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# The relationship between sleep apnea, metabolic dysfunction and inflammation: The gender influence

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ABSTRACT

Obstructive sleep apnea (OSA) has been associated with increased risk of cardiovascular morbidity and mortality. Although inflammatory markers may mediate this association, it is unknown the influence of gender in this mechanism. Thus, we aimed to evaluate the interaction effects between OSA and gender on metabolic and inflammatory profile in a population sample. This study is part of EPISONO cohort, in which 1042 participants underwent polysomnography, answered questionnaires, and had their blood collected for analysis of fasting glucose, total cholesterol and fractions, leptin, ghrelin, liver transaminases, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and C-reactive protein. The results showed that men with OSA had higher leptin levels, shorter sleep latency and lower N3 sleep stage compared to men control (CTRL). They also presented higher apnea index and number of central apneas compared to both CTRL men and OSA women. In women, OSA was related to longer REM sleep latency, higher apnea-hypopnea index (AHI) during REM sleep and increased TNF-α levels compared to CTRL women. A multivariate model showed that male gender, ghrelin and total cholesterol were negatively associated with TNF-α, while IL-6, triglycerides and hypopnea index were positively associated ($R^2 = 0.21$). Additionally, gender (men), body mass index, ghrelin, apnea index and smoking were positive predictors of leptin levels ($R^2 = 0.55$). Of note, postmenopause was associated with changes observed in both TNF-α and AHI during REM sleep in women with OSA. Taken together, our study suggests that OSA consequences may differ between genders and this could indicate a need for different OSA management in women according to their reproductive life's stage.



## 1. Introduction

Obstructive sleep apnea (OSA) is characterized by recurrent episodes of upper airway collapse during sleep, leading to intermittent hypoxia, hypercapnia, sleep fragmentation, and intrathoracic pressure swings (Epstein et al., 2009). Consequently, individuals with OSA present a higher risk for metabolic and cardiovascular diseases as well as neurocognitive deficits and endothelial dysfunction (McArdle et al., 2007; Bhattacharjee et al., 2012). There is evidence that the mechanisms behind this association are related to the increase in total cholesterol, low-density lipoprotein (LDL), triglycerides, and inflammatory markers such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6 and C reactive protein (CRP) (Gozal and Kheirandish-Gozal, 2008; Drager et al., 2010; Wang et al., 2015). However, there are also studies showing no direct relationship between OSA and systemic inflammation, favoring that obesity would be the true link between them (Sharma et al., 2008). In addition to the differences in methodological approaches used among the studies, another aspect that may also explain some of these disparities is the influence of gender.

Around the world, the prevalence of OSA varies from 2.0 to 26.1% in women and 4.0 to 49.7% in men (Young et al., 1993; Tufik et al., 2010; Heinzer et al., 2015). Several factors have been proposed to address these sex-differences, such as upper airway anatomy, fat distribution and testosterone levels (Ye et al., 2009; Andersen et al., 2011; Wittert, 2014). Although polysomnographic criteria for sleep scoring and its associated events are well established, the influence of gender on these parameters is not yet elucidated. It is known that in healthy population, men and women have physiological differences in sleep pattern. Generally, women have higher sleep efficiency, lower percentage of sleep stage 1 (N1) and 3 (N3), and longer REM sleep latency compared to men (Redline et al., 2004; Bixler et al., 2009; Wittert, 2014). In metabolic and inflammatory profile, healthy women tend to present

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higher concentrations of CRP, high-density lipoprotein (HDL), and total cholesterol as well as lower concentrations of IL-6, TNF-α and triglycerides in comparison to healthy men (Johnson et al., 2004; Cartier et al., 2009; Sugiyama and Agellon, 2012).

Considering the importance of sex differences, we hypothesized that OSA and gender would have interaction effects on physiological parameters due to possible sex differences on sleep parameters. Thus, the main objective of this study was to investigate the interaction effects between OSA and gender on metabolic and inflammatory profile. Secondly, we aimed to understand whether sleep parameters would be associated with the physiological consequences differentially modulated by OSA in men and women from a representative population of the city of São Paulo, Brazil.

## 2. Methods

### 2.1. Study design and sample selection

This was a cross-sectional study, whose data were derived from the epidemiological sleep study EPISONO, which was a large population-based survey conducted between July and December of 2007 in the city of São Paulo, Brazil. Briefly, a 3-stage cluster sampling technique was conducted to obtain a representative sample according to age (20–80 years), gender and socioeconomic status. A total of 1042 subjects were enrolled in this study. The full methodology was previously described by Santos-Silva and colleagues (2009). The protocol was approved by the Research Ethics Committee from Universidade Federal de São Paulo (CEP 0593/06).

### 2.2. Socioeconomic and clinical assessment

The socioeconomic class was evaluated by the questionnaire *Critério de Classificação Econômica Brasil* (CCEB, Associação Brasileira de Empresas de Pesquisa, 2003). Volunteers were also asked about the use of medications and medical history in the Pre-sleep questionnaire (Bittencourt et al., 2005). Smoking was considered as positive in case of tobacco use in the last 3 months. The body mass index (BMI) was obtained from the ratio between the body weight and the square of height. Trained personnel also evaluated systolic and diastolic blood pressure (SBP and DBP, respectively), circumference of neck, hips and waist. Reproductive life's stage was evaluated in all women as described before by Hachul et al. (2015).

