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Insomnia Symptoms with Objective Short Sleep Duration are Associated with Systemic Inflammation in Adolescents

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Abstract

Inflammation has been suggested as a potential pathway by which insomnia and short sleep can affect risk of morbidity in adults. However, few studies have examined the association of insomnia with inflammation in adolescents, despite accumulating evidence that pathophysiologic changes may already occur during this critical developmental period. The present study sought to examine the association of insomnia symptoms with systemic inflammation and the role of objective sleep duration in this association. Participants were 378 adolescents (16.9 ± 2.3 y, 45.8% female) from the Penn State Child Cohort, a population-based sample who underwent 9-hour polysomnography (PSG) followed by a single fasting blood draw to assess plasma levels of C-reactive protein (CRP) and other inflammatory markers. Insomnia symptoms were defined by a self-report of difficulties falling and/or staying asleep, while objective sleep duration groups were defined as a PSG total sleep time ≥ 8 , 8–7, and ≤ 7 hours. We assessed the association of insomnia symptoms, objective sleep duration, and their interaction with inflammatory markers, while adjusting for multiple potential confounders. Adolescents reporting insomnia symptoms had significantly higher levels of CRP compared to controls and a significant interaction ($p < 0.01$) showed that objective sleep duration modified this association. Elevated CRP was present in adolescents with insomnia symptoms and ≤ 7 hours of sleep (1.79 mg/L) as compared to controls or adolescents with insomnia symptoms and ≥ 8 hours of sleep (0.90 mg/L and 0.98 mg/L, respectively) or controls with ≤ 7 hours of sleep (0.74 mg/L; all p -values < 0.01). In sum, insomnia symptoms with objective short sleep duration are associated with systemic inflammation as early as adolescence. This study suggests that chronic low-grade inflammation may be a common final pathway towards morbidity in adulthood in this insomnia phenotype.

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Key terms

adolescents; C-reactive protein; inflammation; insomnia symptoms; short sleep duration

1. Introduction

Insomnia, particularly in adults with objective short sleep duration, has been associated with increased physiologic indices of hyperarousal, ranging from increased cortisol and norepinephrine levels, whole-body metabolic rate and impaired heart rate variability to increased high-frequency cortical dynamics, cerebral glucose metabolism and decreased gamma-aminobutyric acid levels. (Bonnet and Arand, 2010; Riemann et al., 2010; Vgontzas et al., 2013b) Furthermore, adults with insomnia and objective short sleep duration have been found to be at increased risk of hypertension, type 2 diabetes, neurocognitive impairment, depression and mortality. (Bathgate et al., 2016; Fernandez-Mendoza et al., 2015; Vgontzas et al., 2013) Thus, there is a need to identify biomarkers to target the diagnosis and treatment of these adverse health outcomes early in the life span. Chronic low-grade inflammation has been proposed as one of the potential paths by which insomnia can lead to adverse health outcomes. (Irwin, 2015; Vgontzas et al., 2002, 2013b) However, little is known about the association of adolescent insomnia with inflammation, despite accumulating evidence that the main pathophysiologic mechanism of insomnia, i.e., hyperarousal, may already be present during this critical developmental period (Fernandez-Mendoza et al., 2014, 2016; Ly et al., 2015; Zhang et al., 2014) This period is also when sleep disorders develop and, therefore, marks a point for potential interventions; indeed, the prevalence of insomnia symptoms increases with the onset of puberty (Calhoun et al., 2014), reaching a peak of 40% in adolescence, similar to the prevalence of insomnia symptoms in adults. (Ohayon, 2002)

An increasing field of research supports that disturbed sleep may play a role in the modulation of circulating inflammatory molecules such as C-reactive protein (CRP) and interleukin-6 (IL-6). A recent meta-analysis by Irwin et al. (2016) of 72 studies comprising 50,000 primarily middle-aged individuals demonstrated that insomnia symptoms were significantly associated with increased levels of CRP (effect size [ES] = 0.12) and IL-6 (ES = 0.20). To a lesser extent, shorter sleep duration was associated with significantly increased CRP (ES = 0.09) and IL-6 (ES = 0.11) levels. Interestingly, the association of shorter sleep duration with CRP and IL-6 was stronger in studies using objective sleep measures (ES = 0.18 and ES = 0.29, respectively) as compared to studies using subjective sleep measures (ES = 0.04 and ES = 0.03, respectively). Tumor necrosis factor alpha (TNF- α) levels were not significantly associated with either insomnia symptoms or shorter sleep duration in the studies covered by this meta-analysis. Despite this promising evidence, these effect sizes are modest and a potential explanation may be the lack of studies examining the joint effect of insomnia symptoms and objective sleep duration on inflammatory markers, as pointed out by Irwin et al. (2016). Furthermore, few studies have examined these associations across the lifespan, which will provide a better understanding of the association of insomnia symptoms and short sleep duration with inflammation early in the development of sleep disorders and other morbidity.

