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Increased inflammation from childhood to adolescence predicts sleep apnea in boys: A preliminary study

Jordan Gaines^a, Alexandros N. Vgontzas^{a,*}, Julio Fernandez-Mendoza^a, Fan He^b, Susan L. Calhoun^a, Duanping Liao^b, Edward O. Bixler^a

^aSleep Research and Treatment Center, Department of Psychiatry, Pennsylvania State University College of Medicine, Hershey, PA, United States

^bDepartment of Public Health Sciences, Pennsylvania State University College of Medicine, Hershey, PA, United States

Abstract

While chronic systemic inflammation in obstructive sleep apnea (OSA) has been traditionally considered a consequence of intermittent hypoxia, several treatment studies targeting inflammation suggest that this process may precede the development of the disorder. A recent cross-sectional study in the Penn State Child Cohort (PSCC) revealed that inflammation largely mediates the association between visceral adiposity and OSA in adolescence. The purpose of this study was to examine for the first time whether, longitudinally, inflammation precedes OSA during this developmental period. A subsample of the PSCC with longitudinal sleep and inflammation data ($n = 51$) was included in this study. Participants underwent 9-h polysomnography (22:00–7:00), physical exam, and fasting morning blood draw at both time points. Plasma C-reactive protein (CRP) was measured via ELISA. At follow-up, visceral, subcutaneous, and total fat area were assessed via dual X-ray absorptiometry. Sex differences in body composition emerged in adolescence, with boys having more visceral adiposity than girls. Longitudinal increases in waist circumference from childhood to adolescence were associated with increases in CRP (CRP) and follow-up CRP in boys, but not girls. Furthermore, in boys, CRP was associated with higher follow-up apnea/hypopnea index (AHI). When CRP was entered into a model predicting follow-up AHI, waist circumference was no longer significant, indicating that inflammation largely explains the association between increasing central obesity and OSA severity. These preliminary findings, in a longitudinal, non-clinical sample of children developing OSA, suggest that inflammation derived from visceral adipose tissue precedes the development of the disorder, suggesting a potential causal mechanism.

Keywords

Obstructive sleep apnea; Inflammation; Children; Adolescents; Obesity

*Corresponding author at: Penn State University College of Medicine, Department of Psychiatry H073, 500 University Drive, Hershey, PA 17033, United States. avgontzas@psu.edu (A.N. Vgontzas).

Conflict of interest

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1. Introduction

It is estimated that 1–4% of the general pediatric population (Bixler et al., 2008; Lumeng and Chervin, 2008) and 4–11% of adolescents (Spilsbury et al., 2015; Bixler et al., 2016) have obstructive sleep apnea (OSA), a prevalent sleep disorder characterized by obstruction of the upper airway during sleep despite breathing effort. While OSA in children is commonly thought to result from upper airway structural abnormalities, central obesity is increasingly recognized as a strong risk factor in children, adolescents, and adults (Bixler et al., 2008; Lumeng and Chervin, 2008; Shinohara et al., 1997; Vgontzas et al., 2000; Tauman and Gozal, 2006; Tsaousoglou et al., 2010; Canapari et al., 2011). Importantly, the prevalence of OSA in adult men and women differs, with 17–24% of men and 5–9% of women demonstrating an apnea/hypopnea index (AHI) of five or more events per hour of sleep (Young et al., 1993; Bixler et al., 1998, 2001); these rates have recently increased between 14% and 55%, depending on the subpopulation studied, in conjunction with the obesity epidemic (Peppard et al., 2013).

Chronic systemic inflammation in OSA has been well-documented over the last two decades and correlates positively with apnea severity (Vgontzas et al., 2000). Traditionally, this inflammation has been largely considered a consequence of intermittent hypoxic episodes resulting from breathing pauses throughout the night (Ryan et al., 2005). Several treatment studies targeting inflammation, however, suggest that inflammation also precedes – and perhaps even plays a causal role – in the development of OSA. For example, compared to placebo, a three-week trial of the tumor necrosis factor alpha (TNF α) antagonist etanercept has been shown to reduce AHI in obese men with OSA (Vgontzas et al., 2004). Combined oral anti-inflammatory and intranasal corticosteroid treatment has also been demonstrated to largely normalize mild OSA in children (Kheirandish-Gozal et al., 2014). On the other hand, while the gold standard treatment, continuous positive airway pressure (CPAP) therapy, reliably reduces AHI, a recent large systematic review and meta-analysis of randomized controlled studies concluded that CPAP does not significantly alter levels of plasma inflammatory cytokines (Jullian-Desayes et al., 2015). Together, these findings suggest that inflammation may be more than simply a result of apnea, but rather also a cause and potential treatment target – though no longitudinal study has yet explored this hypothesis.

