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Gender differences in the association of sleep apnea and inflammation

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ABSTRACT

Over the last 15 years, many studies have established an association of sleep apnea with inflammation and metabolic aberrations. However, no controlled studies have examined potential gender effects in this association. We recruited 120 middle-aged, predominantly non-obese mild-to-moderate sleep apneics and controls (62 males, 58 females). All participants underwent a clinical history, physical examination, and 1-night 8-h polysomnography recording and provided a single fasting blood sample for assessment of interleukin-6 (IL-6), tumor necrosis factor receptor 1 (TNFR1), C-reactive protein (CRP), leptin, and adiponectin levels. Among non-sleep apneics, females had higher levels of TNFR1 ($p = 0.01$), CRP ($p = 0.005$), leptin ($p < 0.001$), and adiponectin ($p < 0.001$) compared to males, independent of age and body mass index. When analyzed separately by gender, sleep apneic men had elevated TNFR1 ($p = 0.04$), CRP ($p = 0.06$) and IL-6 ($p = 0.11$) relative to control men; in sleep apneic females, only CRP was elevated ($p = 0.04$). Furthermore, CRP was associated with apnea severity in a dose–response manner (p-linear = 0.04 in both genders) and was independently associated with comorbid hypertension in apnea (p-linear = 0.005 for women; p-linear = 0.09 for men). In conclusion, although women have naturally higher levels of inflammatory and metabolic markers than men, sleep apneic men appear to have a more severe inflammatory profile compared to women. Our findings suggest that these markers should be analyzed and interpreted separately in men and women, and that a single measure of plasma CRP appears to be a clinically-useful marker of apnea severity and comorbid cardiovascular morbidity.

1. Introduction

Obstructive sleep apnea is a prevalent sleep disorder characterized by obstruction of the upper airway during sleep despite breathing effort, as well as an associated reduction in blood oxygen saturation. Seventeen to 24% of men and 5–9% of women in general population samples demonstrate an apnea-hypopnea index (AHI) of five or more events per hour of sleep, while 4% of men and 2% of women meet the current clinical and polysomnographic criteria for the diagnosis of sleep apnea warranting immediate therapeutic intervention (Bixler et al., 1998, 2001; Young et al., 1993). Sleep apnea has been associated with the elevation of several pro-inflammatory cytokines, independent of obesity (Kritikou et al., 2014; Sahlman et al., 2010; Shamsuzzaman et al., 2002; Vgontzas et al., 1997, 2000, 2008) Among a number of inflammatory pathways, sleep apnea has been especially linked to activation of tumor necrosis factor (TNF)-α receptors, which stimulates secretion of interleukin (IL)-6, in turn triggering the synthesis of C-reactive protein (CRP) in the liver (Akira et al., 1990; Hirano et al., 1990). It is hypothesized that this inflammatory cascade, in addition to insulin resistance, mediates the link between sleep apnea and cardiometabolic complications (Vgontzas et al., 2005a).

Although sleep apnea was traditionally recognized in middle-aged, obese men, its occurrence in women as well as lean individuals is increasingly recognized. The prevalence of sleep apnea increases markedly after menopause, with post-menopausal women having a doubled rate of apnea compared to pre-menopausal women, even after accounting for neck circumference and body mass index (Bixler et al., 2001; Dancey et al., 2001). Also, while the maximum prevalence for obstructive sleep apnea peaks between ages 50 and 59 in men (Bixler et al., 1998), this peak is not seen in females until after age 65 (Bixler et al., 2001). Furthermore, men tend to have a higher AHI than women when matched for body mass index (Kapsimalis and Kryger, 2002), are more likely to exhibit the classical symptoms of excessive daytime sleepiness

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and snoring (Phillips et al., 2008), and the severity of their daytime sleepiness is more likely to be related to lack of regular exercise, depression, and minimum oxygen desaturation than AHI per se (Basta et al., 2008).

In healthy individuals, it has been demonstrated that peripheral levels of CRP (Cartier et al., 2009; Khera et al., 2005; Lakoski et al., 2006; McConnell et al., 2002, Wener et al., 2002), leptin (Couillard et al., 1997; Hellström et al., 2000; Hickey et al., 1996; Kennedy et al., 1997; Ostlund et al., 1996), and adiponectin (Böttner et al., 2004; Saltevo et al., 2009; Song et al., 2014) are naturally higher in women compared to men, independent of age, race, and body mass index. Despite this, although most studies of sleep apneics statistically control for gender in their analyses, very few have expressly investigated possible gender differences in inflammation.

A number of studies in large general population samples, both prospective and cross-sectional, have demonstrated a clear association between sleep apnea and hypertension (Bixler et al., 2000; Nieto et al., 2000; Peppard et al., 2000). Subsequent studies in large community-based cohorts have further demonstrated that men with sleep apnea have a significantly increased risk for hypertension (Hedner et al., 2006; Mohsenin et al., 2009) and stroke (Redline et al., 2010) compared to women. Additionally, a recent large study reported that men with serum CRP > 3.0 mg/L have significantly higher odds of both cardiovascular and all-cause mortality compared to women with the same CRP cut-off (Doran et al., 2013). However, no study to date has explored the link between gender and apnea-associated cardiovascular outcomes, such as hypertension, in the context of the inflammatory response.

