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Whole blood glutathione peroxidase and erythrocyte superoxide dismutase activities, serum trace elements (Se, Cu, Zn) and cardiovascular risk factors in subjects from the city of Ponta Delgada, Island of San Miguel, The Azores Archipelago, Portugal

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Abstract

Activities of whole blood glutathione peroxidase (GSH-Px) and erythrocyte superoxide dismutase (SOD) and serum levels of selenium (Se), copper (Cu) and zinc (Zn) were measured in 118 apparently healthy subjects aged 20–60 years from the city of Ponta Delgada, Island of San Miguel, The Azores Archipelago, Portugal. Data were analysed by age/gender, lipid profile and blood pressure as cardiovascular risk factors searching for their relevance when assessing reference values for antioxidant biomarkers. GSH-Px was in the same range, but SOD was significantly lower than in other Portuguese populations. Neither activity differed with gender. GSH-Px activity increased with age, namely in normolipidemic men versus the hyperlipidemic group in which a decrease was observed. This suggests a progressive impairment of GSH-Px with age caused by an enhanced production of oxidant species in hyperlipidemia. GSH-Px was 30% lower in male hypertensives versus normotensives. SOD activity did not relate to age or blood pressure but was 17% higher in the hyperlipidemic men versus the normolipidemic group, suggesting a better antioxidant protection by SOD than by GSH-Px in hyperlipidemia and hypertension. Se was higher in men versus women, particularly in the older subjects, and partly related to hyperlipidemia. Zn levels showed a similar dependency on gender, not related to age or lipid profile. Cu levels were much higher in women than in men in all age or lipid profile classes and decreased in hyperlipidemia. They were lowered with age in both genders, particularly in normolipidemic women. The present research therefore suggests that hyperlipidemia and hypertension do affect antioxidant status and should be considered when assessing antioxidant biomarkers in blood.

Keywords: *Antioxidant biomarkers, cardiovascular risk factors, The Azores*

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Introduction

Glutathione peroxidases (GSH-Px) and superoxide dismutases (SOD) are two major families of mammalian antioxidant enzymes. The former comprises five isoforms that are all selenoenzymes having the ability to reduce hydrogen peroxide (H_2O_2) and various organic hydroperoxides by using glutathione as an electron donor (Behne & Kriakopoulos 2001). Most of the peroxidase activity in blood is due to cytosolic (in erythrocytes) and plasma (extracellular) isoforms (Robberecht & Deelstra 1994). The latter includes three isoforms that dismutate the superoxide radical anion ($O_2^{-\cdot}$) into H_2O_2 and O_2 (Beyer et al. 1991). The Mn-dependent isoform, present in mitochondria, and the Cu, Zn-dependent isoform, located in the cytosol and nucleus of cells, constitute the largest amount of total SOD activity in cells. The latter isoform is responsible for all activity in erythrocytes. The extracellular form, which is a secretory Cu, Zn-dependent form, is present on the surface of many cells and exhibits a high level of activity in the vessel wall (Stralin et al. 1995). The trace elements Se, Cu and Zn, which are components of the active centres of these enzymes, are consequently deeply involved in their antioxidant function. However, they play many other biological roles that make them essential for humans. Oxidative stress is a situation prone to occur in living organisms: it results from an imbalance between the production of oxidants and their elimination by the complex enzymatic and non-enzymatic defence system. Such a state has been the scope of many studies as it has been involved in the initiation or aggravation of pathogenic processes such as those leading to atherosclerosis (Witztum & Steinberg 1991, Ross 1999) and subsequently to cerebral and cardiovascular diseases. These remain the main causes of morbidity and mortality in western countries including Portugal.

The assessment of GSH-Px and SOD activities, taken as two major biomarkers of the antioxidant status of individuals, as well as of selected trace element levels in biological fluids is therefore of great interest to investigate the role of oxidative stress in health and disease both in the general population and in selected groups of patients. Such an objective implies the determination of reference ranges and of the various factors, both biological and environmental, that are determinants for these parameters of antioxidant status of individuals. Not only do large discrepancies exist in the literature on the variability of these indicators, but also few epidemiological studies actually focus on the relevance of the lipid profile or the blood pressure condition of individuals. Gender has been associated with various different risks for cardiovascular disease in an age-related manner (Reckelhoff 2001). Moreover, age, hyperlipidemia and blood hypertension are other major cardiovascular risk factors that have to be more carefully considered (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001). In fact, there is inadequate knowledge and there are sometimes too many contradictory results published on antioxidant parameters in hyperlipidemia and hypertension.