In adults, levels of proinflammatory cytokines are correlated positively with body mass index and, in particular, visceral adiposity (Vgontzas et al., 2000; Khaodhri et al., 2004). Resident macrophages in visceral fat tissue secrete high levels of cytokines (Fain, 2006) as a result of adipocyte growth, oxidative stress, and endothelial damage (Wellen and Hotamisligil, 2003). Given that adult men tend to have at least twice as much visceral fat tissue as women (Wajchenberg, 2000) and are significantly more likely to present with OSA (Young et al., 1993; Bixler et al., 1998, 2001), inflammation again presents an enticing target. Indeed, a recent study in the general population Penn State Child Cohort demonstrated that inflammation strongly mediates, cross-sectionally, the association between visceral adiposity and OSA in adolescents, with 82% of the association explained by CRP levels (Gaines et al., 2016). No studies to date, however, have explored this association in a longitudinal sample.

Given the well-characterized sex differences in central obesity and OSA prevalence, the association of visceral adiposity with inflammation, and evidence that inflammation may precede the development of OSA, we explored the longitudinal mechanisms underlying the development of incident OSA in a general population sample of children transitioning to adolescence. We hypothesized that OSA severity in adolescence was associated with (a) increases in waist circumference since childhood, particularly in boys, (b) elevations in proinflammatory cytokines since childhood, and (c) that increases in inflammation during this developmental period largely explain the association between accumulating central obesity and sleep apnea severity.

2. Methods

2.1. Participants

The study participants comprised a subsample of the Penn State Child Cohort (PSCC) – a representative general population sample of 700 children (ages 5–12 years) – who served as healthy controls ($AHI < 2$) in a study exploring inflammation and metabolic abnormalities in children with OSA (Tsaoussoglou et al., 2010). Of $n = 82$ participants who were approached and gave a blood sample during childhood, $n = 51$ returned an average of 8.4 years later as adolescents as part of their follow-up visit (Bixler et al., 2008, 2009). All participants have complete longitudinal sleep data and provided fasting morning blood samples. Importantly, the 51 participants in the present study did not differ from the rest of the PSCC participants ($n = 649$) in terms of baseline age (9.14 ± 0.23 years vs. 9.17 ± 0.08 years, respectively, $p = 0.905$), BMI percentile (63.19 ± 4.17 vs. 63.13 ± 1.14 , $p = 0.989$), sex distribution (41.2% boys vs. 53.3% boys, $p = 0.071$), nor ethnic minority distribution (15.70% minority vs. 20.20% minority, $p = 0.284$).

Written informed consents were obtained from participants 18 years and older. Assent was sought for those younger than 18 years, and consent was obtained from their parents or legal guardians. All research protocols were reviewed and approved for compliance with the policy of the human subjects Institutional Review Board at Penn State University College of Medicine.

2.2. Sleep laboratory protocol

At both baseline and follow-up time points, all participants underwent a 9-h, single-night polysomnography (PSG) recording in a sound-attenuated, light- and temperature-controlled room with a comfortable, bedroom-like atmosphere (Fig. 1). Each subject was continuously monitored from 22:00 h until 7:00 h using 14-channel recordings of electroencephalogram (EEG), electrooculo-gram (EOG), and electromyogram (EMG). Respiration was monitored via nasal pressure (Pro-Tech PTAF Lite; Mukilteo, WA), thermocouple (Salter Labs; Lake Forest, IL), and thoracic/abdominal strain gauges (Model 1312, Sleepmate Technologies; Midlothian, VA). Hemoglobin oxygen saturation (SpO_2) was assessed using a pulse oximeter placed on the index finger (Model 3011 Xpod, Nonin Medical, Inc.; Plymouth, MN). Snoring sounds were monitored via a sensor attached to the throat. All data were recorded using Twin Recording & Analysis software (Grass-Telefactor; West Warwick, RI). Visual sleep stage scoring was conducted by a registered polysomnography technologist

