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Oxidative stress in hypercholesterolemia and its association with Ala16Val superoxide dismutase gene polymorphism

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ABSTRACT

Objectives: To investigate the role of the oxidative stress and the antioxidant system as well as the influence of the manganese superoxide dismutase (Ala16Val) polymorphism on hypercholesterolemia.

Design and methods: Levels of glucose, lipid, high-sensitivity C reactive protein (hs-CRP), thiobarbituric acid reactive substances (TBARS), carbonyl protein, thiols, reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and vitamin C, vitamin E, as well as the presence of the manganese superoxide dismutase (Ala16Val) polymorphism were determined in 40 subjects with hypercholesterolemia and 40 controls.

Results: Lipid profile, hs-CRP, glucose, TBARS, carbonyl protein, CAT, and vitamin E were significantly higher in subjects with hypercholesterolemia. In contrast, GSH and SOD were lower. TBARS, carbonyl protein, thiols, CAT, and vitamin E were significantly higher in hypercholesterolemic subjects with VV genotype for MnSOD, while GSH, SOD, and vitamin C were lower in these subjects.

Conclusions: We suggest an association between the genotypes of MnSOD, hypercholesterolemia, and oxidative stress biomarkers.

Introduction

Reactive oxygen species (ROS) produced during normal cellular functions [1] have a high chemical reactivity that leads to the oxidation of lipids, proteins or DNA. The balance between ROS generation and antioxidant activity is critical to the pathogenesis of oxidative stress-related disorders [2]. Hypercholesterolemia has been associated with oxidative stress that results from the increased production of reactive oxygen radicals or impairment of the antioxidant system [3,4]. One of the most consistent hypotheses to explain atherogenesis postulates that it is triggered by *in vivo* LDL oxidation caused by ROS [5,6]. Significant increases in lipoperoxidation products or decreases in some antioxidants in plasma have been reported in hypercholesterolemia, including lipoperoxidation and protein oxidation [7–9].

Superoxide dismutase (SOD) is the primary antioxidant in the mitochondria that converts ROS into oxygen and hydrogen peroxide [10–13]. There are three SOD isoforms, including the mitochondrial SOD manganese dependent (MnSOD). MnSOD is encoded by a single gene containing five exons and it is located on chromosome 6q25 [14]. One of the common polymorphisms of MnSOD results in the replacement of alanine 16 (GCT) with a valine (GTT); the Ala16Val polymorphism). This polymorphism affects the import of MnSOD into the mitochondria by altering the conformation of its leader signal [15]. This mutation may reflect a functional polymorphism of mitochondrial transport of human MnSOD. Ala16Val is implicated in a decreased efficiency of MnSOD transport into target mitochondria in V allele carriers [16]. A study performed by Sutton et al. [17] suggested the Ala-MnSOD precursor generated 30%–40% more of the active, matricial, and processed MnSOD homotetramer than the Val-MnSOD precursor. These results showed that the Ala-MnSOD/mitochondrial-targeting sequence (MTS) allows a more efficient MnSOD import into the mitochondrial matrix than Val variant [17]. The modulation of the redox status influenced by genetic polymorphisms could affect cardiovascular homeostasis [18], and recently a positive association between the ValVal genotype of the MnSOD gene and obesity was observed [19].

It is known that non enzymatic antioxidant mechanisms also protect against oxidative stress. These mechanisms include natural lipophilic antioxidants such as vitamin E and hydrophilic substances like vitamin C and glutathione (GSH), which could act synergistically.

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No protein thiols have a variety of functions in bioreduction and detoxification processes [20–25]. Clinical studies have demonstrated that patients with hypercholesterolemia have markers suggestive of decreased antioxidant activity when compared with normal subjects; however, the relationship between the MnSOD polymorphism and level of oxidant and antioxidant markers is not understood until now. Therefore, the aim of this study was to investigate lipid peroxidation, protein oxidation, and the antioxidant system in hypercholesterolemic patients and healthy subjects. We also evaluated the influence of the manganese superoxide dismutase (Ala16Val) polymorphism on oxidative stress biomarkers.

