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## Original Research Article

# Serum ferritin levels may have a pro-atherosclerotic role in coronary artery disease patients with sleep disordered breathing



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CAD, coronary artery disease

SDB, sleep disordered breathing

AHI, apnea-hypopnea index

CRP, high-sensitivity C-reactive protein

OSA, obstructive sleep apnea

NOS, nitric oxide synthase

## ABSTRACT

Elevated ferritin levels may lead to oxidative stress, and are associated with coronary artery disease (CAD). Sleep disordered breathing (SDB) is frequently present in atherosclerosis patients, and causes endothelial dysfunction leading to atherosclerotic plaque progression. Hypoxic conditions, such as SDB, may upregulate ferritin. The aim of this study was to evaluate ferritin levels in CAD patients and to correlate ferritin levels with parameters related to CAD progression, including SDB. We studied 27 patients with CAD (defined as >30% coronary narrowing) and 29 controls. We found that ferritin was increased in CAD patients, and was positively correlated with the apnea-hypopnea index (AHI), age, C-reactive protein (CRP), transferrin, hemoglobin, and testosterone levels, and was negatively correlated with O<sub>2</sub> saturation. Nitrites and nitrates, an indirect measure of nitric oxide (\*NO) concentration, were lower in CAD patients, and were negatively correlated with ferritin. The increase in ferritin may be related to oxidative stress, suggesting a possible pro-atherosclerotic role of increased ferritin in CAD patients with SDB.

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eNOS, endothelial NOS  
GSH, reduced glutathione  
GSSG, oxidized glutathione  
GsT, glutathione S-transferase  
BMI, body mass index

## Introduction

Coronary artery disease (CAD) is characterized by artery plaque formation, and is considered an oxidative stress-associated and inflammation-associated disorder (Bradley and Floras, 2009; Celen and Peker, 2010; Klein et al., 2010). Morbidity and mortality due to CAD are often associated with sleep disordered breathing (SDB) (Mooe et al., 1996; Luthje and Andreas, 2008; Bradley and Floras, 2009). Obstructive sleep apnea (OSA) is an important form of SDB, a group of disorders characterized by abnormalities of respiratory pattern during sleep. OSA is characterized by repetitive upper airway obstruction, which leads to intermittent hypoxic sleep conditions (Bradley and Floras, 2009). Intermittent hypoxia induces inflammatory pathways and oxidative stress, causing functional impairment of the vascular endothelium (Bradley and Floras, 2009). *In vivo* and *in vitro* experiments have provided evidence of free radical formation in hypoxic conditions (Gozal and Kheirandish-Gozal, 2008).

Ferritin is a globular protein complex comprising 24 subunits. It is an iron depository structure, and is an iron source for iron-dependent proteins (Sheftel et al., 2012). The serum ferritin level correlates with total body iron stores (You and Wang, 2005). The role of ferritin in oxidative stress is controversial. Ferritin has been considered to be either an antioxidant or a pro-oxidant protein complex. Ferritin may reduce  $\text{Fe}^{2+}$  to less reactive  $\text{Fe}^{3+}$  utilizing oxygen ( $\text{O}_2$ ) or hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) avoiding oxidative stress by the Fenton reaction (Chepelev and Willmore, 2011). However, iron can be released from ferritin during oxidative stress. Superoxide radical ( $\text{O}_2^{\bullet-}$ ) mediates the release of  $\text{Fe}^{2+}$  from ferritin and is rapidly oxidized to  $\text{Fe}^{3+}$ , contributing to free radical generation (Paul, 2000).

Elevated serum ferritin levels are associated with high CAD risk (You and Wang, 2005; Ahluwalia et al., 2010; Torti and Torti, 2002), and patients with elevated serum ferritin present increased risk of myocardial infarction (Perez-Lopez et al., 2010). High serum levels of ferritin increase risk for cardiovascular disease in both men and women (Perez-Lopez et al., 2010). The development of atherosclerosis may be related to elevated iron stores, which may increase free radical generation (Perez-Lopez et al., 2010). Moreover, hypoxia-related inflammation may participate in ferritin regulation (You and Wang, 2005; Torti and Torti, 2002). Thus, the role of ferritin in the development of atherosclerosis is unclear and needs to be investigated.

Decreased nitric oxide ( $\text{*NO}$ ) availability is associated with OSA and CAD (Jelic et al., 2008).  $\text{*NO}$  is a potent vasodilator and is a product of nitric oxide synthase (NOS). Endothelial NOS (eNOS) transiently produces ( $\text{O}_2^{\bullet-}$ ) in hypoxic conditions, reducing  $\text{*NO}$  availability and increasing oxidative damage

(Singh and Jialal, 2006). OSA causes a reduction in  $\text{*NO}$ -dependent vasodilatation and may have vascular consequences (Gozal and Kheirandish-Gozal, 2008; Khayat et al., 2009).

CAD may be related to endocrine alterations (Perez-Lopez et al., 2010). Endogenous and exogenous steroid hormones are immunomodulatory, and changes in their levels can modify inflammatory and oxidative stress pathways (Villablanca et al., 2010). Frequently, androgens are considered pro-atherogenic, while estrogens are considered to be anti-atherogenic hormones (Vitale et al., 2010). Men are more susceptible to atherosclerosis than women; however, the role of testosterone in vascular impairment is poorly understood (Vitale et al., 2010).