according to standardized criteria (Rechtschaffen and Kales, 1968). Apnea/hypopnea index (AHI; number of apneas and hypopneas summed per hour) was ascertained. An apnea was defined as a cessation of airflow with a minimum duration of 5 s (for those aged < 16 - years) or 10 s (for those ≥ 16 years at follow-up) and an associated out-of-phase strain gauge movement; a hypopnea was characterized by a reduction of airflow by approximately 50% with an associated decrease in SpO₂ of at least 3% or an associated EEG arousal (Iber et al., 2007).

2.3. Physical assessment

During their baseline and follow-up visits in the laboratory (Fig. 1), all participants underwent a physical examination, during which height (stadiometer Model 242, SECA Corp.; Hanover, MD), weight (Model 758C, Cardinal Manufacturing; Webb City, MO), and waist circumference (via tape measure) were recorded according to Centers for Disease Control (2011) criteria. Body mass index (BMI) was calculated (in kg/m²) and converted to a percentile according to a formula based on the Centers for Disease Control's (2009) sex-specific BMI-for-age growth charts. All participants were deemed to not have any active illness or infection at the time of their visit, nor did anybody have CRP values >10 mg/L. Participants identified their race/ethnicity from one of six options; "ethnic minority status" was re-defined as "non-white/Caucasian" for statistical purposes.

At follow-up, participants also underwent a dual-energy X-ray absorptiometry (DXA) scan using a Hologic Discovery W scanner (Hologic Inc.; Waltham, MA; 195 × 65 cm field of view) to obtain a precise measure of body fat. Regions of interest included visceral, subcutaneous, and total (visceral plus subcutaneous) adipose tissue area. These regions were identified by Hologic APEX 4.0 software (Hologic Inc.; Bedford, MA) and visually verified by an experienced investigator; detailed descriptions of these measures can be found elsewhere (Hologic, Inc., 2010; Kelly et al., 2010).

2.4. Blood draw and assay procedures

Upon awakening (7:00), blood samples were collected in EDTA-containing tubes, then spun for 10 min at 3000 RPM. Plasma was aliquoted into cryotubes and stored at -80 °C until assayed. High-sensitivity C-reactive protein (CRP) was measured via enzyme-linked immunosorbent assay (ELISA; R&D Systems; Minneapolis, MN). The intra- and interassay coefficients of variation were 5.5% and 6.5% for samples collected at baseline, and 5.8% and 5.3% for samples collected at follow-up.

2.5. Statistical analysis

Differences in sociodemographic, sleep, body composition, and CRP characteristics at baseline and follow-up time points were assessed via paired-samples t-tests.

Body composition variables (waist circumference, waist/hip ratio, and DXA-measured body fat variables) were compared in boys and girls at both baseline and follow-up using analysis of covariance (ANCOVA), controlling for age, ethnic minority status, and total fat area (for visceral and subcutaneous fat area analyses). Linear regressions were then used to examine the associations of waist circumference (defined as waist circumference at follow-up minus

that at baseline) with Δ CRP (defined as follow-up CRP minus baseline CRP) and follow-up CRP, adjusting for baseline CRP, follow-up age, and ethnic minority status.

To examine, longitudinally, the relationship between CRP and OSA severity at follow-up, linear regression analyses were performed, with Δ CRP as the predictor. Our primary outcome was AHI; secondary analyses included the sleep variables total sleep time (TST), total wake time (TWT), sleep onset latency (SOL), wake after sleep onset (WASO), number of wakes, sleep efficiency (SE), and percent of stages 1, 2, slow-wave sleep (SWS) and rapid eye movement (REM) sleep. Analyses were controlled for follow-up age, follow-up BMI percentile, and ethnic minority status.