Some years ago, the present authors' group investigated serum Se, Cu and Zn levels in some Portuguese populations (both on the Continent and on the Islands) with different socio-cultural profiles, namely with urban, fishing and rural characteristics, taking into account factors such as gender, age and lipid profile of the subjects (Pavão et al. 1999, 2003, Lopes et al. 2004a). The first survey performed in the Azorean city of Ponta Delgada (Viegas-Crespo et al. 2000) led us to carry out a further investigation more precisely targeted on the antioxidant status of healthy adults in order to enable designs in more specific groups with atherosclerotic disease. The

present study reports for the first time the whole blood GSH-Px and erythrocyte SOD activities, as well as serum Se, Cu and Zn levels in the population of Ponta Delgada, and relates them to age, gender, lipid profile and blood pressure condition. A second purpose of the present study is to compare these parameters with data from other Portuguese populations where some studies were carried out as part of the same project. The population of Lisbon reflects inhabitants of continental Portugal.

Material and methods

Study population

Subjects were 118 volunteers (57 men and 61 women), 20–60 years of age, born and living in Ponta Delgada, the main city (about 63 000 inhabitants) of the Island of San Miguel, The Azores Archipelago, Portugal. They belonged to a middle-high socio-economic class and had urban dietary habits without significant differences. They were recruited and examined by a medical doctor and gave their informed consent for this study. They were apparently healthy and had no chronic diseases, as stated by their clinical reports, which also provided information on their blood pressure condition and intake of medicines. None of the selected individuals consumed any drug, and about 80 and 64%, respectively, neither drank any alcohol nor smoked. The others did not have more than one glass of wine/beer or 15 cigarettes per day, which did not significantly affect the measures as confirmed by statistical comparison tests in relation to non-consumers. Blood samples were collected from January to July 2000.

Blood sampling and storage

Blood samples (about 15 ml) were drawn in the morning in fasting conditions. A total of 3 ml whole blood were transferred into a heparinized propylene tube for the determination of GSH-Px and SOD activities which were performed in the same day. The remainder of the blood collected was allowed to clot and centrifuged at 1800*g* at 4°C for 15 min to obtain serum. One aliquot was immediately used to evaluate the lipid parameters. Another portion was stored at −20°C for the measurement of Se, Cu and Zn levels.

Whole blood glutathione peroxidase activity assay

GSH-Px activity in whole blood was measured by a modification of the Paglia & Valentine (1967) method, using cumene hydroperoxide as substrate (Ransel Kit, Randox Laboratories, Antrim, N Ireland). One unit of GSH-Px activity is equivalent to 1 μmol NADPH oxidized min^{-1} and is expressed as units per gram of haemoglobin (U g^{-1} Hb). Haemoglobin concentration was determined by the cyanomethaemoglobin method (Merckotest, E Merck Darmstadt, Germany). The intra-assay variation for three measurements was <3%.

Erythrocyte superoxide dismutase activity assay

Heparinized blood (1–2 ml) was centrifuged at 3800*g* for 15 min at 0–4°C and the plasma carefully removed. Erythrocytes were lysed and the haemolysate was centrifuged at 3800*g* for 20 min at 0–4°C. SOD activity was determined on the

supernant by the Misra & Fridovich (1972) method. One unit of SOD activity is defined as the amount of SOD that inhibits the rate of epinephrine auto-oxidation by 50% under the defined conditions and activity is expressed as U g^{-1} Hb. The intra-assay variation for three measurements was $<6\%$.

Measurement of serum Se, Cu and Zn levels

Serum Se was determined by the direct electrothermal atomic absorption spectrometric procedure with Zeeman background correction (Nève et al. 1987). Cu and Zn concentrations were determined by flame atomic absorption spectrometry (Nève et al. 1983). The accuracy of the procedures was verified by means of Seronorm, a standard reference material consisting of lyophilized human serum from Nycomed (Roskilde, Denmark). Quality assurance was ensured by participation in interlaboratory trials (Nève et al. 1992). The intra-assay variation for three measurements was $<5\%$.

Determination of serum lipids

Total, HDL and LDL-cholesterol, as well as triglyceride levels in serum were determined by using standard enzymatic methods as described (Pavão et al. 2003).