The aim of this study was to evaluate ferritin levels in CAD patients, and to correlate ferritin levels with parameters related to CAD progression, including SDB. In this logical extension of our previous study (Klein et al., 2010; Hackenhaar et al., 2012), we postulated that increased ferritin levels are related to CAD patients with SDB. Moreover, increased ferritin may be related to oxidative stress and  $\text{*NO}$  decrease in CAD patients, further implicating ferritin as a participant in the pathophysiology of CAD.

## Materials and methods

### Patients and CAD study

The project was approved by ethics committee of the Hospital de Clínicas de Porto Alegre, and all participants signed an informed consent form. A cross-sectional study was conducted by screening patients referred for diagnostic or therapeutic coronary angiography. The exclusion criteria were: age less than 35 or greater than 65 years; smoking in the previous 6 months; clinical diagnosis, dietary, or pharmacological treatment for diabetes mellitus; angina in the previous week; use of anxiolytic medication; treatment for chronic pulmonary disease; use of vitamin supplement; body mass index (BMI)  $\geq 40 \text{ kg/m}^2$ ; any physical, psychological, or social issue that would interfere with conducting the home polysomnographic test; and previous coronary intervention (surgical or percutaneous myocardial revascularization). Hypertension, past history of smoking, and medication use were not criteria for exclusion, and the prevalence of these conditions was similar in both groups. All patients were assessed by coronary angiography using the same equipment (SIEMENS Axiom, Germany) and projections, with the table and image intensifier kept at constant height. A 7 in. magnification was used for all images. Image quantification was carried out by the same investigator, who was blinded to clinical and biochemical variables of the patients. Quantitative

coronary angiography was performed using an automated edge detection system (Siemens, Germany). A guiding catheter was used for calibration. The following variables were analyzed: minimal lumen diameter (mm); reference vessel diameter (mm); lesion extension (mm); and degree of stenosis (%). Patients with luminal narrowing of 30% or higher were considered to have CAD. Patients without narrowing or narrowing less than 30% were included in the control group. Narrowing of 30% is considered a moderate stenosis. A normal artery, presenting no obstruction, is considered narrowing of 0%. Total artery obstruction is considered narrowing of 100%. Previous studies have shown the relevance of 30% narrowing in the study of CAD (Warlow, 1991; Slattery, 1996; Leclerc et al., 1999) and even 20–25% narrowing, considered light stenosis, are relevant in CAD studies (Zhao et al., 2012). Narrowing of 30% was used because of the importance of the diagnosis of atherosclerosis in this stage, whereas the stroke occurrence and life expectancy reduction are significant. However, surgery procedure is still controversial in patients presenting moderate stenosis stage (Slattery, 1996).

### Sleep study

In the polysomnographic study, a level III portable monitor (SomnoCheck, Weinmann, Germany) was used at home by the patients. A nasal cannula connected to a pressure transducer was employed to quantify air flow and snoring. Inspiratory effort, pulse oximetry, heart rate, and sleep position were also verified. The polysomnography respiratory analyses were made by a sleep specialist who was blinded to clinical and biochemical variables. Apnea was defined as airflow reduction to 10% or less of the baseline value persisting for 10 s or more; hypopnea was defined as airflow reduction of 50% or more, associated with a reduction of 3% or more in oxygen saturation (de Oliveira et al., 2009). The AHI was defined as apnea/hypopnea episodes per hour of total sleep time, calculated by dividing the sum of total apneas and hypopneas by the hours of recorded polysomnography.

### Laboratory measurements

Approximately 20 mL arterial blood was collected from each patient from femoral artery puncture for catheterization. Blood was collected in vials containing coagulation inhibitor, EDTA and citrate. Immediately after collection, the samples were refrigerated to 0 °C, centrifuged for 10 min at 0 °C, aliquoted, and stored at –80 °C. Hemolysates were prepared by lysing red blood cells with 2% ethanol (ratio 1:10) followed by centrifugation to obtain crude extracts. High-sensitivity C-reactive protein, total proteins, ferritin, transferrin, hemoglobin, and erythrocytes were quantified in routine clinical laboratory analysis; additional tests were performed at the research laboratory.

### Steroid hormones and assay

Steroid hormones were quantified in routine clinical laboratory analysis. Total testosterone was quantified by radioimmunoassay (Roche Diagnostics), estradiol was quantified by electrochemiluminescence immunoassay (Modular E-170,

Roche), and progesterone was quantified by chemiluminescence immunoassay (Immulite 1000).

