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Association between manganese superoxide dismutase (MnSOD) gene polymorphism and elderly obesity

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Abstract Evidence suggests an association between obesity and oxidative stress caused by superoxide production. Since the dismutation of superoxide is catalyzed by superoxide dismutase enzymes, we tested the association between obesity and Ala16Val manganese-dependent superoxide dismutase gene (MnSOD) polymorphism. We analyzed 815 free-living community subjects (≥60 years old) grouped into subjects who were either obese (BMI ≥ 30 kg/m^2) or non-obese (BMI < 25 kg/m^2). Additionally, we investigated the possible interaction between the Ala16Val MnSOD gene polymorphism and obesity in the modulation of biochemical and nutritional variables. We found a positive association between MnSOD polymorphism and obesity, since higher VV frequency (28.2%) was observed in the obese group ($P = 0.002$, odds ratio 1.949, 95% CI: 1.223–3.008). This result was independent of sex, age, diabetes, dyslipidemia, hypertension, and metabolic syndrome. A possible biological explanation of the association described here could be a chronic state of superoxide enzyme imbalance present in VV carriers, which could affect differential metabolic pathways contributing to the obese state.

Keywords Obesity · Ala16val MnSOD polymorphism · Superoxide · Elderly · Nutrigenetics · Dietary pattern

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Introduction

Obesity is characterized by an increase in total body and/or abdominal fat. Fat tissue is not a simple energy storage organ, but has important endocrine and immune functions, producing regulatory and immunological molecules such as adiponectin, leptin, and cytokines; an imbalance of these molecules in obese subjects can affect vascular (endothelial) function, increasing the production of vasoconstrictor proteins and causing oxidative stress [1, 2]. Therefore, evidence is mounting that human obesity is a state of chronic oxidative stress [3, 4].

Superoxide (O_2^-), a powerful free radical molecule, contributes to the development of atherosclerosis from the inhibitory effect of nitric oxide (NO) [5]. The possible association between obesity and superoxide production could involve leptin, which is present at higher plasma levels in the obese (leptin concentrations in healthy subject are <10 ng/ml and in obese subjects they are in the range of 10–100 ng/ml) [6, 7]. Also, studies have suggested that leptin could likely increase body oxidative stress by

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inducing an interaction between superoxide and NO, resulting in enhanced antioxidative enzyme activity for enzymes such as SOD and GSH-PX [8–10].

Important endothelial antioxidant enzymes include SODs, catalase, the thioredoxin system, glutathione peroxidase, and heme oxygenase. The dismutation of superoxide ions to hydrogen peroxide and oxygen is catalyzed by superoxide dismutases (SOD) which are considered as the first enzymes in the defense against oxidative stress produced by normal metabolism [11]. All SODs efficiently convert O_2^- to H_2O_2. The latter is then degraded to water by catalase or glutathione peroxidase. There are three SOD isoforms manganese SOD (SOD2) in the mitochondria, copper–zinc SOD in the cytosol, and extracellular SOD in extracellular compartments [12]. Genetic studies have described that a single AA polymorphism exists in manganese-dependent superoxide dismutase (MnSOD). A change of alanine (Ala) to valine (Val) at the 16th amino acid (Ala16Val) of the signal sequence of the MnSOD (ninth amino acid from the first amino acid of the mature protein) has been suggested to change the secondary structure of the protein and therefore the mitochondrial targeting of the enzyme [13]. The precursor sequence in the MnSOD protein is called the mitochondrial target sequence (MTS). In vitro investigations performed using the import of chimeric proteins into the mouse showed that the Ala-MnSOD precursor generated 30–40% more of the active, processed MnSOD homotetramer, than the Val-MnSOD precursor. Therefore, the Ala-MnSOD/MTS allows efficient MnSOD import into the mitochondrial matrix, while the Val-variant causes partial arrest of the precursor within the inner membrane and decreased formation of the active MnSOD homotetramer in the mitochondrial matrix [14].

