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High Levels of Iron Status and Oxidative Stress in Patients with Metabolic Syndrome

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Abstract Studies concerning oxidative stress (OxE) parameters have increased, mainly because of its important role in cardiovascular diseases and diabetes complications. The main objective of this study was to evaluate iron nutrition status and oxidative stress parameters in subjects that had developed metabolic syndrome (MetS). Subjects from the Research Program of Risk Factors for Cardiovascular Disease (n=155) were studied (ages ranging from 45 to 65 years old) and classified according to the Adult Treatment Panel III criterion. A blood sample was taken after a 12-h fasting period, and basal glucose, insulin, thiobarbituric acid reactive substances (TBARS), oxidized LDL (oxLDL), heme oxygenase (HO) activity, lipid profile, and iron nutrition status were determined. Eighty-five subjects were classified as MetS, and 70 non-MetS. Individuals with MetS showed higher Fe storage (high levels of ferritin, total body iron and low transferrin receptor), oxLDL, TBARS, and homeostatic model assessment for insulin resistance levels. The MetS group showed high levels of oxidative stress parameters (HO activity, oxLDL, and TBARS). The presence of MetS showed an association with LDL oxidation risk (multiple lineal regression according to sex and age, p<0.001). High levels of triglycerides (p<0.001) and waist circumference (p <0.012) were associated with oxLDL levels, as well as an association between TBARS and oxLDL with ferritin levels. Through logistic regression analyses, the highest quartile of ferritin was associated with a threefold risk of developing MetS compared to the lowest quartile; also, TBARS showed a 21-fold risk for the development of MetS. Finally, elevated levels of oxidative stress parameters such us oxLDL, TBARS, HO, and Fe storage were associated to MetS.

Keyword Iron · Oxidative stress · oxLDL · TBARS · Insulin · Metabolic syndrome

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Introduction

The study of oxidative stress (OxE) parameters has increased in recent years, mainly due to its role in cardiovascular diseases and diabetes complications [1]. Oxidative stress is generally defined as an excess of formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. ROS include free radicals such as superoxide ($O_2^{\cdot-}$), hydroxyl (·OH), peroxyl (RO_2·), hydroperoxyl ($HRO_2^{\cdot-}$), as well as non-radical species such as hydrogen peroxide (H_2O_2) and hydrochlorous acid (HOCl). RNS include free radicals like nitric oxide (NO·) and nitrogen dioxide (NO_2), as well as non-radicals such as peroxynitrite ($ONOO^-$), nitrous oxide (HNO_2), and alkyl peroxinitrates (RONOO). $O_2^{\cdot-}$, NO·, and ONOO are the most widely studied species, and they play core roles in the diabetic cardiovascular disease. It is well known that oxidative stress is difficult to measure due to the presence of short-lived agents; however, oxidative species leave detectable traces of modified oxidative products at the site of atherosclerotic

lesions, such as the oxidized form of low-density lipoprotein (oxLDL) [2].

Steinberg et al. [3] suggested that the original oxidative modification hypothesis was based on the notion that oxidation represents a biological modification comparable to the chemical modification discovered by Goldstein et al. [4]. Also, numerous studies have supported the oxLDL hypothesis, which posits that oxLDL can promote foam cell formation through the so-called scavenger receptor pathways [4, 5]. However, it should be noted that macrophages express more than one scavenger receptor for oxLDL, such as CD36 [6], LOX-1 [7], and SR-PSOX [8], and other proteins that had been discovered after Steinberg proposed the oxLDL hypothesis.

Currently, the syndrome of insulin resistance, central obesity, hypertension, and dyslipidemia—also known as metabolic syndrome (MetS)—is considered as a clinical condition associated with high risk for diabetes and cardiovascular disease development. On the other hand, an active role of oxidative stress and inflammatory processes in the etiology of MetS and type 2 diabetes [9] has been suggested. High levels of inflammation increase the risk of developing atherosclerosis and this could be a possible mechanism for the adverse consequences of the MetS [10]. Moreover, it has been consistently shown that both obesity and MetS are linked with increased oxidative stress [3, 11].

