Influence of Dyslipidemia and Smoking on Redox Markers in Humans - a Critical Study [4]

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ABSTRACT

Experimental research indicates that oxidative processes play a role in susceptibility to a large number of diseases. A better understanding of the parameters affecting redox balance could delay and even prevent such processes.

Objective: The present study aims to investigate blood parameters associated with antioxidant systems in a Portuguese population for the first time, taking into consideration gender, age range, lipid profile and smoking habits as influencing factors.

Design and Participants: One hundred and eighty-three healthy Portuguese subjects of both genders were recruited from the metropolitan area of Lisbon. The group consisted of individuals aged from 20 to 70 years, who gave their informed consent before participating in the study. All subjects were screened to determine eligibility, which was based on a clinical report. Subjects were considered eligible if they had no acute or chronic illness and were not taking any drugs or dietary supplements that could compromise the values of the studied parameters. The subjects were then divided into different subgroups according to gender, age range, lipid profile and smoking habits.

Methods: Whole blood glutathione peroxidase activity and serum albumin, transferrin and uric acid were determined using commercially available kits. Superoxide dismutase activity in erythrocytes and thiobarbituric acid reactive substances in serum were measured using methods published elsewhere.

RESUMO

Influência da Dislipidemia e do Tabagismo em Parâmetros do Balanço Redox - um Estudo Crítico

Na actualidade, é consensual que os processos oxidativos desempenham um papel importante no aparecimento e progressão de inúmeras doenças. O conhecimento do comportamento dos parâmetros do balanço redox poderão surtir efeito na prevenção da ocorrência de tais patologias.

Objetivos: o presente estudo numa perspectiva pioneira para uma população portuguesa (a de Lisboa) visa caracterizar a variação de parâmetros sanguíneos associados ao sistema pró-oxidante/antioxidante tendo em consideração variáveis como o sexo, a idade, o perfil lipídico e hábitos de vida, como o tabagismo.

Conceção do Estudo e Participantes: 183 indivíduos saudáveis de ambos os sexos, com idades compreendidas entre os 20 e os 70 anos e residentes na área metropolitana de Lisboa foram recrutados aleatoriamente no Instituto Nacional de Saúde Dr. Ricardo Jorge, enquanto utentes deste laboratório de referência. A avaliação clínica dos participantes foi efetuada com recurso a questionário. Em presença de doenças agudas/crónicas ou de ingestão de suplementos nutricionais e medicação, os indivíduos foram excluídos do estudo por forma a não haver interferência nos resultados dos marcadores a analisar.

Material e Métodos: A actividade da enzima glutatiano peroxidase no sangue total e as
Results: Glutathione peroxidase activity was not affected by any of the studied variables, but superoxide dismutase activity decreased with smoking. Albumin levels remained unchanged under all conditions. Hyperlipidemia was associated with higher lipid peroxidation as well as higher uric acid levels. Gender was the strongest predictor for transferrin, total iron binding capacity and uric acid variations. Finally, a multivariate statistical model clearly separated genders and lipid profile and genders and smoking.

Conclusions: The present study suggests that hyperlipidemia and smoking should be considered important selection criteria in epidemiological studies focusing on oxidative stress and on the atherosclerotic process.

Key words
Antioxidant defenses; Lipid peroxidation; Gender; Age; Hyperlipidemia; Smoking

INTRODUCTION

There is now a large body of evidence showing that over time, living organisms have not only adapted to the hostile presence of free radicals and other reactive species but have also developed mechanisms to use these entities to their advantage. Both the levels and the biological effects of these species are controlled in vivo by a complex and efficient antioxidant system. The first line of antioxidant defense includes enzymes, such as superoxide dismutases (SOD), glutathione peroxidases (GPx) and catalase, which are reinforced by non-enzymatic antioxidants such as glutathione, protein SH groups, vitamins C and E and, β-carotene and by other molecules present in extracellular fluids, including albumin, transferrin and uric acid. Maximal protection is ensured by cooperative interactions between biological antioxidants, in both cellular and the extracellular compartments.