Epidemiological evidence has shown that the Ala allele (AA), thought to alter enzyme transportation into the mitochondria, is associated with increased risk for breast, prostate, colon cancer, hepatocellular carcinoma in HCV-infected patients, and malignant pleural mesothelioma [15–19]. Surprisingly, additional epidemiological investigations have suggested that the V allele is not a "good genetic variant" either, where it has been associated with carotid atherosclerosis quantified as intima-media thickness (IMT) by ultrasound [20], with non-familial dilated cardiomyopathy in Japanese subjects [21], higher oxidized LDL (ox-LDL) levels in Brazilian subjects with synergic effects in diabetes type II patients [22]. However, alternative data have shown that an A allele variant of the signal peptide that increases mitochondrial MnSOD activity, protects macrophages against ox-LDL-induced apoptosis, and reduces the risk of coronary artery disease and acute myocardial infarction [23].

The above findings suggest a "paradox" in the MnSOD polymorphism, since mainly the homozygous condition (AA and VV) of this variation causes an imbalance in antioxidant modulation, leading to increased superoxide levels in VV subjects or hydrogen peroxide levels in AA subjects. In both cases, environmental interactions could increase or decrease the risk of non-transmissible morbidities such as neoplasias and cardiovascular diseases, as suggested in studies that described an interaction between this polymorphism and antioxidant dietary pattern and other lifestyle variables such as cigarette smoking in breast cancer risk [24, 25].

Given that excessive superoxide production is related to obesity and is a risk factor for several chronic diseases, we examined here if an association existed between uncomplicated obesity and Ala16Val polymorphism in elderly subjects (≥60 years old).

Materials and methods

Subjects and study design

We performed a cross-sectional investigation enrolling 1,058 participants of a population study of the Southern Brazilian Research Program that investigates genetic and environmental interactions in aging and related non-transmissible diseases. Previous studies were published and described in more detail about this research project [26–29]. In this sample, we examined the prevalence of obesity and biological, environmental and health variables.

As the study includes genetic variables, the samples were recruited by random selection of Brazilians of European ancestry from the Health and Social Assistance Program of Gravataí, a city in the Porto Alegre Metropolitan area, Rio Grande do Sul State (RS). Compared with Brazil as a whole, the population of this state is composed mainly of people of European ancestry (82%). A previous study performed by Cruz et al. [30] in different ethnic populations showed different allelic and genotype distributions as well as different associations with diseases or biological characteristics. For this reason, we analyzed just Caucasian subjects who represent the main ethnic group [31, 32].

Additionally, we selected all obese and non-obese subjects without previous diseases such as coronary, stroke, neoplasias, other diseases, or dysfunctions that could influence the obese state, dietary pattern, and genotype distribution. These exclusions are justified because these variables could interfere in the analyses [33]. Therefore, a total of 815 subjects were selected and classified as obese (301) or non-obese (514). Obesity was determined by body mass index (BMI) over 30 kg/m^2, and by central obesity defined as a waist circumference (WC) ≥102 cm for men and ≥88 cm for women [34].

A second analysis was performed to evaluate the interaction effect between obesity and biochemical and nutritional variables. The Research Ethics Committee approved the study protocol (No. 537/02), and informed consent was obtained from all individuals whose information was collected prospectively.