Circulating oxLDL levels are associated to a high risk of cardiovascular disease [12]. Remarkably, oxLDL is directly involved in the initiation and progression of atherosclerosis [13], and circulating oxLDL concentrations are thought to reflect the turnover of oxLDL formed in the vascular wall [14]. Also, subjects with MetS show higher levels of circulating oxLDL, which may be associated to a higher predisposition to atherothrombotic vascular disease [15].

Iron absorption by enterocytes in the duodenum is closely regulated, since iron overload leads to free radical damage by the Fenton reaction [16]. Iron excess is broadly stored in organs including the liver and pancreatic β-cells. Because iron may produce oxidative damage, tissues with high mitochondrial activity are major targets of iron, inducing cellular damage in brain cells, hepatocytes, and pancreatic β-cells among other cells and tissues [17]. Elevated iron storage may increase type 2 diabetes risk through several mechanisms. In the liver, this may induce insulin resistance by reducing its capacity for insulin extraction, thereby resulting in impaired suppression of hepatic glucose production [18]. In adipocytes, it may also deteriorate insulin action and interfere with glucose uptake [19]. In muscle, increased iron deposits may enhance fatty acid oxidation, and therefore could interfere with glucose disposal [20]. Finally, in pancreatic β-cells, iron may affect insulin secretion and also activate apoptotic pathways [21].

Earlier studies on subjects of the Risk Factors Research Program for Cardiovascular Disease (PIFRECV) carried out at the University of Talca have shown that the prevalence of risk factors was higher in the city of Talca than the nationwide average in Chile [22]; 44.6 % presented MetS including the Adult Treatment Panel III (ATP III) and/or International Diabetes Federation criteria, and 75 % of them presented MetS according to both criteria [23]. Our aim was to study the association between oxidative stress markers and iron nutrition status in subjects with metabolic syndrome.

Methods

Subjects We randomly selected 155 subjects between 45 to 65 years of age, belonging to the Research Program of Risk Factors for Cardiovascular Disease of Talca, Chile (PIFRECV), using multistage probabilistic sampling. Fasting glucose concentration (>110 mg/dL) and waist circumference (men >102 cm and women >88 cm) values were used to calculate the sample size for its high prevalence (22 and 28 %, respectively), using an α error of 0.05 and a β error of 0.07. As inclusion criteria, we used metabolic syndrome guidelines provided by the Cholesterol Education Program (NCEP) ATP III [24]: waist circumference (men >102 cm and women >88 cm), triglycerides (>150 mg/dL), HDL cholesterol (men <40 mg/dL and women <50 mg/dL), blood pressure (>130/>85 mmHg, at least three times at rest), and fasting glucose concentrations (>100 mg/dL). All participants were personally contacted 72 h before the study and received dietary instructions. All volunteers conducted a survey of general information and drug use. Volunteers who consumed anti-inflammatory, antihypertensive, and vitamins were removed from the protocol. Venous blood samples (20 mL) were collected from the subjects after a 48-h low-fat diet and a 12-h overnight fast. For all nondiabetic subjects, an oral glucose tolerance test (OGTT) was performed (a two-point curve was used: basal and after 2 h of glucose ingestion). The subjects ingested 75 g of glucose in water (Trutol®). None of the controls resulted in an altered OGTT. Serum aliquots were stored at −70 °C. As exclusion criteria, we discarded individuals who had: (1) a random glycemia >200 mg/dL plus symptoms, (2) treatment for diabetes with oral drugs, (3) fast glycemia >126 mg/dL in at least two independent determinations, or (4) postprandial glycemia >200 mg/dL. All patients signed an informed consent, and the protocol was approved by the University of Talca Ethics Committee.

Glucose, hsCRP, and Insulin Levels Serum glucose concentration was measured using a gluco-quant glucose/HK kit

(Roche Diagnostics Mannheim, Germany). Quantification was carried out in a Hitachi 717 Automatic Analyzer. Highly sensitive C-reactive protein (hsCRP) was measured by immunoturbidimetry methods. As an internal standard, we used the reference preparation CRM 470 and Referent Preparation for Proteins in Human Serum. For quality control, we used CRPT control N, Ref 20766321 (EE.UU. 0766321). Basal blood insulin levels were measured using an Elecsys Insulin assay kit (Roche Diagnostics Mannheim, Germany). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated according to HOMA-IR = [Fasting insulin levels (in micro-units per milliliter) × fasting glucose levels (in milligrams per deciliter)]/405.