Finally, linear regression analyses were conducted to explore whether increased CRP explains the longitudinal association between central obesity and apnea severity, adjusting for baseline AHI, age, and ethnic minority status. Specifically, waist circumference was assessed as a predictor of follow-up AHI, then Δ CRP was added in a second model to observe the attenuation of the β for waist circumference.

The statistical confidence level selected for all analyses was $p < 0.05$. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM Corp., Armonk, NY).

3. Results

3.1. Sociodemographics, sleep, and inflammation characteristics of sample

The longitudinal sample of $n = 51$ participants consisted of 41.2% boys and 15.7% non-white ethnic minority. Participants were 9.14 ± 0.23 years old at baseline and 15.58 ± 0.24 years old at follow-up (Table 1). On average, BMI percentile did not differ between the two time points ($p = 0.267$). No participants were pre-pubertal at the time of follow-up (all Tanner scores ≤ 3 ; Carskadon and Acebo, 1993).

From baseline to follow-up, AHI increased from 0.17 ± 0.03 events/hour to 1.77 ± 0.54 events/hour ($p = 0.004$). While all participants had $\text{AHI} < 2$ at baseline, $n = 5$ (2 boys) had developed $2 \leq \text{AHI} < 5$ at follow-up, while $n = 3$ (all boys) had progressed to $\text{AHI} \geq 5$ at follow-up. TST decreased by 19.3 min ($p = 0.016$), WASO increased by 30.3 min ($p < 0.001$), and SE decreased by 4.9% ($p = 0.002$). In terms of sleep architecture, SWS and REM decreased from 40.43% to 27.79% ($p < 0.001$) and 22.48% to 19.09% ($p < 0.001$), respectively, while stages 1 and 2 increased from 0.62% to 1.06% ($p = 0.014$) and 36.45% to 52.09% ($p < 0.001$), respectively.

Waist circumference and hip circumference increased significantly from baseline to follow-up (increases of 15.14 cm and 15.73 cm, respectively; both $p < 0.001$). Plasma CRP increased by 0.33 mg/L from baseline to follow-up; however, this increase was not statistically significant ($p = 0.193$).

3.2. Body composition in childhood and adolescence

Neither waist circumference ($p = 0.418$) nor waist/hip ratio ($p = 0.475$) differed between boys and girls in childhood (Table 2). Sex differences began to emerge in adolescence, however, with boys having a greater waist/hip ratio (1.01 ± 0.05 vs. 0.87 ± 0.04 , $p = 0.062$). Although adolescent girls had, overall, more total body fat compared to boys ($p = 0.061$), this was driven by more subcutaneous fat area ($236.53 \pm 2.16 \text{ cm}^2$ vs. $203.86 \pm 2.60 \text{ cm}^2$; $p < 0.001$). On the other hand, visceral adipose tissue was significantly elevated in boys compared to girls ($74.48 \pm 2.35 \text{ cm}^2$ vs. $42.12 \pm 2.02 \text{ cm}^2$; $p < 0.001$), and the visceral/subcutaneous fat area ratio was significantly higher in boys (0.53 ± 0.03 vs. 0.18 ± 0.03 , $p < 0.001$).

In assessing the longitudinal association between central obesity and inflammation (Table 3), waist circumference was associated with both greater CRP ($\beta = 0.52$, $p = 0.031$) and follow-up CRP ($\beta = 0.54$, $p = 0.031$) in boys, but not girls (all $p = 0.808$). Further controlling for time elapsed between baseline and follow-up assessments did not affect the results, nor did controlling for health comorbidities that could potentially influence inflammation levels (arthritis, asthma, chronic sinusitis/rhinitis, total number of reported health problems, and use of anti-inflammatory medication). Importantly, overall, boys had greater CRP ($1.07 \pm 0.38 \text{ mg/L}$) and follow-up CRP ($1.30 \pm 0.32 \text{ mg/L}$) than girls ($-0.18 \pm 0.32 \text{ mg/L}$, $p = 0.015$ and $0.52 \text{ mg/L} \pm 0.27 \text{ mg/L}$, $p = 0.068$, respectively).