Genetic analyses

The performance of the MnSOD genotyping was similar to that described in two previous articles in the same population [22, 32]. Blood samples from a peripheral vein were taken using venoclysis system with a disposable vacuum device (Vacutainer, Becton Dickinson Co., Juiz de Fora) and stored in tubes with 0.1% EDTA (final concentration of 1 mg/dL). Afterward, the material collected was kept at 4°C until DNA extraction for up to 24 h. Genomic DNA was isolated from peripheral blood leukocytes using a GFX Genomic Blood DNA Purification kit (Amersham Biosciences Inc., Uppsala, Sweden). MnSOD genotyping was performed using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) techniques. A total volume of 50 µl contained 1.25 µl 10 mM dNTP, 0.5 µl containing 5.0 µl 10× buffer, 1.0 µl 25 mM $MgCl_2$, Taq polymerase (Gibco Inc.), 1.0 µl of 40 pmol of each primer, 3.0 µl genomic DNA (0.25 µg), 34.5 µl. The amplification primers (Gibco Inc., Co.) for a 110-bp fragment of the human MnSOD gene were 5′-ACCAGCAGGC AGCTGGCGCCGG-3′ (sense-strand) and 5′-GCGTTGAT GTGAGGTT CCAG-3′ (antisense-strand) with thermocycler parameters consisting of 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; at 37°C, for 6 h, Gibco Inc., Billings, MO). Digested products (23 and 85 bp) were visualized on a 6% agarose gel (Amersham Biosciences Inc., Sweden) 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, so that the following genotypes were observed −9Ala/Ala (23 and 85 bp), −9Ala/Val (23.85 and 110 bp), and −9Val/Val (110 bp) [22]. All genotypes were analyzed by at least two blinded observers. When a conflict in genotype identification (infrequent cases) was observed, PCR-RFLP was repeated and analyzed again. This procedure was performed to reduce this potential confounding variable.

Biochemical analysis

Blood samples were collected from Gravataí's subjects after an overnight fasting (12 h or more); snacks and coffee were offered afterwards. The following blood tests were performed: biochemical analysis (glucose, total cholesterol, HDL-c, LDL-c, and triglycerides, TG) [35]. Total cholesterol, HDL-c, TG, and glucose were determined by enzymatic colorimetric methods using commercial kits total cholesterol Cod-Ana Labtest® (Cat.76, Lagoa Santa, Brazil), HDL-c precipitant Labtest® (Cat.13, Lagoa Santa, Brazil), TG Gpo-Ana, Glucose PAP Labtest® (Lagoa Santa, Brazil), and LDL-c were calculated according to the Friedwald equation: (LDL-c) = (TG) − (HDL-c + TG/5). Plasma ox-LDL was estimated using thiobarbituric acid-reactive substance formation according to the fluorimetric procedures of Yagi [36].

Nutritional assessment

Nutritional patterns were analyzed as described in a previous study conducted in the same population used here [29]. Spontaneous intake of energy and nutrients were assessed, with the specific goal of recording intake without modifying the diet. Dietary assessment of macro- and micronutrients included a 24-h recall asking if the dietary report was a habitual eating pattern or not. The dietary reports were compared, and the subjects who showed differences were excluded. Subjects were asked to recall the frequency of consumption of individual food items (number of times/d,/wk,/mo, or/y), and the estimated portion size, using weight or natural units (small, medium, or large). A second dietary assessment was made to verify whether the diet record was consistent with the habitual eating pattern in another season, at least half a year or 1 year after the first interview. Additionally, food frequency questionnaires (FFQ) were applied [37, 38]. Volunteers who displayed large differences between these dietary assessments were excluded ($n = 218$). The major food item contributing to intake in this study were compiled, and some items were deleted and other items added according to eating practices common in Brazil [39]. Macro- and micronutrient contents were analyzed using the Brazilian software DietWin Clinic, which is structured from Brazilian food nutritional values previously validated.

Statistical analyses

All the analyses were carried out using the statistical package for social studies SPSS version 12.0 (SPSS Inc., Chicago, IL). In the first analysis investigating the association between Ala16Val MnSOD gene polymorphism, we compared the allele and genotype frequencies by the χ^2 test. Logistic regression (*Forward Wald* method) was used to investigate possible intervening factors. All variables that showed univariate statistical significance ($P \leq 0.1$) were included in the test (sex, age, diabetes, dyslipidemia, hypertension, and metabolic syndrome). Odds ratio values

and interval confidence at 95% were also calculated. In the second analysis, we compared the ox-LDL levels and the other biochemical variables in obese and non-obese subjects to different Ala16Val MnSOD gene polymorphisms using one-way analysis of variance followed by Bonferroni's post hoc test. The α value considered was $P = 0.05$. All P values were two-tailed.