Oxidative Stress Markers Thiobarbituric acid reactive substances (TBARS) were measured according to Jo and Ahn [25]. Briefly, 0.5 mL of each serum was incubated with both 1 mL 0.67 % thiobarbituric acid and 0.3 mL 50 % trichloroacetic acid for 30 min at 90 °C and centrifuged at 1,620×*g* for 15 min. The supernatant was used to measure TBARS at 530 nm in a Metertech SP830 spectrophotometer (Metertech, Inc. Taiwan). oxLDL was measured by a commercial ELISA (Mercodia Oxidized LDL ELISA, SE-754 50 Uppsala, Sweden). The reaction was quantified spectrophotometrically in a Biotek Elx800 microplate reader (BioTek Instruments, Inc.) at 450 nm. The results were expressed as units per liter of oxLDL.

Heme Oxygenase Activity Peripheral mononuclear cells (PMCs) were separated by Ficoll-Histopaque gradient sedimentation (1.119: density, Sigma, St. Louis, MO). The mononuclear layer was removed, washed twice in PBS, and adjusted to 40×10^6 PMCs/mL using RPMI-1640 media with gentamicin. Then, PMCs (1×10^6) were suspended in 1 mL RPMI-1640 (Sigma-Aldrich, St. Louis, MO) and 100 μL of 900 μM H_2O_2 and incubated in 5 % CO_2 for 18 h at 37 °C. Cells were centrifuged for 10 min at 1,500×*g*. A pellet was homogenized in 100 μL of non-denaturing lysis buffer (in millimoles per liter: 20 KH_2PO_4, 135 KCl, 0.1 EDTA, pH 7.4) and centrifuged for 20 min at 10,000×*g*. The supernatant (100 μL) was incubated for 1 h at 37 °C in the dark with 100 μL of 15 μmol/L hemin (Sigma-Aldrich, St. Louis, MO), 100 μL of 100 μg/mL biliverdin reductase isolated from rat liver, and 600 μL of re-suspension buffer (100 mmol/L KH_2PO_4; pH 7,4). The reaction was initiated with 100 μL of 1 mmol/L NADPH (Sigma-Aldrich, St. Louis, MO). Bilirubin was extracted with 1 mL of chloroform by first stirring for 1 h and then centrifuging for 5 min at 200×*g*. Bilirubin concentrations were measured at 530 nm (Shimadzu, model UV-1601). Heme oxygenase (HO) activity was expressed as nanomoles of bilirubin/milligram protein/hour (bilirubin molar extinction coefficient: ε= 43.5 $mM^{-1} \times cm^{-1}$) [26].

Iron Nutritional Status Iron nutritional status was evaluated. The following cutoffs were used: (a) 120 and 135 g/L as the lower normal limit for hemoglobin (Hb) in women and men, respectively [27]; 70 μg/dL as the upper normal limit for free erythrocyte protoporphyrin in red blood cells, measured using a hematofluorometer (model 206D; AVIV Biomed, Lakewood, NJ); and 70 μg/dL as a lower normal limit for serum iron, measured using graphite furnace atomic absorption spectrometry (SIMAA 6100; Perkin-Elmer, Shelton, CT). (b) Depleted iron stores were defined as serum ferritin (SF) concentrations below 15 μg/L measured using an enzyme-linked immunosorbent assay (Dako Corp, Carpinteria, CA). (c) Iron deficiency without anemia was defined as having normal Hb and two other abnormal laboratory results (such as serum Fe or ferritin, for instance), and (d) iron deficiency anemia was defined as below-normal Hb and two other abnormal laboratory results (World Health Organization, 2007). The cutoff for transferrin receptor (TfR) to define iron deficiency was >8.3 mg/L (according to the test kit reference value, Ramco, Laboratories Inc, Houston, TX). (e) Iron overload was defined as having a serum ferritin levels greater than 200 μg/L in women and greater than 300 μg /L in men. Total body iron (TBI) was calculated according to: TBI (in milligrams per kilogram)=−{log (TfR/SF ratio)−2.8229}/0.1207 [28].