Methods

Study population

Eighty subjects were selected from a previous cross-sectional investigation that included 1058 participants. The association between the Ala16Val MnSOD polymorphism and obesity was analyzed [19]. The study showed that genetic frequencies were in Hardy–Weinberg equilibrium and there was an independent association between obesity and the VV polymorphism. Firstly, we selected candidate subjects to include in the study from the database of Montano et al. [19]. Subjects with diseases and dysfunctions that could influence results were excluded. The exclusion criteria were as follows: subjects with previous coronary, stroke, neoplasias, morbid obesity (>35 kg/m^2), diabetes type 2, metabolic syndrome, as well as subjects undergoing hypolipemic treatment or taking anti-inflammatory or other medications that could alter cholesterol levels, smokers, and carriers of other diseases or dysfunctions that could influence the data obtained. Volunteers were invited to participate in the study and were prospectively enrolled at LABIMED, located in Santa Maria-RS, Brazil. Subjects were divided into two groups according to serum cholesterol levels as follows: control group, with 40 healthy subjects with cholesterol levels ranging from 104 to 178 mg/dL (2.69–4.61 mmol/L); and the hypercholesterolemia group, with 40 subjects with high cholesterol levels ranging from >240 to 529 mg/dL (6.47–13.70 mmol/L) and LDL cholesterol ≥160 mg/dL (4.15 mmol/L). We used a maximum value of 529 mg/dL to decrease the possibility of including individuals with familial hypercholesterolemia who typically have very high levels of total cholesterol [27]. Nevertheless, we could not assure that the hypercholesterolemic group studied was indeed truly homogeneous because some subjects could fall into the group of familial polygenic hypercholesterolemia (Frederickson phenotype IIa, b). Possible secondary hypercholesterolemia cases, occurred as a consequence of other disturbances, were also excluded. The LDL-cholesterol cut-point was recommended by the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III Final Report) [26].

All subjects provided written informed consent and this protocol was approved by the Human Ethics Committee of the Federal University of Santa Maria (number 23081.009087/2008).

Laboratory analyses

Blood samples were collected after 12 h overnight fasting by venous puncture into gray and red top Vacutainers®(BD Diagnostics, Plymouth, UK) tubes. Plasma was used to measure the levels of fasting glucose and serum total cholesterol, and triglyceride concentrations were measured using standard enzymatic methods by use of Ortho-Clinical Diagnostics®reagents on the fully automated analyzer (Vitros 950®dry chemistry system; Johnson & Johnson, Rochester, NY, USA). High-density lipoprotein cholesterol was measured in the supernatant plasma after the precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride as previously described [28]. Low-density lipoprotein cholesterol was estimated with the Friedewald equation [29]. High-sensitivity C reactive protein (hs-CRP) was measured by nephelometry (Dade Behring, Newark, DE, EUA).

Serum thiobarbituric acid reactive substances (TBARS) were measured according to the modified method of Jentzsch et al. [30]. The carbonylation of serum proteins was determined by the Levine method with modifications [31]. Whole blood catalase activity was determined by the method of Aebi [32] by measuring the rate of decomposition of H_2O_2 at 240 nm. Whole blood superoxide dismutase activity was measured as described by McCord and Fridovich [33]. Reduced glutathione and non-protein thiols were assayed in plasma by the method of Ellman [34]. Serum vitamin E was estimated by a modified method from Hansen and Warwick [35]. Vitamin C analysis was performed by the method described by Roe [36]. Protein was measured by the method of Bradford using bovine serum albumin as a standard [37].

We provided intra-assay and inter-assay coefficients of variation, as follows: glucose (1.30% and 1.73%); total cholesterol (2.43% and 2.77%); triglyceride (2.37% and 2.49%); HDL cholesterol (3.32% and 3.86%); hs-CRP (1.5% and 1.8%); TBARS (4.8% and 5.2%); carbonyl protein (4.3% and 1.1%); GSH (4.8% and 5.4%); SOD (4.5% and 5.0%), catalase (4.3% and 5.2%), vitamin C (4.6% and 5.4%) and vitamin E (4.1% and 5.0%).

DNA analysis

Genomic DNA was isolated from peripheral blood leukocytes using a DNA Mini Kit Purification (Mo Bio). SOD polymorphism was detected by PCR-RFLP analysis. The method used here to detect the Ala-16Val polymorphism is described in detail by Taufer et al. [38]. PCR amplifications were performed in a total volume of 50 µl containing 5 µl of 10× buffer, 1 µl of 25 mM MgCl2, 1.25 µl of 10 mM dNTP, 0.5 µl of Taq Polymerase (Gibco Inc, Co.), 1 µl of each primer (40 pmol), 3 µl of genomic DNA (0.25 µg), and 34.5 µl of ddH2O. The amplification primers (Gibco Inc, Co.) for a 110-bp fragment of the human MnSOD gene were 5′-ACCAGCAGGCAGCTGGCGCCGG-3′ (sense strand) and 5′-GCGTTGATGTGAGGTTCCAG-3′ (antisense strand) with the following thermocycler parameters: an initial cycle of 95 °C for 5 min followed by 35 cycles at 95 °C for 1 min and 61 °C for 1 min. The final cycle was followed by an extension period of 2 min at 72 °C. The PCR product (10 µl) was digested with Hae III (15 U; 37 °C, 6 h, Gibco. Inc, Co.). Digested products (23 and 85 bp) were visualized on a 4% agarose gel (Amersham Biosciences Inc, Co.) stained with ethidium bromide. A mutation was introduced by a primer mismatch to create a restriction cut site for Hae III in the -9 codon, and the following genotypes were observed: -9Ala/Ala (23 and 85 bp); -9Ala/Val (23, 85 and 110 bp); and -9Val/Val (110 bp).