3.3. CRP predicting adolescent OSA and sleep disturbance

In adolescence, sex differences emerged in terms of OSA severity, with girls having an average AHI of 1.05 ± 0.65 events/hour, and boys an AHI of 2.83 ± 0.75 events/hour ($p = 0.076$). Adjusting for follow-up age, follow-up BMI percentile, and ethnic minority status, CRP significantly predicted higher AHI in boys ($\beta = 0.95$, $p < 0.001$; $R^2 = 0.922$; Fig. 2A), but not girls ($\beta = 0.13$, $p = 0.53$; $R^2 = 0.086$; Fig. 2B). Excluding a male outlier (not shown in the figure) did not significantly affect the results ($\beta = 0.98$, $p < 0.001$, $R^2 = 0.98$). In boys, CRP was also associated with greater number of wakes ($\beta = 0.77$, $p < 0.001$), greater stage 2 sleep ($\beta = 0.70$, $p < 0.001$), and less REM ($\beta = -0.84$, $p < 0.001$); however, these associations were no longer significant after further adjusting for follow-up AHI (all $p = 0.111$), suggesting that lower sleep quality was driven by apneas/hypopneas. No associations of CRP were observed with follow-up apnea severity or sleep quality variables in girls (all $p > 0.116$).

Given the sex differences in Tables 2, and associations observed in Table 3 and Fig. 2, we then tested the hypothesis that CRP largely explains the relationship between waist circumference and follow-up AHI. In both boys and girls, waist circumference predicted follow-up AHI ($\beta = 0.54$, $p = 0.037$ and $\beta = 0.41$, $p = 0.027$), adjusting for baseline AHI, follow-up age, and ethnic minority status. When CRP was added to the model, waist circumference was no longer a significant predictor in boys ($\beta = 0.01$, $p = 0.946$), suggesting that increases in inflammation explain, to a large degree, the relationship between increased waist circumference and AHI at follow-up (Fig. 3). In girls, on the other hand, adding CRP to the model did not alter the association between waist and follow-up AHI ($\beta = 0.43$, $p = 0.027$). In secondary analyses, adding follow-up CRP instead of CRP to the model yielded

similar results ($\beta = -0.06$, $p = 0.215$ for boys; $\beta = 0.42$, $p = 0.025$ for girls). When we tested the alternative hypothesis that AHI accounts for the association between waist circumference and follow-up CRP, however, adding AHI to the model did not significantly alter the association (both $p < 0.05$), indicating that AHI is not driving increases in CRP.

4. Discussion

This is the first study to examine the complex interplay of central obesity, obstructive sleep apnea, and inflammation using a longitudinal study population. First, we report that sex differences in body composition emerge in adolescence, with boys having more visceral fat than girls. Next, we show that increases in waist circumference from childhood to adolescence are positively correlated with increases in CRP from childhood to adolescence in boys. We also describe how increases in CRP since childhood predict sleep apnea and sleep disturbance in adolescence. Taken together, we demonstrate that increases in CRP from childhood to adolescence explain, to a great degree, the relationship between increased waist circumference and sleep apnea severity in boys. These preliminary findings – in a non-clinical, longitudinal sample of young people developing incident sleep apnea – suggest that inflammation derived from visceral fat tissue precedes the development of the disorder.

While many studies have described strong associations between elevated inflammatory cytokines and OSA severity, several lend evidence specifically for a causal role of inflammation. A recent randomized trial reported no changes in CRP after 6 months of CPAP use unless combined with weight loss, suggesting that obesity is a major source of inflammation in OSA (Chirinos et al., 2014). A meta-analysis of 24 sham-CPAP controlled studies corroborates this, concluding that, overall, CPAP therapy alone does not significantly alter inflammation (Jullian-Desayes et al., 2015). More recent work has demonstrated how OSA rates have undergone relative increases of 14–55% over the last two decades, depending on the subpopulation studied, in conjunction with the obesity epidemic (Peppard et al., 2013). Interestingly, the Wisconsin Sleep Cohort has reported that a 10% loss of body weight predicts a 26% decrease in AHI (Peppard et al., 2000). In children, pre- and perinatal factors such as chorioamnionitis, maternal smoking, weight gain during pregnancy, gestational diabetes, and prematurity have been associated with childhood OSA (Hibbs et al., 2008; Calhoun et al., 2010; Tapia et al., 2016); inflammation during this early period has been hypothesized as a possible mechanism. Taken together, these very different studies suggest that systemic inflammation may precede the development – or at least the worsening – of OSA in adults and children.

