JOURNAL OF APPLIED BIOMEDICINE 13 (2015) 289–298

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/jab

Original Research Article

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

Fernanda S. Hackenhaar [a,b,c], *Denis Martinez* [c,d], *Cristini Klein* [a,b,c], *Tássia M. Medeiros* [a,b,c], *Paulo V.G. Alabarse* [a,b,c], *Marco V. Wainstein* [d], *Sandro C. Gonçalves* [d], *Mara S. Benfato* [a,b,c,*]

[a] *Departamento de Biofísica, Brazil*
[b] *Programa de Pós-Graduação em Biologia Celular e Molecular, Brazil*
[c] *Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[d] *Cardiology Unit, Hospital de Clinicas de Porto Alegre (HCPA), Brazil*

ARTICLE INFO

Article history:
Received 10 December 2014
Received in revised form
19 March 2015
Accepted 24 March 2015
Available online 7 April 2015

Keywords:
Atherosclerosis
Ferritin
Nitric oxide
Obstructive sleep apnea
Oxidative stress
Testosterone

Abbreviations:
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_2 saturation. Nitrites and nitrates, an indirect measure of nitric oxide ($^{\bullet}NO$) 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.

* *Corresponding author at*: Departamento de Biofísica, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Prédio 43422, Room 204, Porto Alegre, RS 91501-970, Brazil. Tel.: +55 51 33087603; fax: +55 51 33087003.
E-mail address: mara.benfato@ufrgs.br (M.S. Benfato).

1214-021X/$ – see front matter
http://dx.doi.org/10.1016/j.jab.2015.03.006

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 Fe^{2+} to less reactive Fe^{3+} utilizing oxygen (O_2) or hydrogen peroxide (H_2O_2) avoiding oxidative stress by the Fenton reaction (Chepelev and Willmore, 2011). However, iron can be released from ferritin during oxidative stress. Superoxide radical ($O_2^{\bullet -}$) mediates the release of Fe^{2+} from ferritin and is rapidly oxidized to 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 ($^{\bullet}NO$) availability is associated with OSA and CAD (Jelic et al., 2008). $^{\bullet}NO$ is a potent vasodilator and is a product of nitric oxide synthase (NOS). Endothelial NOS (eNOS) transiently produces ($O_2^{\bullet -}$) in hypoxic conditions, reducing $^{\bullet}NO$ availability and increasing oxidative damage (Singh and Jialal, 2006). OSA causes a reduction in $^{\bullet}NO$-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 $^{\bullet}NO$ 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) ≥ 40 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