Statistical analysis

Data are presented as mean and standard error of the mean (SEM). Statistical differences between groups were evaluated by Students *t* test. Pearson correlation was assessed to evaluate the correlation between variables. We previously excluded patients with diseases and dysfunctions that could influence the results. The differences between groups and correlation to MnSOD polymorphism genotypes of the subjects were evaluated by analysis of variance (one-way ANOVA) followed by Tukey's post hoc test. Statistical significance was assumed at $P<0.05$. We performed a multivariate analysis using a multiple logistic regression method (Backward Wald). We considered BMI and triglycerides as possible intervening variables (16). Statistical analyses were performed where all p-values were two-tailed, and $p<0.05$ was considered statistically significant.

Results

Eighty subjects were investigated in this study, consisting of 40 subjects with hypercholesterolemia and 40 controls. Baseline characteristics of the study subjects are shown in Table 1. Total cholesterol, LDL cholesterol, triglycerides, hs-CRP, and glucose were significantly higher in subjects with hypercholesterolemia, while HDL cholesterol was lower in these subjects. The values of TBARS (10.67 ± 0.32 *vs* 7.07 ± 0.26 nmol MDA/mL serum, $P<0.001$), carbonyl protein (1.07 ± 0.03 *vs* 0.94 ± 0.04 nmol/mg protein, $P<0.05$), thiols (1.27 ± 0.02 *vs* 0.91 ± 0.03 μmol/mL plasma, $P<0.001$), catalase (201.50 ± 7.08 *vs* 109.30 ± 2.44 U/g hemoglobin, $P<0.001$), and vitamin E (12.73 ± 0.40 *vs* 7.92 ± 0.22 μmol/L serum, $P<0.001$) were significantly higher in subjects with hypercholesterolemia in comparison to controls. In contrast, GSH (0.55 ± 0.03 *vs* 1.22 ± 0.04 μmol/mL plasma, $P<0.001$) and SOD (0.52 ± 0.02 *vs* 0.71 ± 0.02 U/mg hemoglobin, $P<0.001$) were lower in the hypercholesterolemia group, as shown in Fig. 1. No statistical differences were observed for vitamin C levels between the study groups. Moreover, we observed significant correlations between total cholesterol, hs-CRP, and oxidative stress biomarkers, as shown in Table 2.

Potential correlation between MnSOD polymorphism genotypes and lipids and inflammatory and oxidative stress biomarkers was also evaluated. We assessed the association of MnSOD genotypes alanine/alanine (AA), alanine/valine (AV) and valine/valine (VV) as reported in Table 3. Distribution of MnSOD polymorphism genotypes in the control group was as follows: AA (65.0%, n = 26), AV (27.5%, n = 11), and VV (7.5%, n = 3). In contrast, the frequency of the alleles Ala/Val and Val/Val in the hypercholesterolemic group was significantly higher as follows: AA (10.0%, n = 4), AV (55.0%, n = 22), and VV (35.0%, n = 14). These data indicate an increase in the oxidative stress in the hypercholesterolemia group, especially in subjects with the VV genotype for MnSOD. TBARS, carbonyl protein, thiols, catalase, and vitamin E levels were significantly higher in hypercholesterolemic subjects with the VV genotype for MnSOD, while GSH, SOD, and vitamin C levels were lower in these subjects. Multivariate analysis showed that these results were independent of BMI and triglycerides levels.

Discussion

In the present study, some indicators of oxidative stress were higher in the subjects with hypercholesterolemia, including TBARS levels. This finding is associated with the damage to lipid structures by free radicals. We also report here a positive correlation between total cholesterol and TBARS. These findings are in agreement with results of other recent investigations, which have showed lipid peroxidation associated with high serum lipids [7,39–41]. TBARS have been shown to be a predictor of cardiovascular events in patients with established heart disease, independent of traditional risk factors and inflammatory markers [42].

Proteins constitute major components of living cells and relatively minor structural modifications often lead to a marked change of function. Under oxidative stress, proteins and lipids are the major targets of ROS. The formation of protein carbonyl seems to be a common phenomenon during oxidation, and its quantification may be used to measure the extent of oxidative damage [9]. The results of the present study indicate an increase in the oxidation of plasma proteins in subjects with hypercholesterolemia, as previously reported [43]. Fu et al. [43] have investigated the roles of radicals in protein oxidation in advanced human atherosclerotic plaques, and they concluded that reactive products of protein oxidation should be considered to be possible contributors to atherogenesis.