Results

The baseline characteristics of the obese and non-obese subjects are shown in Table 1. The obese group had more women and displayed a higher prevalence of diabetes type II and metabolic syndrome than did the non-obese group.

Genotype and allele Ala16Val MnSOD polymorphism frequencies were determined and are presented in Table 2. Genotype frequency of the control group was in agreement with those expected under Hardy–Weinberg equilibrium. We found a positive association between Ala16Val MnSOD genotypes and obesity since higher VV frequency was observed in the obese group ($\chi^2 = 12.774$, $P = 0.002$).

A calculated dose–allele effect confirms this association (VV versus AA + AV) genotypes ($\chi^2 = 4.090$, $P = 0.03$). The odds ratio determined was 1.949 (95% CI: 1.223–3.008), indicating a greater chance of VV demonstrating an obesity pattern compared to other genotypes. Multivariate logistic regression showed that the association between VV genotype and obesity was independent of sex, age, diabetes, dyslipidemia, hypertension, and metabolic syndrome ($P = 0.524$). In this case, the obese group who expressed VV or AA did not display differential proportions of these variables.

Additional analyses comparing some biological and nutritional variables (Tables 3, 4) in obese and non-obese groups to different MnSOD genotypes showed a weak interaction. We found higher VV and AA cholesterol levels compared to AV in the obese group, and VV and AV glucose levels higher than those in AA in the non-obese group. Obese VV showed lower caloric intake than AA and AV obese subjects and lower lipid intake than AA subjects. Additionally, obese VV and AV carriers had lower vitamin E intake than AA subjects. Just protein intake was higher in AA subjects, whereas the other nutritional parameters were similar in non-obese with different Ala16Val MnSOD polymorphism.

Table 2 Genotype and allele frequencies of Ala16Val SOD2 gene polymorphism in obese and control subjects

Genetic	Obese *n* (%)	Non-obese *n* (%)
Genotypes		
AA	30 (10.0)	95 (18.5)
VV	85 (28.2)	110 (21.4)
AV	186 (61.8)	309 (60.1)
Alleles		
A	0.4086	0.4854
V	0.5914	0.5146
Odds ratio (95% CI)		
AA + AV	1	1
VV	1.949 (1.223–3.008)	0.809 (0.720–0.910)

Pearson χ^2 value = 12.774, $P = 0.002$. Control group are in Hardy–Weinberg equilibrium (χ^2 value = 4.115, $P = 0.1277$). 95% CI (interval confidence)

Discussion

We describe here, for the first time, a positive association between obesity and Ala16Val MnSOD gene polymorphism, with weak interaction of these two factors in regard to biological variables related to cardiovascular risk and general dietary pattern. Despite the several limitations, the results obtained here raise an important question: Can oxidative stress caused by chronic exposure to superoxide

Table 1 Baseline characteristics between obese case–control subjects

	Obese ($n = 301$)	Control ($n = 514$)	*P*
Age (years, mean ± SD)	66.0 ± 7.8	66.5 ± 8.7	0.459
Gender			
Male *n* (%)	54 (17.9)	141 (27.4)	0.002
Female *n* (%)	247 (82.1)	373 (72.6)	
BMI (kg/m^2, mean ± SD)	35.79 ± 4.87	25.48 ± 2.51	0.0001
Waist circumference (cm, mean ± SD)	100.10 ± 9.3	88.52 ± 9.17	0.0001
Dislipidemia, *n* (%)	132 (43.9)	225 (43.8)	0.982
Diabetes type II, *n* (%)	49 (16.3)	56 (10.9)	0.027
Hypertension, *n* (%)	159 (52.8)	259 (50.4)	0.502
Metabolic syndrome, *n* (%)	84 (27.9)	11 (2.1)	0.001

BMI body mass index, *P* statistical value from Student *t*-test or χ^2 (categorical) variables, *n* sample number, *SD* standard deviation