An imbalance between oxidant species, such as those derived from oxygen and nitrogen, and
antioxidants in favor of oxidants has been associated with a large number of human diseases. So-called “oxidative stress” is thought to play a role in the pathogenesis of degenerative diseases such as diabetes, cancer and atherosclerosis, HIV infection, and age-related neuropathologies such as Alzheimer’s and Parkinson’s disease. Reactive species can damage essential biological molecules such as proteins, nucleic acids and lipids. Polynaturated fatty acids of blood low-density lipoproteins (LDL) are particularly prone to oxidation, leading to the formation of deleterious molecules such as malondialdehyde and 4-hydroxynonenal, which are common markers of lipid peroxidation.

It has been suggested that hypercholesteremia increases endothelial superoxide production and affects tissue antioxidant status. In hyperlipidemic subjects, the antioxidant/oxidant balance is impaired, but the mechanism underlying this effect has not been clarified. In the past, the protective action of HDL has been mostly attributed to its classical function in removing cholesterol from peripheral tissues and transferring it to the liver through a process known as reverse cholesterol transport. More recently, the antioxidant properties of HDL have been attributed in part to the HDL-associated enzyme paraoxonase, which protects low-density lipoproteins against oxidative modification. Also, there is evidence that HDL-associated lysophospholipids stimulate the production of the potent antiatherogenic signaling molecule NO by the vascular endothelium.

Epidemiological studies in humans have demonstrated that redox balance may be influenced by factors such as age, gender, and cigarette smoking. Tobacco smoke contains numerous compounds emitted as gases and condensed particles that contain high concentrations of free radicals and other oxidants which can cause oxidative injury to human tissues, including lipid peroxidation.

Large-scale surveys describing redox status in humans and associated demographic, physiological, social and nutritional factors are scarce. Parameters related to the antioxidant/pro-oxidant balance in a sample of Portuguese subjects from the city of Lisbon are reported in the present study for the first time. The activity of the antioxidant enzymes SOD and GPx, levels of other molecules with a role in antioxidant defenses such as albumin, transferrin and uric acid, and also thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation, were assessed. Possible associations between these parameters and gender, age range, lipid profile and tobacco consumption were investigated, in order to study selective criteria in oxidative stress related processes.

METHODS

Subjects and study design

A total of 183 Portuguese subjects were recruited from the Lisbon area. The group consisted of healthy individuals aged from 20 to 70 years, who gave their informed consent before participating in the study. Information regarding smoking habits, drug intake and general clinical state was obtained. Subjects were considered eligible if they had no acute or chronic illness and were not taking any drugs or dietary supplements that could compromise the values of the studied parameters. The subjects were divided into different subgroups according to gender, age range, lipid profile and smoking habits.

The Human Ethics Committee of the Dr. Ricardo Jorge National Health Institute approved the protocol of this study.

Blood collection

Blood was collected in the morning after 12 hours fasting. For each sample, an aliquot was stored at 4 °C for assessment of antioxidant enzymes, which was performed within 24 h. Serum was removed by centrifugation (1500 g for 10 min at 4 °C) and divided into various aliquots for lipoprotein separation, lipid profile evaluation, lipid peroxide level determination and assessment of albumin, transferrin and uric acid concentrations. For assessment of lipoperoxidation, aliquots were stored in liquid nitrogen until analysis.

Analytical procedures

Determination of serum lipids

Separation of HDL was performed by addition of polyethylene glycol to the fresh samples in order to precipitate other lipoproteins. Enzymatic methods were used to assay total
cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG), with the CHOD-PAP®, HDL-C Plus® and GPO-PAP® kits, respectively (Roche Diagnostics, Mannheim, Germany). Low-density lipoprotein cholesterol (LDL-C) concentration was calculated by the original Friedewald formula \(29\).

**Determination of enzyme activity**

SOD activity in erythrocytes was assessed by a method based on the autoxidation of epinephrine to adrenochrome \(\text{AD}^3\). One unit (U) of SOD was defined as the quantity of enzyme producing an inhibition of 50% in the rate of adrenochrome production. GPx activity in whole blood was measured by the method of Paglia and Valentine \(30\), with cumene hydroperoxide and reduced glutathione as substrates (Ransel RS 506 kit, Randox Laboratories, UK). The unit (U) of GPx activity was defined based on the rate of NADPH consumption by glutathione reductase, which catalyses the reduction of oxidized glutathione. For both enzymes, results were expressed per g hemoglobin. Hemoglobin concentration was determined by the cyanmethemoglobin method (Randox HG 980 kit).