Statistical methods

The normality of the distribution was evaluated by the Kolmogorov–Smirnov and by the Lilliefors tests. Group mean comparisons were tested for significance by the Student's t-test and correlations were assessed by the Pearson's test. The level of statistical significance was set at $p<0.05$. All analyses were performed using Excel 97 (Microsoft Corp., Seattle, WA, USA) and STATISTICA programme for Windows (release 5, Statsoft, Inc. Tulsa, OK, USA).

Results

Enzyme activities and trace element concentrations according to gender and age

Table I shows whole blood GSH-Px and erythrocyte SOD activities in investigated subjects. No significant differences in either enzymatic activity were found between males and females in all age groups. However, GSH-Px activity increased slightly with age both in men and women ($n=118$, $r=0.29$, $p<0.05$) and in the male subgroup

Table I. Whole blood glutathione peroxidase (GSH-Px) and erythrocyte superoxide dismutase (SOD) activities according to age and gender in subjects from the city of Ponta Delgada, S. Miguel Island, The Azores Archipelago, Portugal.

Age group (years)	Gender	GSH-Px (U g^{-1} Hb)	SOD (U g^{-1} Hb)
All ages	male ($n=57$)	46±14	2875±858
	female (61)	50±15	2767±800
20–39	male (22)	43±14	2941±957
	female (34)	49±15	2832±815
40–60	male (35)	48±14	2830±796
	female (27)	51±16	2669±785

Values are mean±SD.

Table II. Serum selenium, copper and zinc concentrations according to age and gender in subjects from the city of Ponta Delgada, S. Miguel Island, The Azores Archipelago, Portugal.

Age group (years)	Gender	Se ($\mu g\,l^{-1}$)	Cu ($mg\,l^{-1}$)	Zn ($mg\,l^{-1}$)
All ages	male ($n=57$)	90 ± 15*	1.03 ± 0.40***	1.01 ± 0.17*
	female (61)	85 ± 11	1.65 ± 0.73	0.95 ± 0.15
20–39	male (22)	89 ± 15	1.12 ± 0.65***	1.04 ± 0.15
	female (34)	87 ± 11	1.85 ± 0.78^{a}	0.96 ± 0.16
40–60	male (35)	92 ± 14**	0.98 ± 0.45**	1.00 ± 0.18
	female (27)	82 ± 9	1.39 ± 0.63^{a}	0.94 ± 0.12

Values are mean $\pm$ SD. *Significant differences due to gender within the same age group. *$p<0.05$, **$p<0.01$, ***$p<0.001$. Values sharing a common superscript letter are significantly different; [a] $p<0.05$.

($n=57$, $r=0.27$, $p<0.05$). Moreover, it decreased with age within the subgroup of the middle-aged women ($n=27$, $r=-0.45$, $p<0.05$).

Concerning serum trace element concentrations, significant differences due to gender were observed for the three elements (Table II). Means were higher (by 6%) in men than in women both for Se and Zn, namely for Se in the older subjects (12%). Within each gender group, Se and Zn levels did not vary with age. Mean copper levels were much higher (up to 65%) in women than in men in all age groups. Serum Cu concentrations in the females were significantly lower in the older group than in its younger counterpart and slightly decreased with age within the older male subgroup ($n=23$, $r=-0.37$, $p<0.05$).

Enzyme activities and trace element concentrations according to lipid profile

Table III reports the enzyme activities and trace element concentrations classified according to lipid profile. Men and women were divided into two groups: normolipidemic (total cholesterol <250 mg dl^{-1} and/or triglycerides <200 mg dl^{-1}) and hyperlipidemic (one or both parameters above these reference values). About 44% of men and 25% of women were hyperlipidemics (Table III). In this last group, about 68% of

Table III. Whole blood GSH-Px and erythrocyte SOD activities, serum selenium, copper, zinc and total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride concentrations by lipid profile in subjects from the city of Ponta Delgada, S. Miguel Island, The Azores Archipelago, Portugal.