Table 3 Biological variables comparison between obese and non-obese with different Ala16Val polymorphism of the MnSOD gene

Variables	MnSOD	Obese Mean ± SD	Non-obese Mean ± SD
Fasting glucose (mg/dL)	AA	100.4 ± 11.7[a]	95.1 ± 12.1[a]
	VV	97.0 ± 10.5[a]	102.8 ± 16.9[b]
	AV	96.3 ± 10.8[a]	97.2 ± 9.7[ab]
	Total	97.2 ± 10.9	97.7 ± 11.5
Cholesterol (mg/dL)	AA	224.7 ± 41.2[a]	208.6 ± 38.6[a]
	VV	215.2 ± 31.5[ab]	219.6 ± 31.3[a]
	AV	204.7 ± 36.4[b]	220.8 ± 38.8[a]
	Total	210.3 ± 36.4	218.9 ± 37.8
Triglycerides (mg/dL)	AA	149.1 ± 92.6[a]	120.1 ± 37.0[a]
	VV	134.2 ± 62.1[a]	146.8 ± 43.6[b]
	AV	151.9 ± 73.2[a]	154.0 ± 65.8[a]
	Total	146.9 ± 73.3	148.2 ± 60.6
HDL-chol (mg/dL)	AA	47.1 ± 8.8[a]	42.4 ± 6.8[a]
	VV	45.0 ± 8.8[a]	46.6 ± 8.7[a]
	AV	44.6 ± 10.1[a]	44.2 ± 7.4[a]
	Total	45.0 ± 9.5	44.3 ± 7.5
LDL-chol (mg/dL)	AA	147.7 ± 32.6[a]	142.3 ± 34.9[a]
	VV	143.3 ± 29.9[a]	143.6 ± 25.4[a]
	AV	129.7 ± 37.5[a]	145.8 ± 39.1[a]
	Total	135.9 ± 35.6	145.0 ± 36.7
SBP (mmHg)	AA	145.9 ± 38.6[a]	148.9 ± 24.0[a]
	VV	153.1 ± 24.1[a]	144.0 ± 15.1[a]
	AV	149.9 ± 27.3[a]	147.4 ± 31.0[a]
	Total	150.2 ± 28.3	147.1 ± 28.2
DBP (mmHg)	AA	74.1 ± 16.9[a]	77.4 ± 13.0[a]
	VV	80.5 ± 12.7[a]	76.6 ± 8.7[a]
	AV	77.5 ± 12.8[a]	77.6 ± 13.8[a]
	Total	77.8 ± 13.4	77.4 ± 13.0

SD standard deviation, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

Values with the same letter are not statistically significant ($P < 0.05$)

ANOVA multivariate test followed by LSD post hoc test

Table 4 Macro- and micronutrients comparison between obese and non-obese with different Ala16Val polymorphism of the MnSOD gene

Macro- and micronutrients	MnSOD	Obese Mean ± SD	Non-obese Mean ± SD
Calories (Kcal)	AA	1.907 ± 307[ab]	1.216 ± 405[a]
	VV	1.558 ± 403[a]	1.481 ± 555[a]
	AV	1.666 ± 534[b]	1.671 ± 526[a]
	Total	1.594 ± 522	1.456 ± 34
Carbohydrate (%)	AA	53 ± 5[a]	48 ± 7[a]
	VV	60 ± 15[b]	54 ± 11[a]
	AV	56 ± 8[ab]	55 ± 10[a]
	Total	57 ± 11	54 ± 10
Proteins (%)	AA	17 ± 2[a]	25 ± 5[a]
	VV	17 ± 5[a]	20 ± 5[ab]
	AV	20 ± 3[a]	19 ± 5[b]
	Total	19 ± 4	20 ± 5
Lipids (%)	AA	26 ± 0[a]	27 ± 7[a]
	VV	22 ± 8[b]	25 ± 8[a]
	AV	23 ± 7[b]	25 ± 9[a]
Vitamin C	AA	135 ± 144[a]	144 ± 135[a]
	VV	137 ± 164[a]	194 ± 57[a]
	AV	86 ± 66[a]	123 ± 108[a]
	Total	110 ± 114	142 ± 125
Folic acid	AA	377 ± 91[a]	377 ± 238[a]
	VV	197 ± 141[a]	967 ± 0.359[a]
	AV	258 ± 175[a]	306 ± 72[a]
	Total	248 ± 159	470 ± 0.152
Retinol	AA	395 ± 45[a]	339 ± 04[a]
	VV	787 ± 775[a]	819 ± 912[a]
	AV	548 ± 498[a]	1.002 ± 2.113[a]
	Total	620 ± 586	881 ± 0.759
Vitamin E	AA	6.9 ± 1.0[a]	4.1 ± 2.7[a]
	VV	4.1 ± 2.6[b]	6.2 ± 5.7[a]
	AV	3.5 ± 1.7[b]	7.6 ± 9.8[a]
	Total	4.1 ± 2.2	6.8 ± 8.4