Statistical Analysis Variables were analyzed using the Kolmogorov–Smirnov test and were described as mean and standard deviation (or geometric mean and range), for normal or abnormal distribution, respectively. For men and women, a *t* test or Mann–Whitney *U* non-parametric tests was used to compare between no MetS and MetS subjects for normal or non-normal distribution, respectively. A multiple regression analysis adjusted by sex and age was used to associate oxLDL, TBARS, and HOMA and to determine which variables define metabolic syndrome. For ferritin analyses, subjects were classified into quartiles according to their serum ferritin concentrations. In the logistic regression analyses, the dependent variable was subjects with MetS vs no MetS, and the independent variables of interest were ferritin and TBARS. We performed a logistic regression analysis adjusted in the first place by sex and BMI, and in second place by hsCRP. A $p<$ 0.05 was considered as a statistical difference. SAS 9.1.3 for Windows and SPSS 14.0 for Windows software were used for statistical analyses.

Table 1 Anthropometric and biochemical variables associated to metabolic syndrome in subjects with metabolic syndrome (MetS) or without (No MetS)

Variable	Men		p value[a]	Women		p value[a]
	No MetS	MetS		No MetS	MetS	
n	22	26		48	59	
Age (years)	56.8±4.2	57.6±3.9	NS	56.0±4.0	56.6±3.8	NS
Weight (kg)	74.2±9.3	92.9 ±13.4	<0.001	65.7±10.1	78.6±15.5	<0.001
Height (m)	1.66±0.07	1.71±0.06	<0.01	1.56±0.05	1.55±0.05	NS
BMI (kg/m^2)	26.8±2.9	31.7±3.9	<0.001	27.3±4.7	32.8±6.4	<0.001
Waist circumference (cm)	92.4±7.2	108.5±10.4	<0.001	87.1±10.7	98.7±12.0	<0.001
SBP (mmHg)	133.2±19.9	152.2±20.2	<0.001	124.7±16.1	140.9±22.3	<0.001
DBP (mmHg)	79.1±10.1	91.3±13.1	<0.001	73.5±8.8	83.0±10.9	<0.001
Glycemia (mg/dL)	93.5±7.4	124.1±44.0	<0.001	89.9±8.7	107.6±36.6	<0.001
Total cholesterol (mg/dL)	188.6±37.4	198.8±33.9	NS	218.8±35.8	211.9±43.2	NS
LDL cholesterol (mg/dL)	107.5±31.9	112.9±29.8	NS	129.8±32.7	120.0±35.0	NS
HDL cholesterol (mg/dL)	50.8±15.0	44.4±15.2	NS	62.9±17.4	49.1±11.4	<0.001
Triglycerides (mg/dL)	132 (77–226)	209 (116–375)	<0.02	120 (81–177)	177 (111–283)	<0.001
Insulin (μU/mL)	4.32 (1.7–10.4)	10.7 (5.0–22.8)	<0.001	4.3 (2.2–8.5)	7.9 (4.37–13.5)	<0.001
HOMA-IR	1.0 (0.4–2.5)	3.1 (1.3–7.7)	<0.001	1.0 (0.5–2.0)	2.0 (1.1–3.7)	<0.001

Values are expressed as mean±SD, except for triglycerides and insulin that are presented as geometric mean and range

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

[a] p value represent differences between no MetS and MetS by sex

Results

Using the modified ATP III classification (criteria 2004), 85 subjects were classified with metabolic syndrome (MetS) and 70 without the syndrome (no MetS). In the MetS group, 26/85 were males from 52 to 63 years of age and 59/85 were females from 51 to 65 years of age. A similar distribution by sex and age was observed in the no MetS group (22/70 and 48/70 were male and women, respectively). There were no age differences between men and women compared by group (Table 1). However, MetS subjects had higher weight values, glycemia, triglycerides, waist circumference, blood arterial pressure, basal insulin, HOMA, and BMI than no MetS subjects. HDL cholesterol was remarkably lower in women with MetS (Table 1, t test $p<0.001$).