Further evidence for a causal role of inflammation in the development of OSA comes from treatment interventions that specifically target inflammation. We have previously reported that, compared to placebo, a three-week trial of the TNF α antagonist etanercept significantly reduces AHI and IL-6 levels in obese men with OSA (Vgontzas et al., 2004). In patients with spondyloarthritis, those who take TNF-inhibitors have a significantly lower prevalence of OSA than those who do not (Walsh et al., 2012). In children, recent evidence suggests that anti-inflammatory therapy is effective in reducing apnea severity in children with mild OSA (Kheirandish-Gozal et al., 2014).

These findings make sense in the context of our recent paper in the full adolescent sample of the Penn State Child Cohort, in which we demonstrate that inflammation strongly mediates the association between visceral adiposity and OSA (defined as AHI ≥ 5). Specifically, we report that, cross-sectionally, 42% of the association between visceral fat area and OSA is explained by IL-6, while 82% is explained by CRP levels (Gaines et al., 2016). In the current study, we replicated and expanded these findings in a longitudinal subsample of this cohort. We were particularly interested in focusing on changes in CRP, rather than CRP in childhood, as a predictor given that inflammation is a long-term process. Increases in inflammation from childhood to adolescence are strongly influenced by age, sex, and development, making it particularly important for our longitudinal analyses to take these factors into account. As expected, when we used childhood CRP as a predictor in our analyses, we did not observe an association with AHI in adolescence. Our sample of $n = 51$ children was relatively young (9.14 ± 0.23 years) with no OSA (mean AHI = 0.17 ± 0.03 events/h) and healthy CRP levels (0.51 ± 0.14 mg/L) at baseline; thus, it is not surprising that childhood CRP levels were not predictive of adverse outcomes in adolescence. Of interest, when we tested the alternative hypothesis that increases in AHI explain the association between waist circumference and follow-up CRP, our findings were negative.

The emergence of sex differences in OSA prevalence and manifestation have been well-characterized in adults, albeit poorly understood. While 17–24% of men in the general population have OSA (AHI ≥ 5), this figure drops to 5–9% in women (Young et al., 1993; Bixler et al., 1998, 2001). Furthermore, the prevalence of OSA peaks around age 55 years for men and 65 years for women (Bixler et al., 1998, 2001); hormonal and physiological changes associated with menopausal onset may explain the delay in peak prevalence (Bixler et al., 2001; Young et al., 2003). Indeed, even in this relatively young subsample of the Penn State Child Cohort, AHI in boys was nearly three times higher than in girls (2.83 ± 0.75 events/hour vs. 1.05 ± 0.65 events/hour, $p = 0.076$). Of note, studies examining upper airway morphology show no sex differences, suggesting that there are multiple factors involved in susceptibility for upper airway collapse (Ma et al., 2016).

Visceral fat accounts for approximately 5–8% of total body fat in adult women and 10–20% in men (Wajchenberg, 2000), and women tend to have roughly twice as much subcutaneous fat as men (Lemieux et al., 1993). Several recent studies have explored whether these well-characterized differences in body composition may partially explain the sex disparity in OSA prevalence and severity. In a computed tomography (CT) study of relatively nonobese middle-aged men and postmenopausal women, Kritikou et al. (2013) reported that OSA was associated with visceral adiposity in men, but with more global adiposity (both subcutaneous and visceral adiposity) in women. Similarly, using surrogate markers of central and global adiposity, Mazzuca and colleagues (2014) reported strong associations between waist circumference and AHI in middle-aged men with OSA, and between hip circumference and AHI in women. The findings of our study demonstrate that these sex differences in body composition emerge as early as adolescence, with boys having a nearly 3-fold greater visceral-to-subcutaneous fat area ratio compared to girls (0.53 ± 0.03 vs. 0.18 ± 0.03 , $p < 0.001$). This is consistent with data emphasizing the important role of visceral adiposity, specifically, in OSA severity; compared to age- and BMI-matched controls, elevated visceral fat area is the defining feature in adult men with OSA (Vgontzas et al., 2000). Similarly, a

recent small pilot study reported an association of AHI with DXA-measured visceral fat area, but not with BMI or subcutaneous fat area, in adolescents (Hannon et al., 2011).