SD standard deviation

Values with the same letter are not statistic

induce obesity pattern, just as obesity pattern potentially induces oxidative stress?

Epidemiological evidence showed that obesity is the primary causal component in metabolic syndrome, supported by experimental studies showing that adipocytes in obese patients exhibit increased oxidative stress via the activation of reactive oxygen species (ROS)-producing systems and inactivation of antioxidant enzymes. For example, Vassalle et al. [40] recently examined whether obesity and smoking habit may accelerate age-related increase in oxidative stress. However, the question: What came first: obesity and afterward oxidative stress or oxidative stress and later obesity? Unfortunately, the role of organic oxidative stress caused by genetic factors is a point that is not well considered and obscure. In this study, we tried to explore this inverse question. We know that obesity causes oxidative stress and changes adipocyte homeostasis. As obesity is strongly associated with high fat diet and lower antioxidant diet, the idea that oxidative stress could also be a causal factor that induces the obese state is logical and well observed in previous studies. Therefore, we asked here if the subject is a carrier of a genotype that could produce an oxidative stress imbalance; this condition could be associated with obesity risk. Therefore, we chose to study the Ala16Val polymorphism of MnSOD to help us answer this question, since the two homozygous genotypes

(AA and VV) could cause an oxidative imbalance by increasing superoxide production (VV) or hydrogen peroxide production (AA). We observed an association between VV and obesity independent of possible metabolic variations that could influence this association, such as diabetes II. In this case, 37.7% of VV carriers were obese, whereas 23.6% of AA or AV carriers were obese ($P = 0.005$) with a similar odds ratio (1.952, 95% CI: 1.220–3.123). That is why the role of superoxide in obesity genesis needs to be further explored. In vitro effects of natural antioxidants on SW872 liposarcoma cells were recently investigated by Roche et al. [41]. These authors found a direct influence of antioxidant molecules (caffeic acid and quercitin) in adipocyte metabolism, demonstrating that oxidative modulation plays an important role in these cells.

Here, we described an association between a MnSOD polymorphism and obesity independent of other potential intervening factors, and these data permit us to speculate that the association between oxidative stress and obesity is close and could establish a "positive feedback process." In this case, VV carriers or AA carriers could have a biological oxidative imbalance that is genetically determined, and this condition could affect adipocyte metabolism, which in turn could lead to the obese state. In this case, a positive feedback oxidative stress could be established, influencing additional metabolic dysfunctions such as insulin resistance and diabetes type II.

Additional questions related to the results described here include the possible role of superoxide imbalance in the genesis of obesity. Superoxide is believed to play an important role in the pathogenesis of cardiovascular diseases, which are closely related to morbidities such as hypertension, diabetes type II, and obesity. In the vascular system, superoxide inhibits the biological action of NO, known as endothelium-derived relaxing factor, leading to vasoconstriction. Additionally, superoxide directly affects the functions of endothelial cells and vascular smooth muscle cells [42]. This effect is stimulated in some metabolic conditions such as the hyperglycemic state which induces superoxide production and inhibits superoxide dismutase by non-enzymatic glycation, known as the Maillard reaction [43]. Hyperlipidemia also increases endothelial superoxide production [44].