**Quantification of serum antioxidant markers: albumin, transferrin, total iron binding capacity and uric acid**

The quantitative determination of serum albumin was performed using a Randox test (AB 362 kit) and expressed in g/dl. The serum concentrations of both transferrin and TIBC were assessed by an immunoturbidimetric assay (Randox TF 2453 kit). Transferrin was expressed in mg/dl and total iron binding capacity (TIBC) in \(\mu\)g/dl. Serum uric acid was measured by a colorimetric method (Randox UA 1613 kit) and expressed in mg/dl.

**Determination of lipid peroxidation products in serum**

Thiobarbituric acid reactive substances (TBARS) were measured in serum by the method of Satoh \(\text{AD}^3\) modified by Wasowicz et al. \(\text{AD}^3\), using malondialdehyde formed from 1,1,3,3-tetramethoxypropane as standard. The concentration of TBARS was expressed in \(\mu\)mol/l.

**Statistical analysis**

Statistical analysis was performed using STATISTICA 5.0 and SPSS 10.0 software. Data were expressed as mean ± standard deviation (SD). The values were analyzed using the Student’s t test for independent samples with a significance level of p<0.05. The presence of outliers was verified by running boxplots, and when the parameters did not fit a normal distribution, a logarithmic transformation was carried out \((30\)\). Correlation analyses were also applied, using Pearson’s coefficient, to assess linear relationships between age, lipids, antioxidant defense parameters and TBARS. A forward stepwise discriminant analysis was performed, using as grouping variable a new variable combining gender and dyslipidemia or gender and smoking habits to obtain components of variability between enzymatic and non-enzymatic antioxidant markers, lipids and TBARS \(\text{AD}^3\).

**RESULTS**

**Baseline characteristics of the study population**

The characteristics of both groups are shown in Table 1. Most of the subjects (74%) were female. Based on serum cholesterol and/or triglycerides levels, subjects were divided into normolipidemias (with total cholesterol and triglycerides below 200 mg/dl and 150 mg/dl respectively) and hyperlipidemias with one or both parameters above these reference values \(\text{AD}^3\). Lipid parameters (in mg/dl) for normo- and hyperlipidemic subjects for the two genders were as follows: normolipidemic females (n=69): TC=165±21, HDL-C=63±14, LDL-C=89±18, TG=71±28; hyperlipidemic females (n=67):

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the Lisbon study population</th>
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<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
</tr>
<tr>
<td>Normo vs. Hyper (%)</td>
</tr>
<tr>
<td>Non-smokers vs. smokers (%)</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD. Normo: normolipidemias; Hyper: hyperlipidemias.
TC=234±28, HDL-C=66±19, LDL-C=146±28, TG=111±61; normolipidemic males (n=25): TC=166±24, HDL-C=49±13, LDL-C=59±20, TG=82±32; hyperlipidemic males (n=22): TC=234±22, HDL-C=59±21, LDL-C=152±23, TG=116±59. About half of the subjects were hyperlipidemics, and 20% were current smokers consuming on average 15 cigarettes per day (Table I).

Redox parameters according to gender and age

Table II shows enzyme activity, non-enzymatic antioxidant levels and lipid peroxidation index, according to gender and age. The levels of serum transferrin and TIBC were higher in females, while the opposite was found for uric acid concentration. When analyzed according to age range, older females showed higher values of SOD activity and uric acid levels and lower transferrin and TIBC levels than younger ones. No significant variations were found for the other parameters based on gender or age range.

Overall, transferrin correlated negatively with age (r=-0.31; p<0.05) and TBARS correlated negatively with both of the antioxidant enzymes, SOD (r=-0.24; p<0.05) and GPx (r=-0.24; p<0.05).