### GSH assay

GSH is the most abundant intracellular antioxidant, and its deficient levels in erythrocytes leads to oxidative stress (Ellison and Richie, 2012). Total glutathione content (the sum of reduced glutathione (GSH) and oxidized glutathione (GSSG)) was measured in hemolysates (Kondo and Awada, 2000). Glutathione reductase (GR) was used to reduce GSSG to 2GSH. GSH was detected through its reaction with 5,5-dithiobis (acid 2-nitrobenzoic) (DTNB), forming the 5-thio nitrobenzoate chromophore, detected at 412 nm (Monostori et al., 2009). GSH concentration was obtained through the subtraction of GSSG from total glutathione content. Briefly, 50  $\mu$ L of 2 M perchloric acid and 4 mM EDTA were added to 50  $\mu$ L hemolysate. After vortexing and centrifugation, 10  $\mu$ L of 0.25 N-ethylmaleimide was added to 90  $\mu$ L of supernatant (GSSG assay only). After new vortexing and centrifugation, 77  $\mu$ L of 2 M KOH was added, reaching pH 6. Samples were vortexed and centrifuged to complete preparation. Samples were maintained in ice. A standard curve was prepared using 5  $\mu$ M, 10  $\mu$ M, 25  $\mu$ M, and 50  $\mu$ M GSH in 50 mM phosphate buffer, 5 mM EDTA. The microplate assay was performed adding 174  $\mu$ L of 50 mM phosphate buffer, 17  $\mu$ L of 4 mM NADPH (in 0.5% NaHCO<sub>3</sub>), 7  $\mu$ L of 6 U/mL GR (in 10 mM phosphate buffer) and 35  $\mu$ L of sample or standard. Microplates were incubated at 37 °C for 5 min and 18  $\mu$ L of 4 mg/mL DTNB (in 100 mM phosphate buffer) was added. Absorbance at 412 nm was obtained after 2 min incubation at 37 °C.

### GST assay

The enzyme glutathione S-transferase (GST) participates in oxidative stress detoxification through the conjugation of the thiol glutathione (GSH) to various substrates, and its polymorphisms and decreased activity may be related to atherosclerosis (Maciel et al., 2009; Turkanoglu et al., 2010). The glutathione-S-transferase antioxidant assay is based on formation of S-(2,4-dinitrophenyl)-glutathione by GST enzymatic activity through 1-chloro-2,4-dinitrobenzene (CDNB) and GSH conjugation (Tsuchida, 2000). Briefly, hemolysates were centrifuged and maintained on ice. A spectrophotometer cuvette was filled with 500  $\mu$ L 0.2 mM potassium phosphate (pH 6.5), 100  $\mu$ L 10 mM GSH, 300  $\mu$ L sample, and 100  $\mu$ L 10 mM CDNB (in 2 ethanol:3 water, at 37 °C). All assays were performed in triplicate. Absorbance was determined at 340 nm. The S-(2,4-dinitrophenyl)-glutathione molar extinction coefficient at 340 nm ( $\epsilon = 9600 \text{ M}^{-1} \text{ cm}^{-1}$ ) was used to calculate GST activity.

### Nitrites/nitrates assay

The Griess reaction, which measure nitrites and nitrates in the sample, was employed to determine circulating •NO concentration (Bryan and Grisham, 2007). Hemolysates were centrifuged and maintained on ice. A standard curve was made using 1 mM sodium nitrite (NaNO<sub>2</sub>) diluted in 100 mM phosphate buffer to standard curve concentrations of

2.5 nmol/mL, 5 nmol/mL, 15 nmol/mL and 30 nmol/mL. In the microplate (all wells in triplicate) 100  $\mu$ L of sample or standards were added, followed by 50  $\mu$ L of Griess reagent (0.02 g of naphthylenediamine dichloride, 0.2 g of sulfanilamide, 500  $\mu$ L of orthophosphoric acid and 10 mL of MilliQ water). The microplate was protected from light during a 10 min incubation. Absorbance was read at 543 nm (Grisham et al., 1996).

### Statistical analysis

Categorical variables are presented as absolute values and were analyzed by chi-square test. The Kolmogorov–Smirnov test of normality was employed to verify distribution of variables. Variables with normal distribution are presented as mean  $\pm$  standard error (SE); means were compared by student's t-test. Variables without normal distribution are presented as median (minimum–maximum); medians were compared by Mann–Whitney U test. Spearman coefficient was employed to test correlation between variables without normal distribution, and Pearson coefficient was used to test correlation between variables with normal distribution. Data were analyzed at the significance level  $2\alpha = 0.05$ .

## Results

### Clinical and anthropometric data in CAD groups

Table 1 presents anthropometric and clinical data of analyzed patients. Gender and body mass index (BMI) did not differ between groups (Table 1). CAD patients were significantly older than controls (Table 1). AHI was significantly higher in the CAD group, and lowest O<sub>2</sub> saturation was decreased in the CAD group (Table 1).

### Biochemical data in CAD groups

Table 2 presents biochemical data from the analyzed patients.