In recent years, visceral adipose tissue has been increasingly regarded as a metabolically-active organ. Compounding the health risk is the fact that visceral fat is localized around rich vasculature, innervation, and critical organs such as the liver, making this tissue type relatively more dangerous to overall health (Ibrahim, 2010). Indeed, elevations in visceral fat have been strongly linked to development of the metabolic syndrome, cardiovascular events, and cardiovascular and all-cause mortality, particularly in men (Dobbelsteijn et al., 2001; Kuk et al., 2006). Compared to subcutaneous fat, visceral fat contains proportionally more adipocytes that are capable of growing quite large before dividing (Ibrahim, 2010). As they grow, these adipocytes adopt a higher rate of glucose uptake, become insulin resistant, begin secreting low levels of TNF α , and recruit macrophages which, in turn, lead to further elevations in proinflammatory cytokines (Mårin et al., 1992; Xu et al., 2003). Synthesis of the acute-phase reactant CRP in the liver is triggered by chronic inflammation and activation of the innate immune system, and is regarded as a good nonspecific marker of overall inflammation (Seo, 2012). In our sample, both CRP and follow-up C-reactive protein levels were positively associated with waist circumference in boys, but not girls, suggesting that central obesity – even at this young age – is contributing to the development of systemic inflammation.

The mechanisms linking increased inflammation to the development of OSA are not well-understood, but are likely a combination of several factors. First, because a significant amount of lymph tissue is located in the head and neck, swelling of glands or lymphatic vessels due to chronic systemic inflammation may contribute to upper airway narrowing (Baluk et al., 2005). Second, increased inflammatory gene expression in skeletal muscle (Poelkens et al., 2013) may contribute to dysfunction of airway dilator muscles during sleep, leading to upper airway collapse (Vgontzas et al., 2016). Finally, inflammation originating from fat tissue and other sources launches a vicious cycle of cardiovascular and metabolic sequelae, including leptin resistance (Campo et al., 2007), elevated lipids and triglycerides, endothelial dysfunction, and elevated blood pressure; through various mechanisms, these conditions may worsen obesity and contribute further to respiratory depression, soft tissue edema, or other mechanisms.

There are several limitations to the current study. For one, only a morning (7:00) blood sample was collected at both time points. Fortunately, CRP lacks circadian rhythmicity, with levels remaining relatively stable regardless of sample collection time (Meier-Ewert et al., 2001). Secondly, even though we excluded those with AHI > 2 at baseline, all subjects included in our study actually had AHI $\leq$ 1 at baseline, which means there is a relatively low range in both apnea severity and inflammation levels. Despite this, however, it is interesting to have the opportunity to observe all cases of mild or moderate OSA at follow-up, highlighting the important mechanistic role of central obesity and inflammation in the development of the disorder. Furthermore, the sample size ($n = 51$) is relatively low, particularly when stratified by sex; importantly, however, this is the first study to examine longitudinal changes in blood biomarkers as they relate to OSA in this young age group, and our sample did not differ significantly from the original general population sample of $n =$

700 children in terms of age, BMI percentile, sex, or ethnic minority distributions. Finally, our model is limited in that it was not based on a temporal separation between waist circumference, inflammation, and AHI. Physiologically, however, the association of increased waist circumference from childhood to adolescence – not simply baseline waist circumference *per se* – with elevated AHI in adolescence is a more parsimonious model, given that there are major changes and sex differences in body composition from prepuberty to adolescence (Table 2). Future studies should directly test this model using mediation analysis.

In sum, our preliminary findings in a non-clinical, longitudinal sample of children developing sleep apnea in adolescence, suggest that (a) sex differences in body composition emerge in adolescence; (b) increases in waist circumference from childhood to adolescence are associated with increased CRP in boys; (c) increases in CRP since childhood predict sleep apnea in adolescence; and (d) inflammation explains, to a great degree, the relationship between increased waist circumference and sleep apnea severity, adding to our understanding of the pathogenesis, sex differences, and potential treatments for OSA.