Redox parameters according to lipidemia characterization

Table III shows that hyperlipidemic females had higher serum uric acid and TBARS levels than normolipidemic ones. For males, the highest values for SOD activity in erythrocytes were observed in the hyperlipidemic group. GPx activity in whole blood and albumin, transferrin and TIBC levels in serum, appeared not to be affected by lipidemia (Table III).

In normolipidemic subjects, TBARS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Females</th>
<th></th>
<th>Males</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>All n=136</td>
<td>20-44 n=75</td>
<td>45-70 n=61</td>
<td>All n=47</td>
</tr>
<tr>
<td>SOD (U/g Hb)/100</td>
<td>41±17</td>
<td>37±14</td>
<td>46±19 **</td>
<td>41±19</td>
</tr>
<tr>
<td>GPx (U/g Hb)</td>
<td>33±13</td>
<td>33±14</td>
<td>33±12</td>
<td>31±11</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.15±0.35</td>
<td>4.11±0.38</td>
<td>4.19±0.32</td>
<td>4.24±0.31</td>
</tr>
<tr>
<td>Transferrin (mg/dl)</td>
<td>303±58</td>
<td>324±61</td>
<td>279±43 **</td>
<td>274±39 *</td>
</tr>
<tr>
<td>TIBC (μg/dl)</td>
<td>355±73</td>
<td>41±77</td>
<td>335±54 **</td>
<td>348±59</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>4.2±1.3</td>
<td>4.0±0.9</td>
<td>4.2±1.5 **</td>
<td>5.1±1.6 *</td>
</tr>
<tr>
<td>TBARS (μmol/L)</td>
<td>9.5±0.42</td>
<td>9.6±0.48</td>
<td>9.4±0.34</td>
<td>9.7±0.51</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD. * p<0.05 between genders for all subjects; ** p<0.05 between the two age groups within each gender.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Females</th>
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<th>Males</th>
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<tbody>
<tr>
<td></td>
<td>Normo n=69</td>
<td>Hyper n=57</td>
<td></td>
<td>Normo n=25</td>
</tr>
<tr>
<td>SOD (U/g Hb)/100</td>
<td>41±16</td>
<td>40±18</td>
<td>35±13</td>
<td>49±21 *</td>
</tr>
<tr>
<td>GPx (U/g Hb)</td>
<td>33±13</td>
<td>33±13</td>
<td>33±14</td>
<td>29±7</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.17±0.38</td>
<td>4.12±0.32</td>
<td>4.25±0.34</td>
<td>4.22±0.28</td>
</tr>
<tr>
<td>Transferrin (mg/dl)</td>
<td>312±61</td>
<td>294±53</td>
<td>278±55</td>
<td>278±48</td>
</tr>
<tr>
<td>TIBC (μg/dl)</td>
<td>356±73</td>
<td>375±67</td>
<td>343±45</td>
<td>333±55</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>3.9±1.0</td>
<td>4.7±1.4 *</td>
<td>5.8±1.4</td>
<td>6.4±1.7</td>
</tr>
<tr>
<td>TBARS (μmol/L)</td>
<td>0.79±0.38</td>
<td>1.02±0.44 *</td>
<td>0.84±0.45</td>
<td>1.12±0.55</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD. Normo: normolipidemics; Hyper: hyperlipidemics; Hb: hemoglobin. * p<0.05 between normo- and hyperlipidemic groups, within the same gender.
Table IV. Erythrocyte SOD activity, whole blood GPx activity, and serum levels of albumin, transferrin, total iron binding capacity (TIBC), uric acid and thiobarbituric acid reactive substances (TBARS) of subjects from the Lisbon study population, according to smoking habits for both genders

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Smoking habits</th>
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<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-smokers n=111</td>
<td>Smokers n=25</td>
<td>Non-smokers n=36</td>
</tr>
<tr>
<td>SOD (U/g Hb)/100</td>
<td>42±18</td>
<td>34±11*</td>
<td>43±20</td>
</tr>
<tr>
<td>GPx (U/g Hb)</td>
<td>33±13</td>
<td>33±13</td>
<td>31±11</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.15±0.35</td>
<td>4.11±0.36</td>
<td>4.24±0.31</td>
</tr>
<tr>
<td>Transferrin (mg/dl)</td>
<td>306±56</td>
<td>294±64</td>
<td>276±41</td>
</tr>
<tr>
<td>TIBC (mg/dl)</td>
<td>307±72</td>
<td>373±61</td>
<td>350±52</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>4.4±1.3</td>
<td>3.9±1.1</td>
<td>6.3±1.6</td>
</tr>
<tr>
<td>TBARS (nmol/l)</td>
<td>0.89±0.41</td>
<td>0.99±0.49</td>
<td>1.06±0.53</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD. * p<0.05 between smokers and non-smokers, within the same gender.