	Males ($n=57$)		Females (61)	
Parameter	nl (32)	hl (25)	nl (46)	hl (15)
GSH-Px ($U\,g^{-1}$ Hb)	45 ± 13	49 ± 15	51 ± 15	46 ± 14
SOD ($U\,g^{-1}$ Hb)	2643 ± 671^{i}	3097 ± 987^{i}	2736 ± 836	2858 ± 698
Se ($\mu g\,l^{-1}$)	87 ± 15^{a}	96 ± 14***^{a}	87 ± 12	81 ± 6
Cu ($mg\,l^{-1}$)	1.19 ± 0.55***^{b}	0.83 ± 0.48**^{b}	1.79 ± 0.80	1.40 ± 0.50
Zn ($mg\,l^{-1}$)	1.02 ± 0.16	1.01 ± 0.18	0.95 ± 0.14	0.97 ± 0.13
CHo ($mg\,dl^{-1}$)	198 ± 27^{c}	264 ± 28^{c}	196 ± 32^{d}	255 ± 49^{d}
HDL-CHo ($mg\,dl^{-1}$)	47 ± 13**	46 ± 13**	57 ± 12	62 ± 15
LDL-CHo ($mg\,dl^{-1}$)	129 ± 27^{e}	177 ± 34^{e}	121 ± 28^{f}	166 ± 44^{f}
TG ($mg\,dl^{-1}$)	107 ± 46^{g}	199 ± 99^{g}	93 ± 32^{h}	169 ± 83^{h}

Values are mean $\pm$ SD in normolipidemic (nl) or hyperlipidemic (hl) subjects. *Significant differences due to gender within the same class: *$p<0.05$, **$p<0.01$, ***$p<0.001$. Values sharing a common superscript letter are significantly different; [a] $p=0.02$, [b,i] $p<0.05$, [c–h] $p<0.001$.

men and 60% of women were hypercholesterolemics and 60% in both genders had triglyceride levels above the reference values. According to the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001), the hypercholesterolemic subjects were at a high-risk level for coronary heart disease.

GSH-Px activity was not related to lipid profile but SOD activity was significantly higher (17%) in hyper- than in the normolipidemic men. Concerning serum trace elements, the difference in Se levels due to gender evidenced in the whole population (Table II), was confirmed in the hyperlipidemic subjects but not in the normo- ones (Table III). This is in accordance with the increased Se concentration (by 10%) found in the hyperlipidemic men (but not in women). Concerning Cu, the difference due to gender was exhibited in both lipid profiles. In addition, a drop of 30% in Cu levels was observed in the hyperlipidemic men as compared with the respective normo- group and a similar tendency was observed in the hyperlipidemic women albeit not statistically significant. Finally, the difference due to gender observed in the whole population for Zn levels (Table II) was not related to lipid profile (Table III).

Enzyme activities and trace element concentrations according to blood pressure

Men and women were divided into normotensive and hypertensive groups by considering hypertensive those with systolic blood pressure $\geq$140 mm Hg, diastolic blood pressure $\geq$90 mm Hg or taking antihypertensive medication (National Institutes of Health 1997). As indicated in Table IV, 12% of males but fewer than 2% of women were hypertensive, which did not allow them to be taken into account for further statistical analysis. GSH-Px activity was significantly decreased (by 30%) in the hypertensive subgroup but SOD activity was not related to blood pressure.

Concerning trace elements, gender related differences similar to those observed in the whole population (Table II) were confirmed in the normotensive subjects (Table IV). A non-significant tendency for increased serum Cu levels was observed in hypertensive men but no significant differences were found for the other trace elements.

GSH-Px activities and serum Cu levels as related to age, lipid profile and blood pressure

As already reported, when considering the whole male group, a weak positive correlation was found between GSH-Px activity and age, which was confirmed in both

Table IV. Whole blood GSH-Px and erythrocyte SOD activities, serum selenium, copper and zinc concentrations according to blood pressure in subjects from the city of Ponta Delgada, S. Miguel Island, The Azores Archipelago, Portugal.

	Males ($n=57$)		Females (61)	
Parameter	nt (50)	ht (7)	nt (59)	ht (2)
GSH-Px (U g^{-1} Hb)	48 ± 14^{a}	35 ± 11^{a}	50 ± 15	55
SOD (U g^{-1} Hb)	2870 ± 881	2722 ± 616	2732 ± 791	3763
Se ($\mu g\,l^{-1}$)	91 ± 15*	87 ± 13	85 ± 10	94
Cu ($mg\,l^{-1}$)	0.99 ± 0.52**	1.23 ± 0.63	1.67 ± 0.70	2.42
Zn ($mg\,l^{-1}$)	1.02 ± 0.17*	0.95 ± 0.18	0.96 ± 0.14	1.02

Values are mean $\pm$SD or mean (in the cases of only two values) in normotensive (nt) or hypertensive (ht) subjects. *Significant differences due to gender within the same class: *$p<0.05$, **$p<0.001$. Values sharing a common superscript letter are significantly different; $^{a}p<0.05$.

the normolipidemic ($n=35$, $r=0.34$, $p<0.05$) and normotensive ($n=50$, $r=0.29$, $p<0.05$) groups. However, a decrease in enzyme activity with age was observed in the hyperlipidemics ($n=25$, $r=-0.46$, $p<0.05$). The significant decrease in serum Cu levels with age in women (Table II) was further supported by the negative relationship between these parameters in the normolipidemic group ($n=46$, $r=-0.34$, $p<0.05$) but not in the respective hyper- group. No such relationships were observed in either normo- or hyperlipidemic men.