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Fig. 1. Design of the baseline and follow-up examinations. Participants in the present study ($n = 51$) have complete 9-h polysomnography, body composition, and CRP data at both time points.

Fig. 2. Scatterplot depicting the association of ΔCRP with follow-up AHI in boys (A) and girls (B).

Fig. 3.

Increased inflammation largely explains the association between increased central obesity and follow-up AHI in boys, while increased central obesity is independently associated with follow-up AHI in girls. Data are standardized regression coefficients adjusted for follow-up age, race, and baseline predictor values. * $p < 0.05$; *** $p < 0.001$.

Table 1

Sociodemographic, sleep, body composition, and inflammation characteristics of the Penn State Child Cohort subsample (n = 51) at baseline and follow-up.

	Baseline	Follow-up	P
Age (years)	9.14 (0.23)	15.58 (0.24)	<0.001
BMI percentile	63.19 (4.17)	66.10 (4.13)	0.267
AHI (events/h)	0.17 (0.03)	1.77 (0.54)	0.004
TST (min.)	465.46 (4.14)	446.12 (7.79)	0.016
SOL (min.)	24.25 (2.06)	21.85 (1.91)	0.346
WASO (min.)	45.54 (3.45)	75.86 (6.76)	<0.001
SE (%)	87.20 (0.71)	82.25 (1.43)	0.002
Stage 1 (%)	0.62 (0.09)	1.06 (0.16)	0.014
Stage 2 (%)	36.45 (1.41)	52.09 (1.13)	<0.001
SWS (%)	40.43 (1.42)	27.79 (1.02)	<0.001
REM (%)	22.48 (0.57)	19.09 (0.73)	<0.001
Waist circumference (cm)	65.01 (1.55)	80.15 (2.11)	<0.001
Hip circumference (cm)	73.06 (1.88)	88.79 (2.05)	<0.001
Total fat area (cm ²)	-	279.62 (25.95)	-
Visceral fat area (cm ²)	-	56.54 (5.63)	-
Subcutaneous fat area (cm ²)	-	223.08 (21.20)	-
CRP (mg/L)	0.51 (0.14)	0.84 (0.21)	0.193

Data presented as mean (SEM). BMI = body mass index; AHI = apnea/hypopnea index; TST = total sleep time; SOL = sleep onset latency; WASO = wake after sleep onset; SE = sleep efficiency; SWS = slow-wave sleep; REM = rapid eye movement; CRP = C-reactive protein.

Table 2

Body composition at baseline and follow-up, stratified by sex.

	Boys (n = 21)	Girls (n = 30)	P
<i>Baseline</i>			
Waist circumference (cm)	63.59 (2.24)	65.95 (1.83)	0.418
Waist/hip ratio	0.89 (0.06)	0.95 (0.05)	0.475
<i>Follow-up</i>			
Waist circumference (cm)	83.40 (2.96)	77.64 (2.47)	0.144
Waist/hip ratio	1.01 (0.05)	0.87 (0.04)	0.062
Total fat area (cm ²)	223.38 (38.03)	318.99 (31.77)	0.061
Visceral fat area (cm ²)	75.76 (2.60)	43.09 (2.16)	<0.001
Subcutaneous fat area (cm ²)	203.86 (2.60)	236.53 (2.16)	<0.001
Visceral/subcutaneous fat area ratio	0.53 (0.03)	0.18 (0.03)	<0.001

Data presented as mean (SEM). Adjusted for baseline age (for baseline waist circumference) or follow-up age (for follow-up waist circumference and DXA variables) and ethnic minority status. Visceral fat area, subcutaneous fat area, and their ratio further adjusted for total fat area.

Table 3

Change in waist circumference as a predictor of CRP, stratified by sex.

	Boys (n = 21)		Girls (n = 30)	
	CRP	Follow-up CRP	CRP	Follow-up CRP
Waist circumference	0.52 *	0.54 *	0.01	0.04

Standardized β reported. Adjusted for baseline CRP, follow-up age, and ethnic minority status. CRP = C-reactive protein.

* $p < 0.05$.