The inflammatory marker, high-sensitivity C-reactive protein (CRP), as well as the analyzed steroid hormones testosterone, estradiol and progesterone were not significantly different between groups (Table 2). Hematocrit and hemoglobin concentration were significantly increased in CAD patients, while erythrocytes presented no difference between groups (Table 2). CAD patients presented significantly increased ferritin levels, however there was no difference in transferrin or free iron concentrations (Table 2). GsT activity,

**Table 1 – Patient clinical and anthropometric data.**

	Control group (n = 29)	CAD group (n = 27)
Gender (male)	12 (41%)	19 (70%)
Age (years)	51.62 $\pm$ 1.28	56.96 $\pm$ 1.11 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	27.30 $\pm$ 0.74	27.79 $\pm$ 0.74
AHI (events/h)	7 (1–48)	17 (1–56) <sup>a</sup>
Lowest O <sub>2</sub> saturation (%)	87.5 (63–93)	85.0 (77–90) <sup>a</sup>
Number of coronaries with >30% stenosis	0 (0–0)	1.95 ( $\pm$ 0.83)
Number of coronary segments with >30% stenosis	0 (0–0)	2.3 ( $\pm$ 1.42)

Categorical data are presented as n (%). Variables with normal distribution are presented as mean  $\pm$  SE. Variables without normal distribution are presented as median (range).

<sup>a</sup> Statistically significant as compared with control group. BMI, body mass index (weight divided by the square of height, kg/m<sup>2</sup>); AHI, apnea-hypopnea index (apnea-hypopnea/hour of sleep).

**Table 2 – Patient biochemical data.**

	Control group (n = 29)	CAD group (n = 27)
CRP (mg/L)	1.59 (0.19–21.50)	2.00 (0.16–18.40)
Erythrocytes (million erythrocytes/mL)	4.47 $\pm$ 0.06	4.65 $\pm$ 0.07
Hemoglobin (g/dL)	13.04 $\pm$ 0.24	13.77 $\pm$ 0.25 <sup>a</sup>
Ferritin (ng/mL)	144.00 (8.3–720.3)	302.35 (45.50–685.80) <sup>a</sup>
Transferrin (mg/mL)	255 (183–371)	236 (160–296)
Total testosterone (ng/mL)	0.63 (0.11–8.06)	3.55 (0.11–6.93)
Estradiol (pg/mL)	18.10 (5.00–411.60)	25.00 (5.00–624.70)
Progesterone (ng/mL)	0.29 (0.15–6.11)	0.26 (0.15–25.01)
Total glutathione ( $\mu$ mol/g Hb)	438.44 (52.63–5564.45)	440.30 (57.85–3854.17)
GSH ( $\mu$ mol/g Hb)	329.67 (34.44–5445.85)	403.04 (85.30–3654.09)
GSSG ( $\mu$ mol/g Hb)	78.62 (8.86–558.50)	86.65 (19.37–880.63)
GsT (U/mL)	0.054 $\pm$ 0.004	0.047 $\pm$ 0.003
Nitrites/nitrates (nmol/mg Hb)	0.76 (0.39–1.11)	0.57 (0.26–1.22) <sup>a</sup>

Variables with normal distribution are presented as mean  $\pm$  SE. Variables without normal distribution are presented as median (range).

<sup>a</sup> Statistically significant as compared with control group. Total glutathione, GSH + GSSG erythrocyte content; GSH, reduced glutathione; GSSG, oxidized glutathione.

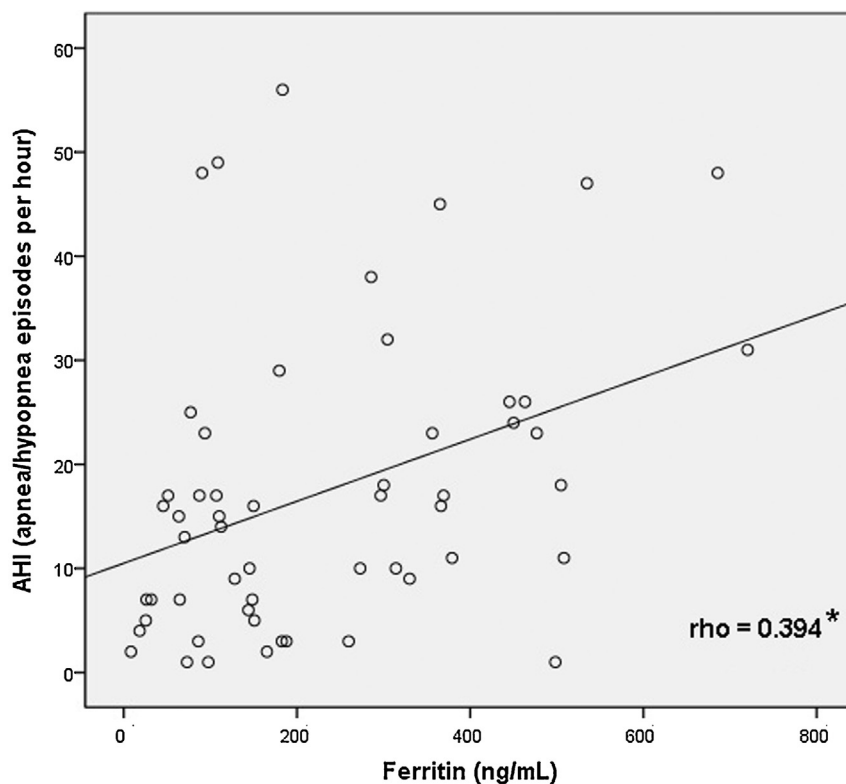


Fig. 1 – Ferritin vs. apnea-hypopnea index (AHI). Spearman's correlation analysis of apnea-hypopnea index (AHI) and serum ferritin concentration. \*Statistically significant. Positive rho value: positive correlation between variables.