correlated negatively with GPx (r=-0.25; p<0.05) and with albumin (r=-0.26; p<0.05). In hyperlipidemias, GPx presented a positive correlation with transferrin (r=0.30; p<0.05) and albumin correlated negatively with total cholesterol (r=-0.31; p<0.05).

**Redox parameters according to smoking habits**

Female smokers had lower SOD activity than non-smokers (Table IV). Cigarette consumption in males decreased serum uric acid levels. The other antioxidant parameters were not affected by smoking. TBARS levels in males were lower in the smoker group but in females, smokers had slightly higher TBARS concentrations than non-smokers (Table IV).

In non-smokers, total cholesterol correlated positively with triglycerides (r=0.32; p<0.05) and TBARS (r=0.31; p<0.05) and negatively with GPx (r=-0.20; p<0.05). For the same individuals, GPx also correlated negatively with TBARS (r=-0.21; p<0.05) and triglycerides correlated negatively with HDL-C (r=-0.34; p<0.05). In smokers, the only positive correlation observed was between total cholesterol and triglycerides (r=0.32; p<0.05).

**Multivariate analysis**

Variations in the parameters measured in the Lisbon study population based on gender, dyslipidemia and smoking habits were assessed by discriminant analysis. Gender, lipidemia and cigarette consumption were used as grouping variables. Discriminant analyses based on both enzyme activity (superoxide dismutase and glutathione peroxidase), TBARS, lipids (total cholesterol, HDL cholesterol and triglycerides) and non-enzymatic antioxidant markers (albumin, transferrin and uric acid) showed their utility as discriminant variables.

Discriminant analysis for lipemia characterization using these parameters revealed that 98% of the variation between groups was accounted for by the first two discriminant functions (Figure 1). The first function (p=0.000), to which the variables total cholesterol and triglycerides were related (discriminant loadings 0.935 and 0.286 respectively), explained 78% of the variations and clearly separated dyslipidemia. The second discriminant function (p=0.000) was associated with uric acid, HDL cholesterol and albumin (discriminant loadings -0.780, 0.462, and -0.179 respectively) and segregated the genders.

Analysis of Figure 1 indicates that variations in lipid profile and antioxidant markers were less pronounced in hyperlipidemic subjects, since the genders overlapped more in this group.

With regard to cigarette smoking, discriminant analysis showed that 96% of the variations between smoking habits and genders were accounted for by the first two discriminant functions (Figure 2). The first function (p=0.000), to which the variables uric acid and albumin were related (discriminant loading 0.827 and 0.159 respectively), explained 78% of the variations, and segregated the genders (Figure 2). The second discriminant function (p=0.047) was related to HDL cholesterol, superoxide dismutase.
DISCUSSION

In this study, data on the influence of gender, age range, hyperlipidemia and smoking habits on redox balance parameters in Portuguese subjects is presented and discussed for the first time in a systematic and integrated fashion.

Redox balance according to gender and age

Among the various theories attempting to explain the ageing process, the free radical theory has received increasing recognition in recent decades. This theory postulates that the accumulation of oxidative damage caused by reactive oxygen species produced as normal by-products of aerobic life underlies the fundamental changes found in senescence. In the present study, no differences in enzyme activity were found between genders, either for SOD in erythrocytes or for GPx in whole blood, which agrees with the findings of Pavão et al. and also of Kasapoglu and Özben, who measured both enzymes in erythrocytes. However, a study on a healthy population with a similar age range reported lower GPx activity in females. When the two age ranges were considered within each gender, our data revealed that SOD activity in females increased in the oldest group, but GPx activity was unchanged in both genders across the age range. Although the activity of the two enzymes was determined in different biological compartments (SOD in erythrocytes and GPx in whole blood), the results are comparable because the activity of GPx in whole blood is largely determined by erythrocytes. Higher SOD activity without a parallel increase in GPx activity could therefore lead to enhanced hydrogen peroxide levels. However, other antioxidant agents could partly compensate for this imbalance. The increase in SOD activity in erythrocytes with age is in agreement with results reported by Merocci et al. and Kasapoglu and Özben, but not with others that found lower SOD activity in elderly humans. This increase could be an adaptive response to ageing. The glutathione peroxidase activity and its maintenance with age observed in this study is similar to that found for Portuguese populations from the Azores archipelago but differs from the positive correlation with age observed in whole blood.