An association between obesity and superoxide production has been evidenced mainly considering hormones and molecules related to fat tissue and energy metabolism such as leptin, ghrelin, orexin B, and adiponectin. Perhaps, the possible association between Ala16Val polymorphism of MnSOD gene and obesity found here involves the modulation of these molecules. We consider this hypothesis based on experimental studies published in the last years involving these substances.

The evidence obtained in these studies conducted in experimental models suggests an association between obesity and oxidative stress related to superoxide production. Unfortunately, we could not find any published study that has investigated the influence of oxidative stress in obesity development. For this reason, the results described here need to be confirmed by additional experimental model investigations, case–control studies in other populations and clinical assays including nutrigenetic designs. We believe in the biological plausibility of these results. Production of hormones and regulatory molecules such as leptin and adiponectin which trigger oxidative stress, caused by chronic superoxide accumulation and potentially occurring in VV carriers, could act in the regulation of these molecules, inducing a state of obesity. In this case, a positive feedback circuit could be established. An initial imbalance of superoxide production could modulate appetite and regulatory molecules in energy metabolism, increasing susceptibility to obesity. As obesity did not occur in all VV carriers, we think that there are biological development and environmental variables that influence this process. To minimize these confounding environmental factors, we investigated apparently healthy obese and non-obese subjects, 50 years old and older, who had a well-established lifestyle, and included a dietary pattern analysis. As we did not find a strong association between the Ala16Val polymorphism and macro- and micronutrient intake, we could speculate that the association between obesity and the polymorphism studied here does not necessarily involve appetite regulation.

However, our data did not support evidence that confirm this suggestion, and we need to develop additional investigations (1) to reproduce the data described here; (2) to perform case–control investigations, including other metabolic disorders (diabetes type II, hypertension, dyslipidemia, and metabolic syndrome) and Ala16Val polymorphism; (3) to perform a prospective study and/or clinical trials that consider nutrigenetic effects and the analysis of hormonal and biochemical factors associated with obesity and atherosclerosis; and (4) to analyze younger subjects.

Finally, it is important to ponder some considerations associated with our methodological design. Since obesity is strongly related to other morbidity investigation studies, researchers looking for an association between gene and non-morbid obesity need to consider these morbidities as intervening variables. In fact, many investigations prefer to include all subjects in the study and perform further exclusions when the statistical analyses are conducted. Of course, that approach increases the sample number and permits more consistent statistical power. However, with the great quantities of variables included in the study and the large number of positive statistical associations

sometimes found in these studies, it becomes very difficult to discuss the results in biological terms.

Another situation that makes it difficult to interpret data from a large sample that shows higher variability in terms of morbidities, risk factors, lifestyle, etc. is the systemic effect of these variables, including possible synergic and additional interactions that we can separate by statistical analysis. For example, in a previous study we found that a positive association between ox-LDL levels [22] and VV genotypes of the MnSOD genotypes was strongly influenced by diabetes type II. However, we could not determine if this influence was due to an interaction between diabetes and obesity or hypertension and dyslipidemia which are highly prevalent in diabetic patients. As obesity is a risk factor for the development of diabetes type II, we designed an additional study that first considered moderate obesity as a possible metabolic condition associated with Ala16Val MnSOD gene polymorphism.

Despite the limitations of the study, the results described could be considered important in clinical terms, since they raise the possibility that antioxidant-rich foods (vegetables and fruits) play an anti-obesity role.

In conclusion, the results described here, despite methodological limitations and previous incipient evidence, suggest an association between VV and obesity. A possible biological explanation for this association could be a chronic state of superoxide imbalance present in VV carriers, which could affect differential metabolic pathways and hormonal factor modulations caused by higher superoxide levels, triggering a positive feedback: oxidative stress–obesity–oxidative stress.

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