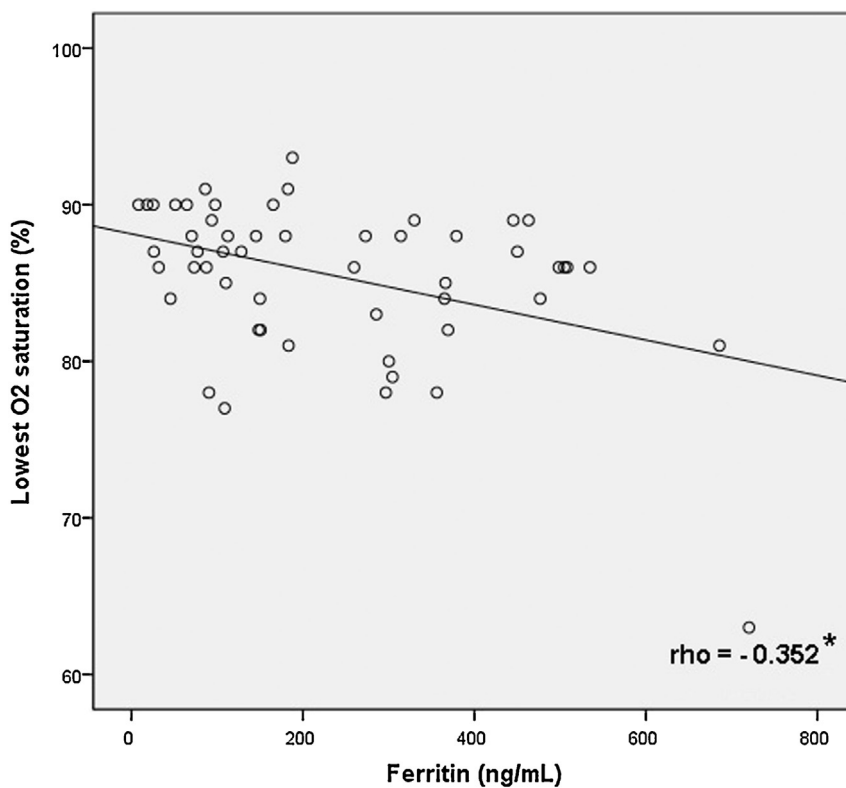


Fig. 2 – Ferritin vs. lowest O<sub>2</sub> saturation (%). Spearman's correlation analysis of lowest O<sub>2</sub> saturation (%) and serum ferritin concentration. \*Statistically significant. Negative rho value: negative correlation between variables.

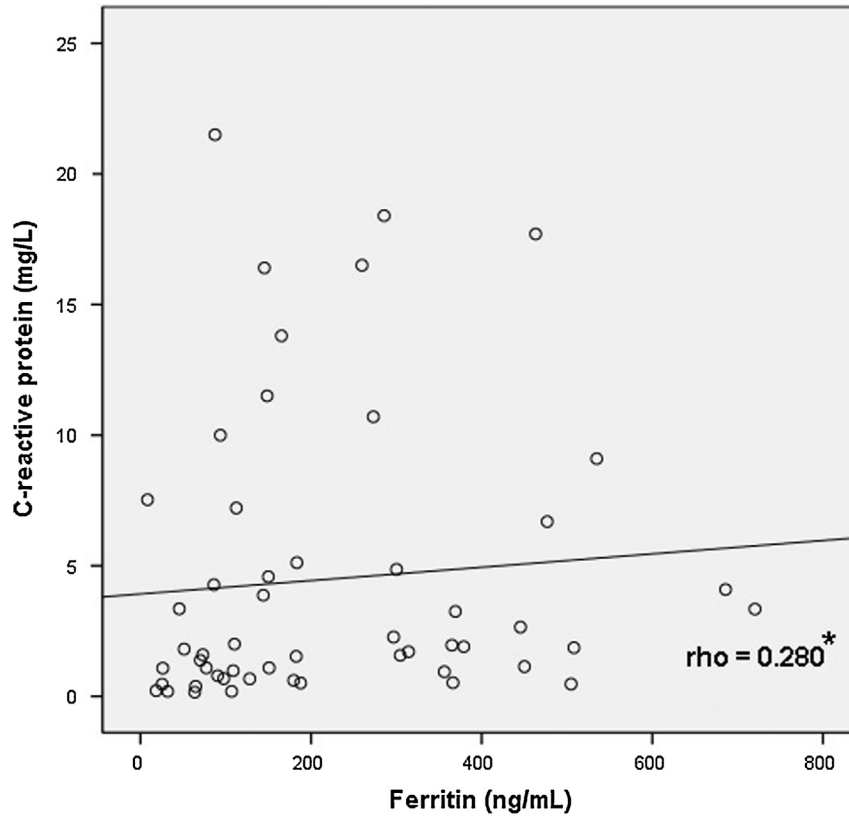


Fig. 3 – Ferritin vs. C-reactive protein. Spearman's correlation analysis of serum C-reactive protein concentration and serum ferritin concentration. \*Statistically significant. Positive rho value: positive correlation between variables.