and total cholesterol (discriminant loadings 0.735, 0.347 and 0.217 respectively), and clearly discriminated smokers from non-smokers. Figure 2 shows that the variations in biochemical parameters were greater within genders.
and in erythrocytes by other authors. Different dietary habits, lifestyles and age range could explain the discrepancies for these enzymes.

The slight increase in uric acid with age in females could enhance antioxidant capacity against possible increased oxidative stress in elderly individuals. Low concentrations of this compound are associated with lipid oxidation and alpha-tocopherol consumption, while a protective role is observed at higher concentrations. Experimental studies have demonstrated that uric acid may have beneficial functions (acting as an antioxidant), but also detrimental actions (stimulating vascular smooth muscle cell proliferation and inducing endothelial dysfunction), depending on its concentration. Nevertheless, serum uric acid levels remained within the normal physiological range found in apparently healthy subjects.

Transferrin has an antioxidant role due to its iron binding capacity. Serum transferrin levels were diminished in older females and correlated negatively with age for all the subjects studied. However, the mean values of this parameter remained within the normal range reported in the literature. The decreased transferrin levels in older females could be related to hormonal factors. Furthermore, the negative correlation found for transferrin with age might reflect the ageing process and could contribute to LDL oxidation, which is known to be a primary step in atherosclerosis.

**Redox balance according to lipidemia characterization**

Hyperlipoproteinemia is a primary risk factor for atherosclerosis. With respect to antioxidant enzyme activity, different behavior was observed between hyperlipidemic subjects according to gender, with SOD activity in erythrocytes increased only in males, suggesting a possible imbalance in antioxidant/oxidant status. Experimental studies have demonstrated that hypercholesterolemia increases cellular superoxide anions in endothelial cells and in platelets. Greater production of this species induces LDL modification and accumulation of lipids within the vascular wall.

In hyperlipidemic subjects, increased serum TBARS reflects oxidative stress. Similar observations have been reported in subjects with a comparable health condition. MDA-modified proteins are thought to cause damage in humans with familial hypercholesterolemia, as demonstrated in the Watanabe rabbit. The oxidative modification of LDL has been shown to increase the ability of LDL to bind the extracellular matrix, thereby increasing its retention within the intima, where it can contribute to the formation of atherosclerotic lesions. Another step is the accumulation of oxidized LDL in macrophages and endothelial cells to form the foam cells that comprise much of the atherosclerotic plaque. Decreased antioxidant enzyme activity in neutrophils occurs in hyperlipoproteinemic subjects and lower antioxidant enzyme activity in erythrocytes has been reported in patients with different types of hyperlipoproteinemia. The discrepancy between our results and those of other studies could be related to the moderate modification in the hyperlipidemic group.

The similar SOD and GPx activity of normo- and hyperlipidemic females, but not of matched males, could be linked to the antioxidant-like effects of estrogens at the non-genomic level. In addition, the increased serum uric acid levels in hyperlipidemic females could be a result of a compensatory mechanism to counteract oxidative damage. This metabolite is a scavenger of peroxynitrite, aqueous peroxyl radicals and hydroxyl radicals, but it may act as a pro-oxidant during LDL oxidation promoted by copper or hydrophilic peroxyl radicals.

Albumin is a circulating molecule which binds a large number of metabolites such as bilirubin and metal ions such as Cu, thus limiting oxidative damage. It is also involved in the scavenging of oxygen free radicals, which is probably related to its high concentration and rapid turnover. In this study, albumin correlated negatively with TBARS in normolipidemies and with total cholesterol in hyperlipidemias, which is in agreement with its antioxidant properties.