The aim of our study was to examine potential gender differences in the association of sleep apnea with inflammation and metabolic markers, as well as the synergistic effect of apnea and hypertension on these outcomes in a sample of middle-aged, predominantly non-obese sleep apneics and controls. We hypothesized that the association of these markers with sleep apnea would be stronger in men than in women.

2. Methods

2.1. Participants

The study sample consisted of 120 middle-aged, predominantly non-obese mild-to-moderate sleep apneics and controls (62 males, 58 females; mean age = 54.67 ± 0.54 years). Participants were recruited through advertisements in the local community and screened according to research protocols by the Sleep Research and Treatment Center at Penn State Milton S. Hershey Medical Center (Hershey, PA, USA). All women in the study were postmenopausal (self-reported absence of menses for at least 12 months or total hysterectomy). Exclusion criteria included a history of diabetes mellitus, use of antiglycemic agents and/or fasting blood glucose levels >126 mg/dL, ongoing infections, rheumatoid arthritis, insomnia, narcolepsy, and use of certain medications (psychotropics, steroids, sympathomimetics, sympatholytics, or hormone therapy for females). The study was approved by the Institutional Review Board at Penn State University College of Medicine and all participants provided written informed consent.

2.2. Sleep laboratory protocol

During their visit in the laboratory, all participants underwent a clinical history and physical examination, during which height and weight were recorded and body mass index (BMI) calculated (in kg/m^2). Blood pressure was also assessed, with hypertension defined as systolic blood pressure $\geqslant$ 140 mmHg and/or diastolic blood pressure $\geqslant$ 90 mmHg, or as the use of antihypertensive medication.

Sleep laboratory recordings were conducted in a sound-attenuated, light- and temperature-controlled room with a comfortable, bedroom-like atmosphere. Each subject was monitored continuously for one night for 8 h (22:30–23:00 until 6:30–7:00) using 16-channel polygraph recordings of EEG, electrooculogram (EOG) and electromyogram (EMG). Polysomnography (PSG), respiration (via thermocouple and thoracic strain gauges), and oximeter data were collected using Grass-Telefactor Gamma Sleep Recording software (Middleton, WI, USA). Visual sleep stage scoring was conducted by a registered polysomnography technologist blind to participant characteristics based on Rechtschaffen and Kales criteria (Rechtschaffen and Kales, 1968). Apnea-hypopnea index (AHI; number of apneas and hypopneas summed per hour) was also ascertained. An apnea was defined as cessation of airflow for $\geqslant$10 s and an out-of-phase strain gauge movement; a hypopnea was defined as a 50% airflow reduction and associated decrease in SaO_2 of at least 4%. In stratifying our study sample, "the presence of sleep apnea" was defined as an AHI $\geqslant$ 5 events/hour of sleep.

2.3. Blood sampling

A single fasting blood draw (via venipuncture) was performed at 7:00 immediately after the end of the PSG recording. Blood was collected in EDTA-containing tubes and refrigerated until centrifugation (within 3 h). Blood was stored at –80 °C until assay.

2.4. Assays

Plasma interleukin-6 (IL-6), tumor necrosis factor receptor 1 (TNFR1), and high-sensitivity C-reactive protein (hsCRP) were measured via enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). The intra- and inter-assay coefficients of variation (CVs) were 7.4% and 7.8% for IL-6, 4.4% and 6.1% for TNFR1, and 5.5% and 11.6% for hsCRP. The lower detection limits were 0.094 pg/mL, 0.043 pg/mL, and 0.124 ng/mL for IL-6, TNFR1, and hsCRP, respectively. Leptin and adiponectin were assessed by commercially-available radioimmunoassays with CVs below 10%.

2.5. Statistical analysis

Two-tailed independent-samples t-tests were used to compare demographic and PSG variables between males and females (between-gender), or between controls and sleep apneics (within-gender). To examine differences in inflammatory and metabolic characteristics between more than two groups (e.g. increasing apnea severity), analyses of covariance (ANCOVA) with Bonferroni correction were conducted. Polynomial linear analysis was also performed to examine the association between increasing apnea severity (i.e. AHI < 5, 5 $\leqslant$ AHI < 15, and AHI $\geqslant$ 15) and inflammatory markers. Finally, to assess the association of sleep apnea associated with the comorbid cardiovascular outcome (i.e., hypertension) and inflammation, we examined differences between three groups: controls without hypertension, sleep apneics without hypertension, and sleep apneics with hypertension. Effect size was also assessed by calculating Cohen's d statistic. Statistical significance was determined using the criterion $p < 0.05$. All analyses were adjusted for the confounders age and BMI. Analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY).

3. Results

Demographic and PSG parameters of sleep apneic and control males and females are presented in Table 1. Within gender, the control and sleep apneic groups did not differ in age (all $p > 0.05$), but

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