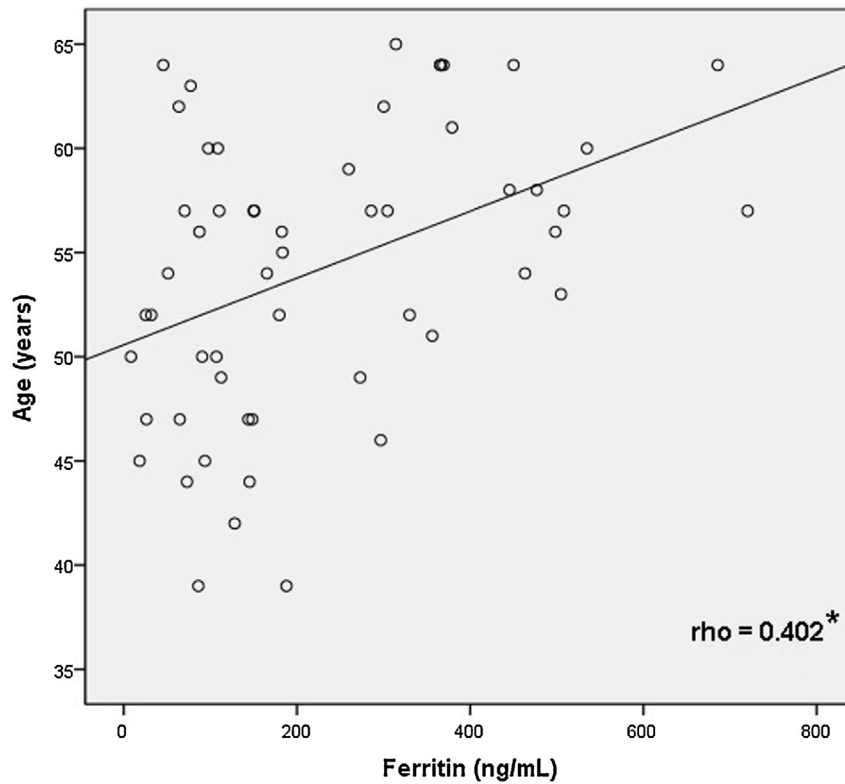


Fig. 4 – Ferritin vs. age. Spearman's correlation analysis of age (years) and serum ferritin concentration. \*Statistically significant. Positive rho value: positive correlation between variables.

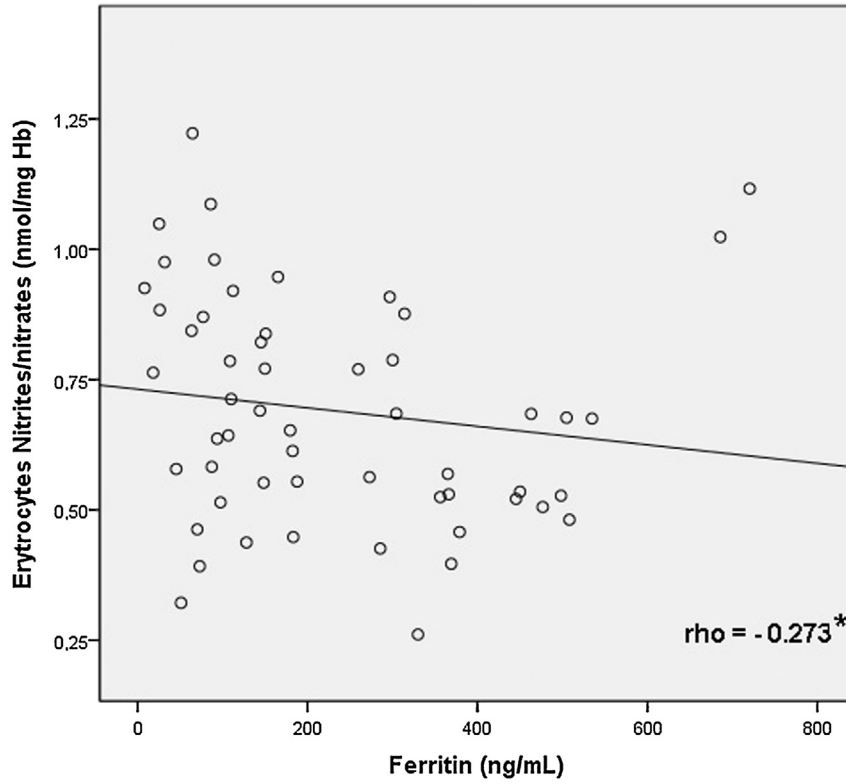


Fig. 5 – Ferritin vs. nitrites/nitrates. Spearman's correlation analysis of erythrocytes nitrites/nitrates concentration and serum ferritin concentration. \*Statistically significant. Negative rho value: negative correlation between variables.