In this study it was demonstrated that SOD activities were decreased in hypercholesterolemic subjects. SOD is the first line of cellular defense against oxidative injury which is involved in the disposal of superoxide anions and hydrogen peroxide. Thus, the insufficient detoxification of these ROS by antioxidant enzymes may lead to an imbalance between antioxidant and oxidant systems. Low SOD activity could also be attributed to enzyme inactivation by ROS-induced damage to proteins [44]. However, catalase activities were higher in hypercholesterolemic subjects. Here it is assumed that oxidative stress acts primarily by increasing the production of $O^{2\cdot-}$ and H_2O_2. Any increased production of catalase is presumed to be secondary in these cases, reflecting an attempt to partially compensate for the increased oxidative stress. Interestingly, there is evidence that even this second method of inducing antioxidant enzymes in response to an oxidative insult can be protective as a way of developing "tolerance" to a subsequent larger insult [11,45].

Our study also revealed decreased GSH levels in hypercholesterolemic subjects. GSH has also been shown to play a role in detoxifying oxygen radicals and therefore may prevent cellular damage from oxidative stress [13]. Data reported in the literature have shown that patients under chronic disease states such as heart disease, arthritis, diabetes, and malignancies have lower plasma levels of GSH than control subjects, suggesting that GSH has a protective role against such diseases [46]. We also observed reduced plasma thiol level in hypercholesterolemic subjects. According to Uzun and colleagues [47], erythrocyte thiol concentrations were significantly lower in morbidly obese patients than in controls. Therefore, thiol levels are not sufficient to prevent oxidative stress in hypercholesterolemia.

In our study, we have shown increased levels of plasma vitamin E. Although an increase in plasma vitamin E seems to be inconsistent with the higher oxidative stress, vitamin E, which is a lipid soluble vitamin, correlates directly with lipid and beta-lipoprotein levels [48]. The increased vitamin E level was likely to partially compensate for the increased oxidative stress because it can act as a peroxyl radical scavenger. Vitamin E could regulate smooth muscle cell proliferation and ROS monocyte production by mechanisms involving protein kinase C (PKC) inhibition, which is largely dependent on vitamin E isoforms and stereoisomers [39].

Here we observed a significant correlation between total cholesterol, hs-CRP, and oxidative stress biomarkers. A growing body of evidence supports the concept that hypercholesterolemia elicits a cascade of proinflammatory reactions that are known to lead to the production of ROS. The oxidation of lipids and proteins leads to a progressive increase in hs-CRP levels. Several reports have suggested that CRP may play a direct pathophysiological role in the development and progression of atherosclerosis [49].

This study demonstrates an association between hypercholesterolemia and the VV genotype for MnSOD as well as increased oxidative stress represented by the increase in TBARS and carbonyl protein levels. The alanine variant of MnSOD is thought to have an α-helical mitochondrial-targeting domain, whereas the valine variant of MnSOD appears to have a β-pleated sheet conformation [50]. This

Table 1
Baseline characteristics of study subjects.

	Control	Hypercholesterolemia
n	40	40
Sex (male/female)	20/20	19/21
BMI (Kg/m^2)	23.28 ± 6.71	23.94 ± 8.92*
Glucose (mmol/L)	4.56 ± 0.060	4.73 ± 0.056*
Total cholesterol (mmol/L)	3.80 ± 0.073	8.23 ± 0.29**
LDL cholesterol (mmol/L)	1.98 ± 0.047	5.89 ± 0.31**
HDL cholesterol (mmol/L)	1.31 ± 0.042	1.17 ± 0.058*
Triglycerides (mmol/L)	1.09 ± 0.076	2.55 ± 0.25**
hs-CRP (mg/L)	0.18 ± 0.01	1.96 ± 0.24**

BMI: body mass index. Data are expressed as mean ± standard error of the mean (SEM).
* $P<0.05$.
** $P<0.001$.