Discussion

The study reports for the first time data on the whole blood GSH-Px and erythrocyte SOD activities in apparently healthy subjects from the city of Ponta Delgada, establishes reference values for several biomarkers related to antioxidant status and examines their relationships with major cardiovascular risk factors.

Enzyme activities in relation to gender, age, lipid profile and blood pressure

The GSH-Px activities in the inhabitants of Ponta Delgada were in the same range as those of two other Azorean populations. The absence of differences due to gender confirmed the observation made in a rural Azorean population (Pavão et al. 2003). The reported positive relationship of enzyme activity with age agreed with some studies (Artur et al. 1992, Bolzán et al. 1997, Erden-Inal et al. 2002) but disagreed with others that evidenced no significant relationships (Andersen et al. 1997) or found a biphasic evolution in erythrocyte GSH-Px with age, reaching the lowest values at 40–50 years and rising later on (Rodriguez-Martinez & Ruiz-Torres 1992).

On the other hand, GSH-Px activity in whole blood did not differ significantly between hyperlipidemics and normolipidemics, which was to some extent in agreement with studies reporting no change in erythrocyte GSH-Px activity in hypercholesterolemic subjects aged about 50 years (Özdemirler et al. 1997). However, the increase in GSH-Px activity with age found in men from Ponta Delgada appeared to be more pronounced when excluding the hyperlipidemic group, which experienced a decrease in enzyme activity with age. Consistently, a similar age-related negative correlation was observed in the middle-aged female group in which hyperlipidemia had a higher incidence than in their younger counterparts (37 and 15%, respectively).

These findings suggested the occurrence of a compensatory response in GSH-Px activity to a moderate increase of peroxides with age, as expected in normolipidemic men aged 20–60 years (Harman 1992), and a progressive age-related inability of the enzyme to fight against the oxidative stress due (at least partly) to hyperlipidemia both in men and in the middle-aged women. This may be the consequence of a parallel progressive impairment of the enzyme caused by an increased production of oxygen- and nitrogen-derived reactive species (ROS and RNS). Some authors reported an inhibition of antioxidant enzymes by high levels of ROS (Lawler & Song 2002).

Concerning blood pressure, hypertensive men from Ponta Delgada exhibited a marked and significant reduction in whole blood GSH-Px activity in comparison with the normotensive group, in agreement with some previous reports (Pedro-Botet et al. 2000, Redón et al. 2003). Several pathological conditions, including hypertension, were shown to be associated with an increase in the production of ROS in vessels (McIntyre et al. 1999, Munzel et al. 1999). Superoxide (and other ROS) in particular

can react with nitric oxide (NO) at a diffusion-limited rate to form peroxynitrite anion, a potent oxidant (Darley-Usmar et al. 1995). Depending on the levels of RNS, they may cause a decrease in GSH-Px activity. In this context, Fu et al. (2001) reported that cytosolic GSH-Px was more effective in protecting mouse hepatocytes against oxidative injuries mediated by ROS alone than by ROS and RNS together.

In both men and women, means of erythrocyte SOD activity were in the same range as in other European populations (Artur et al. 1992). However, they were very significantly lower than those found in subjects from Lisbon, which were about 4000 U g^{-1} Hb in both genders (data not shown). In the present study and in accordance with literature, SOD activity did not depend on gender or age (Artur et al. 1992, Ceballos-Picot et al. 1992, Andersen et al. 1997, Bolzán et al. 1997, Preziosi et al. 1998). In turn, it was higher in hyperlipidemic than in normolipidemic men, which suggested an adequate defence response of SOD to the increased production of superoxide anions associated with hyperlipidemia and not reflected in GSH-Px activity. In addition, enzyme activity in erythrocytes was unchanged in hypertensive men, which generally agrees with some authors (Özdemirler et al. 1997) but disagrees with others who found a lower activity in essential hypertension (Russo et al. 1998). In addition, the activity of SOD in whole blood was significantly lower in hypertensive subjects as compared with normotensive ones (Redón et al. 2003).