The negative correlation found between GPx activity and TBARS in normolipidemic but not in hyperlipidemic subjects is indicative of better regulation of redox status in normolipidemias. In turn the positive correlation between GPx and transferrin in hyperlipidemic subjects could reveal an attempt to adjust antioxidant defenses.
The different correlations between normo- and hyperlipidemic subjects are reinforced by the discriminant analysis, which clearly segregates gender in hyperlipidemia. This result illustrates compensatory antioxidant mechanisms in healthy subjects and emphasizes the relationship between gender-related lipid profile and oxidative status in humans. It also corroborates the putative antioxidant role of HDL-associated paraoxonase 1 (PON1) \(^\text{(39)}\), which preserves the functional integrity of high-density lipoproteins and protects low-density lipoproteins from oxidation \(^\text{(38)}\).

**Redox balance according to smoking habits**

Many pro-oxidants from tobacco smoke can trigger oxidant damage to human tissues, including lipid peroxidation in cell membranes \(^\text{(36)}\). Increased oxidative stress in smokers may lead to antioxidant status breakdown \(^\text{(69)}\, \text{38}\) such as observed by us with α-tocopherol \(^\text{(39)}\).

The activity of SOD was significantly lower in female smokers and a similar tendency was observed in males, in agreement with Codandabany \(^\text{(99)}\), but not with other authors \(^\text{(99, 100)}\). This discrepancy may be related to the number of cigarettes smoked per day, as well as to time of exposure to cigarette smoke. Concerning GPx activity, no difference based on smoking habits was found, which is in accordance with the findings of Pavão et al. \(^\text{(102)}\) and Bolzán et al. \(^\text{(103)}\), but not with others \(^\text{(22, 29)}\). Tobacco is an important risk factor for diseases such as chronic obstructive pulmonary disease (COPD). In a recent study conducted in Lisbon, disruption of the antioxidant enzymatic system was observed in smokers and also in COPD patients who were ex-smokers \(^\text{(75)}\), which was associated with the pathology itself, smoking being the main causative agent.

In the present study, comparison of data on lipoperoxidation between smokers and non-smokers within each gender appeared to be inconclusive. In contrast to slightly higher TBARS levels in female smokers, as also found by Miller et al. \(^\text{(109)}\) and Codandabany \(^\text{(99)}\), lower levels were found in males. This could be explained by the small sample size of males and the fact that most of these subjects were young and normolipidemic. As such, the higher TBARS levels in male non-smokers will be dependent on their lipid profile.

Serum albumin was similar in smokers and non-smokers, which agrees with some epidemiological findings \(^\text{(73, 74)}\), but not with others \(^\text{(94, 95)}\). Similarly, serum transferrin and total iron binding capacity were not affected by smoking habits, which is in agreement with Galdston et al. \(^\text{(77)}\).

For both genders, decreased SOD activity could be indicative of the influence of smoking on the antioxidant/oxidant balance in apparently healthy people. Also, the correlations found between parameters in non-smokers practically disappeared in smokers, which is reinforced by the discriminant analysis that clearly separates the genders according to smoking habits.

**FINAL REMARKS**

No gender-related differences were found in antioxidant enzyme activity. Redox status was slightly affected by age within the age range studied, although in the overall population inverse correlations were observed between a lipid peroxidation marker and each of the two antioxidant enzymes. Hyperlipidemia led to higher lipoperoxidation levels, not followed by variations in the studied antioxidant enzymes, suggesting that further studies should focus on other metabolic antioxidant routes, such as HDL-associated paraoxonase. In turn, smoking negatively affected cellular homeostasis, by leading to an imbalance in redox status, suggested by decreased superoxide dismutase activity in smokers. The antioxidant/pro-oxidant balance does indeed seem to be affected by different factors related to age, tobacco consumption and certain physiological conditions, which generally tend to tip the balance in favor of pro-oxidants. Thus, it is strongly recommended that researchers working in this field include smoking and hyperlipidemia as important discriminatory criteria in epidemiological studies on oxidative stress.

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