	14.4±5.4*
LDL cholest. (mg d l ⁻¹)	W	117.2±20.0	175.5±54.4*
	M	118.6±20.9	182.7± 54.8*
Triglycerides (mg d l ⁻¹)	W	77.0±34.4	276.4±82.8*
	M	103.5±35.8	400.6±120.0*
Se (µg l ⁻¹)	W	89.7±13.6	99.7±18.4**
	M	97.7±23.2	103.3±17.0**

Values represent the mean ± S.D..

W - women (n=18 in normolipidaemic group; n=21 in hiperlipidaemic group);

M - men (n=28 in normolipidaemic group; n=34 in hiperlipidaemic group).

Asterisks denote the significance of the t test of differences between means for the hiperlipidaemic and nor-molipidaemic groups(*p<0.01;**p<0.05).

Table 3 . Lipid parameters and selenium concentration in serum of the subjects from the population of Ponta Delgada - Azores.

Parameter	Sex	Normolipidaemic	Hiperlipidaemic
Cholesterol (mg d l ⁻¹)	W	175.2±20.1	243.8±27.7*
	M	174.4±26.4	237.6±44.2*
HDL cholest. (mg d l ⁻¹)	W	52.4±11.3	46.1± 7.3**
	M	37.5±10.8	40.7±11.4
HDL cholest./ cholesterol,%	W	29.9±7.1	19.2±2.8*
	M	21.6±7.2	17.1±4.9*
LDL cholest. (mg d l ⁻¹)	W	108.2±22.8	170.2±33.0*
	M	114.0±27.2	156.0±42.0*
Triglycerides (mg d l ⁻¹)	W	82.3±39.0	165.6±91.9*
	M	114.0±32.3	255.0±83.9*
Se (µg l ⁻¹)	W	85.6±15.2	93.1±12.9
	M	98.4±16.0	98.1±16.3

Values represent the mean ± S.D..

W - women (n=38 in normolipidaemic group; n=12 in hiperlipidaemic group);

M - men (n=20 in normolipidaemic group; n=28 in hiperlipidaemic group).

Asterisks denote the significance of the t test of differences between means for the hiperlipidaemic and normolipidaemic groups(*p<0.01;**p<0.05).

Table 4. Lipid parameters and selenium concentration in serum of the subjects from the population of Salvaterra de Magos.

Parameter	Sex	Normolipidaemic	Hiperlipidaemic
Cholesterol (mg d l ⁻¹)	W	166.9±26.2	223.9±15.2
	M	173.1±21.4	204.1±30.6
HDL cholest. (mg d l ⁻¹)	W	53.1±17.0	43.1±4.0
	M	44.8±5.2	24.3±4.2*
HDL cholest./ cholesterol,%	W	32.4±10.1	19.3±2.3*
	M	26.4±5.4	11.8±3.2*
LDL chol. (mg d l ⁻¹)	W	103.1±15.4	138.3±8.3*
	M	112.0±13.6	132.1±19.8
Triglycerides (mg d l ⁻¹)	W	109.9±25.8	110.0±37.6
	M	90.7±30.1	143.5±64.0
Se. (µg l ⁻¹)	W	82.6±14.3	84.7±14.4
	M	83.8±17.4	87.1±15.1

Values represent the mean±S.D..

W - women (n=10 in normolipidaemic group;n=9 in hiperlipidaemic group);

M-men (n = 13 in normolipidaemic group;n= 3 in hiperlipidaemic group).

Asterisks denote the significance of the t test of differences between means for the hiperlipidaemic and normolipidaemic groups(*p<0.01).

For the three populations no significant correlations were found between the several lipid parameters and the serum selenium concentrations,either considering the sexual difference or not.

3.1.3. Association between serum selenium concentration and drinking and smoking habits. Concerning alcohol consumption, no statistical difference in the serum selenium concentration was found among individuals for the three studied populations.

However, there is a strongly significant statistical difference (p<0.001) of serum selenium levels between male smokers (82.9±4 µg l⁻¹,n=15) and non-smokers (102.1±4 µg l⁻¹,n=41) in the Lisbon population.

No differences were found in other populations, as well as between smoking and non-smoking women within the same population.

3.2. Discussion

3.2.1. Comparison of the obtained serum selenium concentrations with data from other portuguese regions and other european countries. The similarity of serum selenium concentrations in the urban populations of this study (one from the portuguese mainland and the other from the Azores' Archipelago) with data from fishing portuguese populations (104.2±21.3 µg l⁻¹, n= 59 men - Câmara de Lobos - Madeira Island) ¹⁸ is observed.

The most striking result of this comparative study is the significantly lower mean serum selenium concentration of the rural population of Salvaterra de Magos when compared to the serum selenium concentration found in fishing ¹⁸ and in the two studied urban populations of the portuguese territory. This fact, added to the very low serum selenium concentration found in another rural population (59.8±17.0 µg l⁻¹, n= 16 men - Curral das Freiras - Madeira Island) ¹⁸, suggests that the selenium status is related to the feeding habits, with the serum selenium concentration being directly related to the consumption of animal proteins

A deeper study on the diet and its nutrient composition in these populations is essential to answer these questions.

However, present data for the three portuguese populations are in the same range of values than those obtained in other countries of Europe 19,20. Nevertheless, they seem to be higher than the values obtained in southern european countries, according to data observed in Greece 20 and Yugoslavia 21, including the ones from Barcelona - Spain 22, but they are similar to those found in some populations from Italy according to Cauwenbergh et al 20.

3.2.2. *Association of the serum selenium concentration with other factors.* The increase of selenium serum levels with age observed in the azorean population, taking into account the both sexes, is in accordance with results obtained by other authors for the same range of age 23,24. However, conclusions about age-dependency are questionable, because the studied groups are sometimes poorly defined and the age-range is too small or too large 23.

The tendency observed in portuguese populations concerning the sexual differences in serum selenium levels agrees with data reported by some authors 23,24. However, most of them have found no significant variations in selenium correlated with sex 23,24. According to Robberecht et al 23 the race and hormonal status may jeopardize the conclusions.

The finding of no correlation between serum selenium and lipid parameters agrees with data of Crespo et al in normolipidaemic Portuguese individuals 25 and with the data of Bukkens et al in healthy Dutch subjects 26. But it disagrees with results presented by Salonen et al. 27, who reported a weak positive correlation between serum selenium and HDL cholesterol in Eastern Finnish men.

The weak increase of serum selenium levels observed in most hiperlipidaemic individuals from the population of Lisbon had not been reported so far. So, a more detailed study about hiperlipidaemic subjects considering serum lipid parameters and antioxidant indicators, as well as nutritional, metabolic and genetic factors should be faced in further studies.

A significant difference between smokers and non smokers was observed in men from the population of Lisbon. This result agrees with the referred by some authors 26, but differs of data reported by others 23. Probably lack of registration of type and amount of cigarettes smoked is partly responsible for the discrepancies in literature data 23.

3.2.3. *Final remark.* The present results encourage further investigations on the selenium dietary intake as well as on the environmental selenium in portuguese regions. On the other hand, a further study considering not only selenium, but also other parameters, namely indicators of oxidative stress and their relationship with cardiovascular risk factors should be made for the portuguese populations.

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