Serum trace elements in relation to age and gender

The study confirms the relationship to gender observed in the first survey on the population of Ponta Delgada (Viegas-Crespo et al. 2000). Se and Cu levels in women exhibited significantly ($p < 0.001$) higher values than women from Lisbon (77 ± 13 μg l^{-1} and 1.32 ± 0.50 mg l^{-1}, respectively), while the difference in Zn levels did not reach statistical significance (Lopes et al. 2004b). Similar gender-related differences were observed in both urban Portuguese populations and also in some other Azorean populations (Pavão et al. 1999). In both genders, Se was not significantly correlated to age. This seems in contradiction with data from Lisbon where Se slightly increased with age (regardless of gender) but was significantly higher in both younger men (20–44 years) and in women aged 45–70 years as compared with their respective counterparts. Differences in either the age ranges or the age groups considered could be partly responsible for the disparate results in the two urban Portuguese populations. According to some authors, Se status fluctuates throughout the female life cycle and is related to oestrogen status (Smith et al. 2000). A tendency to decreased concentrations in serum Cu with age was also observed in the Lisbon population (Lopes et al. 2004b). This is probably related to the parallel loss of estrogens in women (namely during and after menopause), since these hormones induce the synthesis of ceruloplasmin in the liver leading to increased serum Cu levels (Versieck & Cornelis 1989).

Serum trace elements in relation to the lipid profile

The lipid profile was differently related to serum Se and Cu levels. Concerning Se, hyperlipidemia appeared to be at least partly responsible for the differences observed between genders in the whole population. Serum Se was actually higher in the male hyperlipidemic subjects, but was unchanged in the female countergroup as compared with the normogoups. This could be related to the binding of Se to lipoproteins,

namely LDL (Ducros et al. 2000), which are particularly high in this male dislipidemic group. Moreover, the increased serum Se level could also be the consequence of an increased binding of Se to selenoproteins other than GSH-Px, but only the determination of selenoprotein P (Nève 2000) could clarify this point. Lopes et al. (2004b) also reported high serum Se levels in hyperlipidemic subjects from Lisbon.

Concerning Cu, a difference due to gender in the whole population was observed in both lipid profiles. A drop in Cu levels was actually observed in both male and female hyperlipidemic groups as compared with the respective controls, although with different statistical significance (Table III). An opposite tendency was observed for the relationships between serum Cu and hyperlipidemia in the Lisbon population (Lopes et al. 2004b) where, however, dislipidemia was far less pronounced than in the present population. Studies on this relationship in apparently healthy populations remain scarce (Abiaka et al. 2003). Among other manifestations, hypercholesterolemia is one of the systemic cardiovascular signs of dietary Cu deficiency in man (Saari 2000). However, some authors have suggested that increased serum Cu is a risk factor for cardiovascular disease through oxidative modification of LDL (Salonen et al. 1991, Klipstein-Grobush et al. 1999). In the present population serum Cu levels depended in a non-predictive manner on the degree of dislipidemia in individuals, possibly involving different requirements of the element by its dependent enzymes and proteins, namely ceruloplasmin (Yu 1994). Finally, no significant differences in serum Zn levels based on lipid profile were observed, which is in agreement with data found in the Lisbon population (Lopes et al. 2004b).

Serum trace elements in relation to blood pressure

Larger groups of hypertensive men and women would be necessary to confirm or clarify the relevance of hypertension in determining the serum levels of these three trace elements. In fact, Nasirudin & Khan (2002) reported increased serum levels of both Cu and Zn in hypertensive subjects, but a significant relationship between Se and blood pressure has not yet been demonstrated (reviewed in Loyke 1997).

The present results suggest that hyperlipidemia and hypertension do affect antioxidant status in blood, as reflected by alterations in SOD or GSH-Px activities. They are therefore significant variables of enzymatic activities even in apparently healthy subjects and therefore influence the relationships of enzymatic activities with age and gender. As a consequence, dislipidemia as well as hypertension should be taken into consideration when assessing reference values in epidemiological studies. Both GSH-Px and SOD activities respond (albeit differently) to the oxidative challenge created by hyperlipidemia and hypertension. However, as other factors can also affect GSH-Px and SOD activities, alterations of these antioxidant biomarkers should not be taken alone as early predictors of cardiovascular disease. A similar statement can be made on the significance of the changes observed in serum selenium or copper levels in hyperlipidemic subjects. Further studies are needed to clarify the potential value of the simultaneous occurrence of altered values in all or in some of those parameters in the assessment of cardiovascular disease.

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