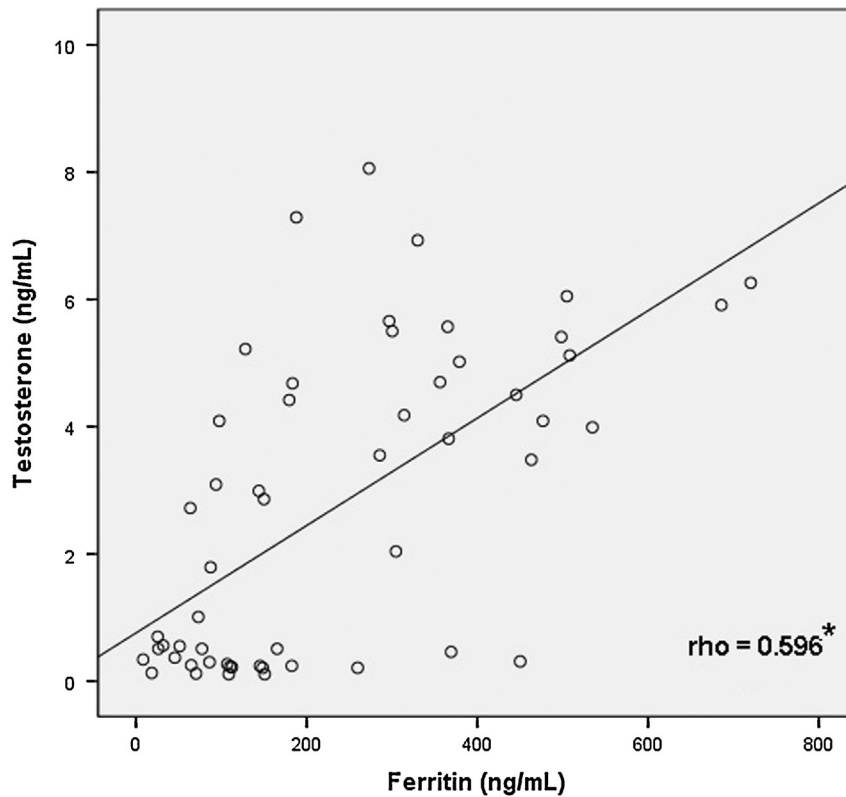


Fig. 6 – Ferritin vs. testosterone. Spearman's correlation analysis of serum testosterone concentration and serum ferritin concentration. \*Statistically significant. Positive rho value: positive correlation between variables.

total glutathione (GSH + GSSG), GSH, and GSSG levels did not differ between controls and CAD patients (Table 2). Nitrites and nitrates were significantly lower in CAD patients than controls (Table 2).

### Correlation analysis

In Spearman's correlation analysis, ferritin correlated with AHI ( $\rho = 0.394$ ,  $P = 0.003$ , Fig. 1), lowest O<sub>2</sub> saturation ( $\rho = -0.352$ ,  $P = 0.010$ , Fig. 2), CRP ( $\rho = 0.280$ ,  $P = 0.042$ , Fig. 3), age ( $\rho = 0.407$ ,  $P = 0.002$ , Fig. 4), nitrites/nitrates ( $\rho = -0.273$ ,  $P = 0.044$ , Fig. 5), transferrin ( $\rho = -0.279$ ,  $P = 0.039$ ) and hemoglobin ( $\rho = 0.500$ ,  $P = 1.003 \times 10^{-4}$ ), however no correlation was found between AHI and CRP ( $\rho = 0.104$ ,  $P = 0.449$ ) or AHI and nitrites/nitrates ( $\rho = -0.066$ ,  $P = 0.624$ ). Spearman's analysis of total testosterone levels showed correlation with ferritin ( $\rho = 0.596$ ,  $P = 2 \times 10^{-6}$ , Fig. 6), transferrin ( $\rho = -0.344$ ,  $P = 0.010$ ), hemoglobin ( $\rho = 0.579$ ,  $P = 2.882 \times 10^{-6}$ ), erythrocytes ( $\rho = 0.544$ ,  $P = 1.446 \times 10^{-5}$ ) and hematocrit ( $\rho = 0.544$ ,  $P = 5.0568 \times 10^{-6}$ ), however no correlation was found between testosterone and available iron ( $\rho = -0.233$ ,  $P = 0.086$ ).

## Discussion

CAD patients exhibited increased ferritin, which was positively correlated with the apnea-hypopnea index (AHI) and was negatively correlated with O<sub>2</sub> saturation and \*NO levels. CAD patients exhibited decreased \*NO levels. The increase in ferritin may be related to oxidative stress, suggesting a possible pro-atherosclerotic role in CAD patients with SDB.