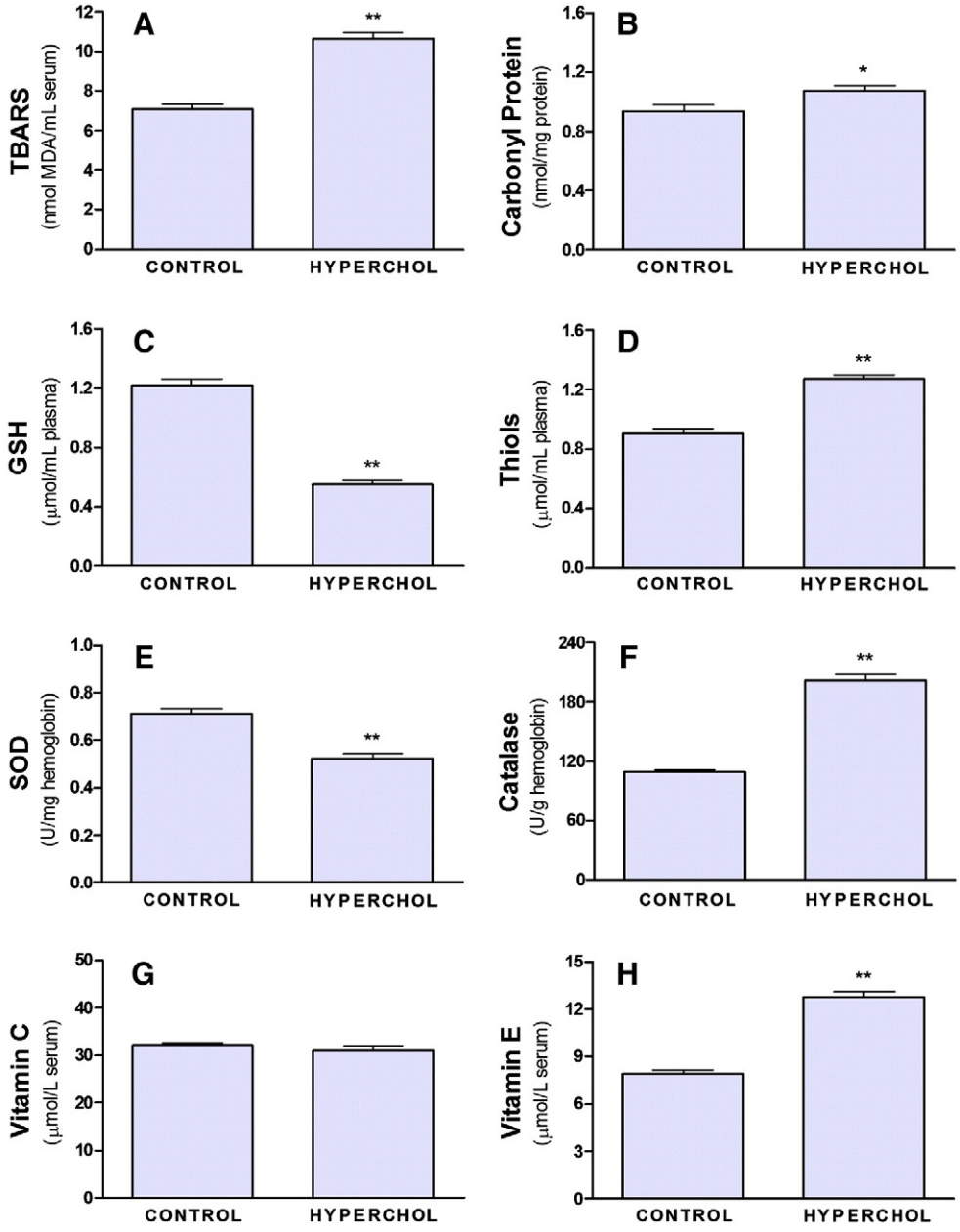

Fig. 1. The values of TBARS (A), carbonyl protein (B), GSH (C), thiols (D), SOD (E), catalase (F), vitamin C (G), and vitamin E (H) in control and hypercholesterolemia groups. $^{*}P<0.05$, $^{**}P<0.001$.

conformational difference is thought to result in more efficient transport of the alanine variant of MnSOD into mitochondria than the valine variant [51]. The Val variant of the MnSOD may be present at a lower concentration in the mitochondria. If this is the case, patients with Val/Val homozygosity should have lower resistance to oxidative stress. The increased production of catalase, thiols and vitamin E is presumed to be secondary to partially compensate for the increased oxidative stress. In agreement with our results, several studies have suggested that MnSOD polymorphism is associated with certain kinds of diseases induced by oxidative damage including atherosclerosis. Dedoussis et al. [52] showed higher oxLDL cholesterol levels for middle aged men with the Val/Val genotype, compared to the other allele (Ala/Ala and Ala/Val) carriers. Recently, Fujimoto et al. [53] found an association between the alanine variant of the signal peptide and increased mitochondrial MnSOD activity, which protects macrophages from the oxLDL-induced apoptosis and reduces the risk of acute coronary syndromes and cardiovascular diseases.

Table 2
Correlations between total cholesterol, hs-CRP and oxidative stress biomarkers.

	Total cholesterol		hs-CRP	
	r	*P*	*r*	*P*
TBARS	0.6873	<0.0001	0.4879	<0.0001
Carbonyl protein	0.2358	0.0352	0.1178	0.2982
GSH	−0.6923	<0.0001	−0.5584	<0.0001
Thiols	0.5786	<0.0001	0.5050	<0.0001
SOD	−0.4561	<0.0001	−0.3448	0.0017
Catalase	0.7377	<0.0001	0.5268	<0.0001
Vitamin C	−0.0918	0.4181	−0.0292	0.7965
Vitamin E	0.7054	<0.0001	0.4486	<0.0001

Finally, it is important to note some considerations associated with our methodological design. This study investigated the possible interaction between the Ala16Val MnSOD polymorphism,

Table 3
MnSOD polymorphism genotypes and baseline levels of biochemical and oxidative stress parameters of study subjects.