oxLDL levels were higher in men and women with MetS than in no MetS subjects (t test $p<0.001$; Fig. 1) (Table 2). By using a multiple lineal regression model adjusted by sex and age, we determined that the presence of MetS exerts an influence on the levels of oxLDL ($p<0.001$). There were no differences in sex or age. Also, high levels of triglycerides and waist circumference were associated to oxLDL levels ($p<0.001$ for both).

TBARS levels in women and men were higher in MetS individuals than in no MetS subjects (t test, $p<0.02$ and $p<0.01$, respectively; Fig. 2 and Table 2). There were no differences in sex or age. When we analyzed the components of MetS as an independent variable to explain the TBARS levels, triglycerides ($p<0.001$) and waist circumferences ($p<0.006$) were significant. There were no differences in sex or age (Table 2). Heme oxygenase enzymatic activity was higher in individuals with MetS (men or women; t test $p<0.001$). These results showed that subjects with MetS present high levels of oxidative stress markers.

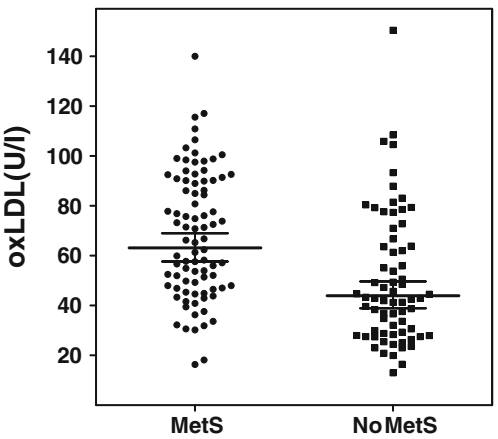

Fig. 1 oxLDL levels in metabolic syndrome and control subjects. Graph is represented as a geometric mean plus 95 % CI. There were no statistical differences between men and women. Unpaired t test, two tails between MetS and no MetS subjects: $p<0.001$

Table 2 Iron nutrition and oxidative stress parameters in subjects with metabolic syndrome (MetS) or without MetS (No MetS)

Variable	Men		*p* value[a]	Women		*p* value[a]
	No MetS	MetS		No MetS	MetS	
Hb (g/L)	152.2±10.3	145.2±13.2	<0.02	133.7±12.9	135.7±14.2	NS
Zn PP	50.7±17.1	64.0±22.5	<0.01	60.9±30.2	65.7±17.2	NS
Fe (μg/dL)	142.3±44.3	141.0±51.0	NS	108.8±43.1	122.9±54.4	<0.03
Ferritin (μg/L)[b]	55.4 (35.6–96.3)	72.4 (46.8–111.5)	<0.001	27.4 (12.6–59.5)	53.9 (34.1–84.8)	<0.001
TfR (μg/mL)	5.8±1.0	4.8±2.1	<0.03	7.2±2.9	6.0 ±1.8	<0.004
TBI (mg/kg)	7.0±2.1	8.3±2.8	<0.04	4.1±2.8	6.3±2.3	<0.001
HO activity[b]	0.25 (0.09–0.75)	0.79 (0.34–1.85)	<0.001	0.21 (0.07–0.64)	0.96 (0.41–2.23)	<0.001
oxLDL (U/L)[b]	41.6 (24.4–71.2)	65.4 (40.9–101.9)	<0.002	45.6 (27.7–75.0)	62.4 (41.9–93.0)	<0.001
TBARS (nmol/mL)[b]	1.27 (0.93–1.73)	1.65 (1.26–2.17)	<0.002	1.33 (1.08–1.64)	1.65 (1.28–2.12)	<0.001

Values are presented as mean±SD

Hb hemoglobin, *Zn PP* Zn protoporfitina (in micrograms red blood cells/deciliter), *TfR* transferrin receptor, *TBI* total body iron, *HO activity* heme oxygenase enzymatic activity (in nanomoles bilirubin/milligrams protein/hour), *oxLDL* oxidized LDL, *TBARS* thiobarbituric acid reactive substances