Serum ferritin levels are related to cardiovascular diseases in both men and women (Berge et al., 1994; Olesnevich et al., 2012). In male patients, a serum ferritin level of 200 ng/mL is associated with a 2.2-fold increase in the risk for myocardial infarction (Olesnevich et al., 2012), which is consistent with our results showing that the median serum ferritin level in CAD patients was 302 ng/mL, significantly higher than the control group (median 144 ng/mL) (Table 2). Ferritin serum concentration is also related to coronary artery calcium levels in male patients, an early biomarker of atherosclerosis (Sung et al., 2012). The majority of CAD researchers consider ferritin to be an antioxidant, and consequently, anti-atherosclerotic because of its role in iron sequestration (You and Wang, 2005). However, our results are consistent with a pro-oxidant and pro-atherosclerotic role of ferritin. As discussed by Halliwell and Gutteridge (2007), ferritin is more likely to cause, rather than ameliorate, oxidative stress (Halliwell and Gutteridge, 2007), possibly by participating in LDL oxidation in atherosclerotic lesions (Ahluwalia et al., 2010). We suggest ferritin participates in CAD progression, contributing to oxidative stress. Hypoxic conditions, such as SDB, produce superoxide radical through changes in the activity of endothelial NOS (eNOS) (Singh and Jialal, 2006) and xanthine dehydrogenase (XDH) (Cuzzocrea et al., 2001). Since iron is released from ferritin by superoxide radical (O<sub>2</sub><sup>•-</sup>) (Paul, 2000), we suggest this may be a pro-oxidant mechanism responsible for the participation of ferritin in the progression of atherosclerosis. Moreover, the release of iron by superoxide radical occurs to ferritin-bound iron but not to transferrin-bound iron

(Paul, 2000). These findings are consistent with our results, where increased ferritin levels are related to CAD (Table 2) and SDB (Figs. 1 and 2) and not to transferrin (Table 2).

The pro-atherosclerotic role of ferritin is reaffirmed by the significant, albeit relatively weak, positive correlation found between serum ferritin levels and the inflammatory biomarker CRP (Fig. 2). Moreover, ferritin correlated negatively with \*NO levels (Fig. 5). CRP, which is an acute phase protein usually expressed in the liver during inflammation, is expressed in smooth muscle cells from atherosclerotic arteries (Kampoli et al., 2012). The majority of patients (including controls) in this study presented higher CRP levels compared to clinical reference levels. Consequently, no relationship between CRP levels and CAD (Table 2) or AHI (data not shown) was found. A related CAD study did not identify an association between ferritin levels and CRP (Grisham et al., 1996).

We observed a positive correlation between ferritin levels and AHI (Fig. 1), and a negative correlation between ferritin levels and lowest O<sub>2</sub> saturation (Fig. 2), which corroborated a previous report of ferritin upregulation by hypoxia (Torti and Torti, 2002). Moreover, the positive correlation with AHI suggests that ferritin levels are related to SDB in CAD patients.

Exogenous testosterone increases erythropoiesis and hemoglobin levels; however, associated differences in transferrin receptor, erythropoietin, or ferritin levels have not generally been reported (Coviello et al., 2008; Rushton and Barth, 2010). In our study, testosterone levels were positively correlated with ferritin levels (Fig. 6), erythrocytes numbers, and hemoglobin levels, and were negatively correlated with transferrin levels (see section "Correlation analysis"). However, no significant correlation was found between testosterone and available iron (see section "Correlation analysis"). It is important to highlight that healthy men present higher baseline levels of ferritin compared to women (Perez-Lopez et al., 2010), and this may be the main cause of the correlation found between testosterone and ferritin, as well as the correlation with erythrocyte numbers and hemoglobin concentration. Ferritin serum levels are upregulated by heme (Chepelev and Willmore, 2011), and there is a well known relationship of ferritin with hemoglobin and hematocrit (Chepelev and Willmore, 2011; Sheftel et al., 2012), as was observed in our patients (see section "Correlation analysis").

Our findings (shown in Fig. 4) are consistent with the well-known relationship between ferritin levels and age (Berge et al., 1994).

\*NO inhibits plaque formation, arterial constriction, platelet aggregation, and macrophage adhesion and penetration (Vanhoutte, 2009). Reduced \*NO availability is associated with CAD and SDB (Jelic et al., 2008). The level of nitrites and nitrates, an indirect measure of \*NO, was significantly lower in our CAD patients (Table 2), indicating that \*NO decrease may be related to atherosclerosis progression. Oxidative stress generated by hypoxia in SDB alters \*NO-dependent vasodilatation (Atanasiu et al., 2007). In this regard, our patients exhibited a negative correlation between \*NO and ferritin (Fig. 5). However, nitrite and nitrate levels did not correlate with AHI in our study (see section "Correlation analysis"). Thus, further work will be necessary to clarify the role of ferritin in CAD progression.

There are important limitations regarding the patients included in this study. The control group in this study



comprised patients presenting with less than 30% luminal narrowing. The included patients and controls were all referred for diagnostic or therapeutic coronary angiography by a physician prior to participation in the study. All patients presented CRP levels that were higher than clinical reference levels for healthy people. The selection of controls was driven, in part, by the fact that healthy patients are not referred for coronary angiography, which is considered a moderate risk procedure. Another limitation was the absence of SDB groups, since AHI was measured as a continuous variable in all patients. Despite the fact that AHI was significantly lower in the control CAD group, not all controls were SDB free.

## Conclusions

The findings reported here provide evidence of the relevance of increased ferritin levels in CAD patients with SDB. Ferritin may cause oxidative stress in CAD patients, thereby participating in progression of atherosclerosis. \*NO decrease is correlated with ferritin levels and may be related to atherosclerosis progression in CAD patients with SDB.

## Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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