Genotypes	Control			Hypercholesterolemia		
	AA	AV	VV	AA	AV	VV
n	26	11	3	4	22	14
Glucose (mmol/L)	4.60 ± 0.08^{a}	4.46 ± 0.09^{a}	4.64 ± 0.048^{a}	4.84 ± 0.16^{a}	4.75 ± 0.06^{a}	$4.80 \pm 0{,}12^{a}$
Total cholesterol (mmol/L)	3.79 ± 0.90^{a}	3.79 ± 0.17^{a}	3.88 ± 0.27^{a}	8.02 ± 0.61^{b}	8.14 ± 0.44^{b}	8.42 ± 0.46^{b}
HDL cholesterol (mmol/L)	1.27 ± 0.047^{a}	1.34 ± 0.09^{a}	1.58 ± 0.17^{a}	1.25 ± 0.16^{a}	1.12 ± 0.08^{a}	1.21 ± 0.11^{a}
LDL cholesterol (mmol/L)	2.00 ± 0.64^{a}	1.97 ± 0.07^{a}	1.88 ± 0.21^{a}	5.90 ± 0.59^{b}	5.85 ± 0.47^{b}	5.95 ± 0.49^{b}
Triglycerides (mmol/L)	1.12 ± 0.10^{a}	1.05 ± 0.14^{a}	0.92 ± 0.23^{a}	1.92 ± 0.37^{a}	2.54 ± 0.42^{b}	2.74 ± 0.31^{b}
hs-CRP (mg/L)	0.20 ± 0.02^{a}	0.14 ± 0.01^{a}	0.15 ± 0.03^{a}	1.58 ± 0.83^{b}	2.05 ± 0.33^{b}	1.92 ± 0.44^{b}
TBARS (nmol MDA/mL serum)	6.22 ± 0.25^{a}	8.55 ± 0.26^{b}	9.06 ± 0.03^{b}	8.97 ± 0.19^{b}	9.84 ± 0.30^{b}	12.45 ± 0.49^{c}
Carbonyl protein (nmol/mg protein)	0.84 ± 0.04^{a}	1.05 ± 0.08^{b}	1.33 ± 0.11^{b}	0.80 ± 0.06^{a}	0.99 ± 0.04^{b}	1.28 ± 0.02^{c}
GSH (μmol/mL plasma)	1.07 ± 0.04^{a}	1.42 ± 0.05^{b}	1.74 ± 0.12^{c}	0.90 ± 0.07^{a}	0.57 ± 0.02^{d}	0.43 ± 0.03^{d}
Thiols (μmol/mL plasma)	0.84 ± 0.04^{a}	1.03 ± 0.04^{b}	1.13 ± 0.09^{b}	1.09 ± 0.05^{b}	1.20 ± 0.01^{b}	1.44 ± 0.03^{c}
SOD (U/mg hemoglobin)	0.79 ± 0.01^{a}	0.59 ± 0.02^{b}	0.45 ± 0.07^{b}	0.71 ± 0.03^{a}	0.53 ± 0.03^{b}	0.46 ± 0.03^{b}
Catalase (U/g hemoglobin)	100.10 ± 1.34^{a}	124.40 ± 3.84^{b}	133.60 ± 2.69^{b}	142.70 ± 3.26^{b}	184.40 ± 7.74^{c}	245.20 ± 4.26^{d}
Vitamin C (μmol/L serum)	31.08 ± 0.62^{a}	33.15 ± 0.43^{a}	38.48 ± 1.82^{b}	40.92 ± 2.03^{b}	31.35 ± 0.91^{a}	27.76 ± 1.27^{a}
Vitamin E (μmol/L serum)	7.32 ± 0.25^{a}	8.73 ± 0.19^{a}	10.21 ± 0.22^{a}	12.12 ± 0.85^{b}	11.76 ± 0.15^{b}	14.44 ± 0.96^{c}

Data are expressed as mean ± standard error of the mean (SEM). Different letters indicate statistical differences at $P<0.05$.

hypercholesterolemia and oxidative stress biomarkers. As these conditions are influenced by many factors, for this study we chose to exclude subjects with diseases and dysfunctions that are highly prevalent in hypercholesterolemic subjects and other diseases and dysfunctions that could influence the levels of oxidative biomarkers analyzed here. This exclusion is the reason for the small number of subjects included in the study. In addition, we could not assure that the hypercholesterolemic group studied was indeed truly homogeneous because some subjects could fall into the group of familial polygenic hypercholesterolemia (Frederickson phenotype IIa, b). We also excluded all possible secondary hypercholesterolemia that occurred as a consequence of other disturbances including diseases, metabolic processes, excess hormones, alcohol consumption, as well as use of drugs such as thiazides and oral contraceptives. However, it is important to note that surprisingly, the number of VV genotypes in the control group and the number of AA genotypes in the hypercholesterolemic group were small, which suggests a genetic association between the polymorphism and hypercholesterolemia that warrants investigation in further studies. This is a preliminary report and to our knowledge it is the first report of an association between the Ala16Val polymorphism and hypercholesterolemia in oxidative stress modulation until now. Further epidemiological studies are required to better understand MnSOD polymorphisms and oxidative stress parameters.