[a] *p* values represent differences between no MetS and MetS by sex

[b] Values are expressed as geometric mean and range

Sample distribution among the quartiles of baseline ferritin, and logistic regression analyses are shown in Table 3. Compared to the lowest quartile of ferritin, there was a threefold higher risk to develop MetS in the highest quartile of ferritin in analyses adjusted for sex, BMI, and hsCRP ($p<0.0001$). With respect to the TBARS, these species raised in 21-fold the risk of developing MetS (Table 3) in analyses adjusted by sex, BMI, and hsCRP (OR=21.18; CI=3.75–119.42; $p=0.001$).

Individuals with MetS (men or women) showed higher levels in iron nutrition parameters than no MetS subjects. There were only 2/48 women in the no MetS group with Fe deficiency anemia. In both, men and women with MetS showed low levels of transferrin receptor and high concentrations of serum ferritin and total body iron (Table 2).

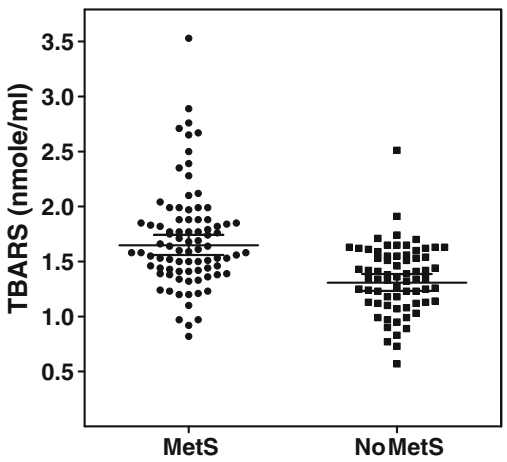

Fig. 2 TBARS levels in metabolic syndrome and controls subjects. Graph is represented as a geometric mean plus 95 % CI. There were no statistical differences between men and women. Unpaired *t* test, two tails between MetS and no MetS subjects: $p<0.001$

Discussion

Recent evidence had been shown in regard to the association between oxidative stress and the development of metabolic syndrome [15, 29]. The direct measurement of oxidative species is difficult because they have a very short life (just a few seconds). Therefore, it is more useful to measure the damage produced by these species over biomolecules, including lipids as main oxidation targets. The main finding in the present study was that the elevated plasma concentration of oxidative stress parameters such as oxLDL, TBARS, and Fe storage were associated to metabolic syndrome. The HOMA-IR index was higher in MetS than in no MetS; also, insulin resistance, overweight, and obesity were characteristic in MetS, which was accompanied by a low response to insulin. In this condition, the pancreas releases more insulin to improve glucose uptake, but blood glucose remains high since insulin resistance has been developed. Insulin resistance and high-fast glucose levels are predictors of type 2 diabetes mellitus [30], and obesity is a risk factor that is strongly associated to oxidative stress with a high prevalence in both women and men [31].

Levels of oxLDL were highly associated to triglycerides and waist circumference and were higher in men and women with MetS than without MetS. Covas et al. [32] found that circulating oxLDL was related to waist circumference and to different body mass index categories. On the other

Table 3 Logistic regression analysis of the association of ferritin and MetS

	Ferritin group				*p* value
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Ferritin (μg/L)					
Men	<28.65	28.65–56.95	56.95–77.80	77.80–137.20	
Women	<45.80	48.50–60.90	60.90–81.90	81.90–112.10	
OR (95 % CI) adjusted for:					
Sex, BMI	1	0.51 (0.06–4.34)	1.12 (0.11–10.84)	3.03 (0.005–1.19)	0.0001
Sex, BMI, hsCRP	1	0.54 (0.06–4.63)	0.99 (0.10–9.41)	3.36 (1.14–4.20)	0.0001

hand, an increased amount of abdominal fat (defined by waist circumference) was associated to oxidative stress biomarkers and inflammation, such as oxLDL, hsCRP, uric acid [33], and pro-insulin [34] among others, which are known risk factors of atherosclerosis development. OxLDL represents the accumulation of LDL modified by a milieu under oxidative stress [35], and Holvoet et al. [15] found that oxLDL showed an association to carotid plaque and coronary artery calcium.