### 2.3. Sleep assessment

All participants underwent a full-night polysomnography (PSG) in a sleep laboratory assessed by a polysomnographic technologist using a digital system (EMBLA® N7000, Embla Systems Inc., Broomfield, CO, USA). The exam was scheduled according to the volunteers' availability trying to respect their habitual sleep schedule. Physiological variables evaluated during PSG included: electroencephalogram (4 channels: C3-A2, C4-A1, O1-A2, O2-A1); electrooculogram (2 channels: EOG-Left-A2, EOG-Right-A1); surface electromyogram (4 channels: submentonian region, masseter region, anterior tibialis muscle and seventh intercostal space); electrocardiogram (1 channel: modified V1 derivation); air flow (2 channels: thermocouple and nasal pressure); respiratory effort (2 channels: thorax and abdomen) by inductance plethysmography belts; snoring and body position (1 channel each) by EMBLA® sensors; and oxygen saturation ($SaO_2$) by EMBLA® oximeter. The exam was performed according to specific criteria for the definition of sleep stages (Rechtschaffen and Kales, 1968). The American Academy of Sleep Medicine Manual for Scoring Sleep and Associated Events (Iber et al., 2007) was the reference to score leg movements, sleep-related respiratory events, and arousals. Hypopneas were scored following the alternative rule: decrease of 50% in the respiratory flow associated with arousal or 3% oxygen desaturation (Iber et al., 2007). OSA was diagnosed following the criteria of the International Classification of Sleep Disorders (American Academy of Sleep Medicine, 2005). Participants were diagnosed with OSA if they exhibited AHI between 5.0 and 14.9 in addition to at least 1 of the following complaints: breathing interruption during sleep, loud snoring, fatigue and/or daytime sleepiness. Individuals with AHI higher than 15.0 were diagnosed with OSA independently of additional complaints (Tufik et al., 2010). Those that did not fit OSA group were considered as controls (CTRL). To assess subjective sleepiness and sleep quality, we used the Epworth Sleepiness Scale (Johns, 1991) and the Pittsburgh Sleep Quality Index (Buysse et al., 1989) instruments.

### 2.4. Metabolic and inflammatory markers

Participants had their blood collected in the following morning of the PSG, after 10–12 h of fasting. The blood was centrifuged to obtain plasma and serum samples for metabolic and inflammatory markers assays. The enzymatic colorimetric assay was used to assess the concentrations of glucose, cholesterol, triglycerides (Advia® 1650/2400/Siemens Healthcare Diagnostics Inc., USA) and HDL (Advia® 1650/2400/Kovalent, Brazil). LDL was assessed by Friedewald formula (Advia® 1650/2400/Siemens Healthcare Diagnostics Inc., USA). Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were assayed by kinetic enzyme kit (Advia® 1650/2400/Siemens Healthcare Diagnostics Inc., USA). TNF-α and IL-6 were assessed by chemiluminescence method (Immulite® 2000/Siemens Healthcare Diagnostics Inc., USA). CRP was assessed by nephelometry (IMMAGE® Beckman Coulter, USA), while ghrelin and leptin levels were evaluated by radioimmunoassay (LINCO® Research Inc., USA).

## 3. Statistical analysis

Normality was determined by Kolmogorov-Smirnov's test. All variables showed non-normal distribution and were standardized by logarithm scale ($Log_{10}$) before we could run the statistical analysis. Chi-square test was used to verify possible associations between categorical variables, and consequently to identify confounding factors. Differences were observed by the adjusted residual. Multiple analysis of variance (MANOVA) was used to assess BMI, age, SBP and DBP, circumference of neck, hips and waist, using OSA and gender as fixed factors. PSG, biochemical, hormonal and inflammatory parameters were analyzed by multivariate analysis of covariance (MANCOVA) with OSA and gender as fixed factors and the following covariates: BMI, age, social class, thyroid problem, cardiovascular disease, alcohol consumption in the day of exam, and use of medications for diabetes, dyslipidemia, central nervous system (antidepressants, anxiolytics, anticonvulsants or neuroleptics) and sleep problems (hypnotics). Linear regression models were constructed for TNF-α and leptin levels using the stepwise procedure. For leptin levels, we considered as independent variables: age, gender, BMI, waist-to-hips ratio, hypertension, social class, ghrelin, smoking, IL-6, TNF-α, CRP, glucose, arousal index, AHI, hypopnea index, apnea index, mean $SaO_2$, sleep efficiency, AHI during REM sleep, cardiovascular, antilipidemic and antidiabetic medication, triglycerides, HDL cholesterol, and LDL cholesterol. For TNF-α levels, we considered as independent variables: age, gender, BMI, waist-to-hips ratio, hypertension, social class, ghrelin, leptin, smoking, IL-6, CRP, glucose, arousal index, AHI, hypopnea index, apnea index, mean $SaO_2$, sleep efficiency, AHI during REM sleep, cardiovascular, antilipidemic