In conclusion, despite the limitations, the current study has demonstrated an increase in lipid peroxidation and protein oxidation in hypercholesterolemia as well as a decrease in some antioxidant mechanisms. Moreover, we suggest an association between the genotypes of MnSOD, hypercholesterolemia and oxidative stress biomarkers. However, more studies are required to investigate this association in a larger population.

References

[1] Kowaltowski AJ, Souza-Pinto NC, Castilho RF, Verces A. Mitochondria and reactive oxygen species. Free Radical Biol Med 2009;47:333–43.
[2] Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Phys India 2004;52:794–804.
[3] Duarte MMMF, Rocha JBT, Moresco RN, Duarte T, Da Cruz IB, Loro VL. Association between ischemia-modified albumin, lipids and inflammation biomarkers in patients with hypercholesterolemia. Clin Biochem 2009;42:666–71.
[4] Prasad K, Kalra J. Oxygen free radicals and hypercholesterolemic atherosclerosis. Am Heart J 1993;125:958–71.
[5] Osipov RM, Bianchi C, Feng J, Clements RT, Liu Y, Robich MP. Effect of hypercholesterolemia on myocardial necrosis and apoptosis in the setting of ischemia-reperfusion. Circulation 2009;120:S22–30.
[6] Sam F, Kerstetter D, Pimental D, Mulukutla S, Tabaee A, Bristow MR. Increased reactive oxygen species production and functional alterations in antioxidant enzymes in human failing myocardium. J Card Fail 2005;11:473–80.
[7] Duarte MMMF, Loro VL, Rocha JBT, Leal DB, Bem AF, Dorneles A. Enzymes that hydrolyze adenine nucleotides of patients with hypercholesterolemia and inflammatory process. FEBS J 2007;274:2707–14.
[8] Cherubini A, Ruggiero C, Polidori MC, Mecocci P. Potential markers of oxidative stress in stroke. Free Radical Biol Med 2005;39:841–52.
[9] Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta 2003;329:23–38.
[10] Michiels C, Raes M, Toussaint O, Remale J. Importance of Se-glutathione peroxidase, catalase and Cu/Zn-SOD for cell survival against oxidative stress. Free Radical Biol Med 1994;17:235–48.
[11] Matés JM, Pérez-Gómez C, Núñez CI. Antioxidant enzymes and human diseases. Clin Biochem 1999;32:595–603.
[12] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44–84.
[13] Xu Y, Liu B, Zweier JL, He G. Formation of hydrogen peroxide and reduction of peroxynitrite via dismutation of superoxide at reperfusion enhances myocardial blood flow and oxygen consumption in postischemic mouse heart. J Pharmacol Exp Ther 2008;327:402–10.
[14] Church SL, Grant JW, Meese EU, Trent JM. Sublocalization of the gene encoding manganese superoxide dismutase (MnSOD/SOD2) to 6q25 by fluorescence in situ hybridization and somatic cell hybrid mapping. Genomics 1992;14:823–5.
[15] Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radical Biol Med 2002;33:337–49.
[16] Suh YA, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D. Cell transformation by the superoxidegenerating oxidase Mox1. Nature 1999;401:79–82.
[17] Sutton A, Imbert A, Igoudjil A, Descartoire V, Cazanave S, Pessayre D. The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. Pharmacogenet Genomics 2005;15:311–9.
[18] Gottlieb MG, Schwanke CH, Santos AF, Jobim PF, Müssel DP, Cruz IBM. Association among oxidized LDL levels, SOD2, apolipoprotein E polymorphisms, and cardiovascular risk factors in a south Brazilian region population. Genet Mol Res 2005;4:691–703.
[19] Montano MAE, Gottlieb MG, Barrios-Lera JP, Schwanke CH, da Rocha MI, Manica-Catani MF. Association between manganese superoxide dismutase (MnSOD) gene polymorphism and elderly obesity. Mol Cell Biochem 2009;328:33–40.
[20] Arrigo AP. Gene expression and thiol redox state. Free Radical Biol Med 1999;27:936–44.
[21] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. fourth ed. Oxford: Clarendon Press; 2007.
[22] Sesso HD, Buring JE, Christen WG, Kurth T, Belanger C, MacFadyen J. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. JAMA 2008;18:2123–33.
[23] Qiao M, Zhao Q, Lee CF, Tannock LR, Smart EJ, LeBaron RG. Thiol oxidative stress induced by metabolic disorders amplifies macrophage chemotactic responses and accelerates atherogenesis and kidney injury in LDL receptor-deficient mice. Arterioscler Thromb Vasc Biol 2009;29:1779–86.
[24] Hasty AH, Gruen ML, Terry ES, Surmi BK, Atkinson RD, Gao L. Effects of vitamin E on oxidative stress and atherosclerosis in an obese hyperlipidemic mouse model. J Nutr Biochem 2007;18:127–33.
[25] Pryor WA. Vitamin E and heart disease: basic science to clinical intervention trials. Free Radical Biol Med 2000;28:141–64.
[26] Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–421.
[27] Austin MA, Hutter CM, Zimmern RL, Humphries SE. Familial hypercholesterolemia and coronary heart disease: a HuGE association review. Am J Epidemiol 2004;160:421–9.