Epidemiological studies have shown that high levels of ferritin are predictors of type 2 diabetes mellitus, and it is also thought that ferritin can play a role in MetS development [36, 37]. In this study, we found that the highest level of ferritin was associated in threefold to the development of MetS. In a case–control study nested in a Finnish cohort, men with high iron stores, as measured by the ratio of transferrin receptor to ferritin, had 2.4-fold elevated risk of incident diabetes compared with those in the lowest quintile [38]. In a recent investigation within the Data from Epidemiological Study on the Insulin Resistance Syndrome cohort (469 men and 475 French women), relatively high levels of both ferritin and transferrin were not only positively associated to the incidence of MetS at baseline but were also positively associated to the incidence of MetS at the end of the 6-year follow-up period [39].

Since MetS and obesity are characterized by chronic mild inflammation, it is difficult to elucidate if high levels of ferritin are due to iron overload or as a consequence of the inflammatory state. For this reason, we used TfR determination as an iron biomarker (which is not influenced by inflammation). Results showed that subjects with MetS had higher levels of iron storage than no MetS. Iron may amplify the inflammation through the formation of free radicals that trigger oxidation of proteins and DNA and may also unleash lipoperoxidation, especially in adipose tissue, inducing insulin resistance not only in adipocytes but also in muscle and hepatocytes [16]. We also showed that HO activity was increased in individuals with MetS, which demonstrates that these subjects are under metabolic stress, since HO is a stress-responsive enzyme and is induced by a great number of agonistic stimuli. The main biological function of HO is to degrade heme, generating biologically active molecules such as CO, Fe^{+2}, and biliverdin [40]. The end products of heme degradation are potentially antioxidant and anti-inflammatory [41].

The oxidation of LDL is a marker of oxidative stress specific to LDL particles. In this study, we found that individuals with MetS had elevated levels of oxLDL compared to subjects without MetS. [15] In the observational CARDIA study conducted by Holvoet et al. (The Coronary Artery Risk Development in Young Adults), it was found that oxidized LDL was associated to incident metabolic syndrome and with obesity. This association could be explained by the occurrence of small dense LDL that is more prone to oxidation. Another possible explanation is that the adipose tissue, which is elevated in obese subjects, contributes to the oxidation of LDL by two biochemical actions: (1) elevated adipose tissue may increase arachinodtae-5-lipooxygenase production, which catalyzes LDL oxidation and (2) increased adipose tissue may decrease the production of superoxide dismutase, which prevents LDL oxidation [37].

There is increasing evidence indicating that oxidative stress and inflammatory processes actively participate in the damage of the β pancreatic cell and in the development of type 2 diabetes and metabolic syndrome [30, 42]. Based on such data, we suggest that individuals with MetS and with altered levels of oxidative stress parameters could have an increased risk to develop type 2 diabetes in their early stages of life.

According to Rolo and Palmeira [43], type 2 diabetes is typically accompanied by an increased production of free radicals and/or impaired antioxidant defense capabilities, indicating a central contribution for reactive oxygen species in the onset, progression, and pathological consequences of diabetes. Increased oxidative stress, measured by the lipid peroxidation index and by protein oxidation are directly associated to an increase in different types of diabetes. Also, an association between oxLDL and hyperglycemia was described, where a decreased insulin signaling and glucose uptake was observed. Moreover, increased levels of oxLDL may cause islet β-cells death in the pancreas [15, 44].

In summary, we observed that elevated plasma concentrations of oxidative stress parameters such us oxLDL, TBARS, ferritin, and iron were associated to metabolic syndrome, and since oxidative stress has been shown to be a risk factor for tissue damage, and therefore for developing type 2 diabetes, we posited that the studying of the oxidative status is crucial in order to prevent type 2 diabetes development and its complications because it is installed long before the disease actually appears.

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Conflict of Interest The authors declare that they have no conflict of interests.

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