[28] Bachorik PS, Albers JJ. Precipitation methods for quantification of lipoproteins. Methods Enzymol 1986;129:78–100.
[29] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[30] Jentzsch AM, Bachmann H, Fürst P, Biesalski HK. Improved analysis of malondialdehyde in human body fluids. Free Radical Biol Med 1996;20:251–6.
[31] Levini RL, Garland D, Oliver CN, Amiei A, Climent I, Lenz A. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990;186: 464–8.
[32] Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.
[33] Mc Cord JM, Fridovich I. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049–55.
[34] Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70–7.
[35] Hansen LG, Warwick WJ. A fluorometric micromethod for serum vitamins A and E. Am J Clin Pathol 1969;51:538–41.
[36] Roe JH. Methods of biochemical analysis. In: Glick D, editor. New York: Interscience; 1954. p. 115–39.
[37] Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[38] Taufer M, Peres A, de Andrade VM, de Oliveira G, Sá G, do Canto ME. Is the Val16Ala manganese superoxide dismutase polymorphism associated with the aging process? J Gerontol A Biol Sci Med Sci 2005;60:432–8.
[39] Serdar Z, Aslan K, Dirican M, Sarandol E, Yesilbursa D, Serdar A. Lipid and protein oxidation and antioxidant status in patients with angiographically proven coronary artery disease. Clin Biochem 2006;39:794–803.
[40] Vassalle C, Pratali L, Boni C, Mercuri A, Ndreu R. An oxidative stress score as a combined measure of the pro-oxidant and anti-oxidant counterparts in patients with coronary artery disease. Clin Biochem 2008;41:1162–7.
[41] Yang RL, Shi YH, Hao G, Li W, Le GW. Increasing in oxidative stress with progressive hyperlipidemia in human: relation between malondiladeyde and atherogenic index. J Clin Biochem Nutr 2008;43:154–8.
[42] Walter MF, Jacob RF, Jeffers B, Ghadanfar MM, Preston GM, Buch J, et al. Serum levels of thiobarbituric acid reactive substances predict cardiovascular events in patients with stable coronary artery disease: a longitudinal analysis of the PREVENT study. J Am Coll Cardiol 2004;44:1996–2002.
[43] Fu S, Davies MJ, Stocker R, Dean RT. Evidence for roles of radicals in protein oxidation in advanced human atherosclerotic plaque. Biochem J 1998;333:519–25.
[44] Nelson S, Bose SK, Grunwald GK, Myhill P, McCord JM. The induction of human superoxide dismutase and catalase in vivo: a fundamentally new approach to antioxidant therapy. Free Radical Biol Med 2006;40:341–7.
[45] Repine JE. Oxidant–antioxidant balance: some observations from studies of ischemia–reperfusion in isolated perfused rat hearts. Am J Med 1991;91:45–53.
[46] Shimizu H, Kiyohara Y, Kato I, Kitazono T, Tanizaki Y, Kubo M. Relationship between plasma glutathione levels and cardiovascular disease in a defined population: the Hisayama study. Stroke 2004;35:2072–7.
[47] Uzun H, Konukoglu D, Gelisgen R, Zengin K, Taskin M. Plasma protein carbonyl and thiol stress before and after laparoscopic gastric banding in morbidly obese patients. Obes Surg 2007;17:1367–73.
[48] Tietz NM. Clinical guide to laboratory tests. second ed. Philadelphia: Saunders; 1990.
[49] Dohi Y, Takase H, Sato K, Ueda R. Association among C-reactive protein, oxidative stress, and traditional risk factors in healthy Japanese subjects. Int J Cardiol 2007;115:63–6.
[50] Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagava-Hattori Y, Shimizu Y, Mizuno Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene: a predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. Biochem Biophys Res Commun 1996;226:561–5.
[51] Rosenblum JS, Gilula NB, Lerner RA. On signal sequence polymorphisms and diseases of distribution. Proc Natl Acad Sci USA 1996;93:4471–3.
[52] Dedoussis GV, Kanoni S, Panagiotakos DB, Louizou E, Grigoriou E, Chrysohoou C. Age-dependent dichotomous effect of superoxide dismutase Ala16Val polymorphism on oxidized LDL levels. Exp Mol Med 2008;40:27–34.
[53] Fujimoto H, Taguchi JI, Imai Y, Ayabe S, Hashimoto H, Kobayashi H. Manganese superoxide dismutase polymorphism affects the oxidized low-density lipoprotein-induced apoptosis of macrophages and coronary artery disease. Eur Heart J 2008;29:1267–74.