Indeed, the literature examining the association of insomnia symptoms or short sleep duration with inflammatory markers in adolescents is sparse. A study in 143 adolescents aged 13–18y found that shorter average sleep duration, as measured by actigraphy, was significantly correlated ($r = -0.29$) with increasing levels of CRP.(Larkin et al., 2005) Another recent study conducted in 188 healthy adolescents found that shorter sleep duration, as measured by self-report, was significantly correlated ($\beta = -0.17$) with increasing CRP levels only after controlling for age, sex and pubertal status.(Martinez-Gomez et al., 2011) However, there are no studies examining the association of insomnia symptoms with inflammation in adolescents and the role of objective sleep duration in this age group.

The overall aim of the present study is to gain a better understanding of the association between adolescent insomnia symptoms and objective short sleep duration with systemic inflammation. CRP was the primary outcome in the present study, while we also examined IL-6, TNF- α , leptin and adiponectin levels as secondary outcomes. We hypothesized that adolescents with insomnia symptoms, as measured by self-report, would have elevated CRP levels compared to adolescents without insomnia symptoms. We also hypothesized that adolescents with objective short sleep duration, as measured by polysomnography (PSG), would have elevated CRP levels compared to adolescents with normal sleep duration. Finally, we tested whether objective sleep duration modifies that association between insomnia symptoms and increased CRP levels in adolescents.

2. Materials and Methods

2.1. Participants

The Penn State Child Cohort (PSCC) is a general population sample of 700 children between ages 5–12 years, of whom 421 were followed up 8.4 years later as adolescents (mean age 17.0 ± 2.2 years, 53.9% male, and 21.9% ethnic minority). Baseline demographic characteristics were similar between those who did and did not participate in the follow-up study.(Bixler et al. 2016) The study protocol was approved by the Penn State University College of Medicine Institutional Review Board. 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.

2.2. Physical assessment

During their laboratory visit, adolescents underwent a physical examination and height (stadiometer Model 242, SECA Corp.; Hanover, MD) and weight (Model 758C, Cardinal Manufacturing; Webb City, MO) were measured.(CDC, 2011) Body mass index (BMI) was calculated (in kg/m^2) and converted to a percentile according to a formula based on the Centers for Disease Control's sex-specific BMI-for-age growth charts.(CDC, 2009) Pubertal development (Tanner staging) was determined via a self-administered rating scale. (Carskadon and Acebo, 1993) Participants identified their race/ethnicity from one of six options, as part of the clinical history. Based on the clinical history and physical examination, a composite variable of number of chronic health conditions was created, which included a history of conditions known to be associated with inflammation such as asthma, chronic sinusitis/rhinitis, arthritis/tendonitis, colitis, or hypertension. Furthermore,

parents or adolescents also reported whether the subject was taking any anti-inflammatory medication, which included NSAIDS, asthma or colitis medications.

2.3. Sleep laboratory protocol

All participants underwent a single-night, 9-hour PSG recording in a sound-attenuated, light- and temperature-controlled room with a comfortable, bedroom-like atmosphere. Each subject was continuously monitored from 22:00 h until 07:00 h using 14-channel recordings of electroencephalogram (EEG), electrooculogram (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 (SpO₂) 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), the number of apneas and hypopneas summed per hour of sleep, was ascertained.

Sleep parameters derived from PSG included sleep continuity variables such as sleep onset latency (SOL; the number of minutes to fall asleep since lights off), wake after sleep onset (WASO; the amount of time awake after the onset of sleep), total sleep time (TST; the total number of minutes slept since lights off until lights on), and sleep efficiency (SE; the amount of time spent asleep divided by the amount of time spent in bed) as well as sleep architecture parameters defined as the amount of time spent in each sleep stage (i.e., stage 1, stage 2, slow wave sleep [SWS] and rapid eye movement sleep [REM]) divided by total sleep time. From the PSG-measured TST, we split the overall sample into 3 ordinal groups based on the population quartiles: 8 hours (i.e., top 25% of the sample or 75th percentile), 7–8 hours (i.e., middle 50% of the sample), and 7 hours (i.e., bottom 25% of the sample or 25th percentile). Thus, we defined objective short sleep duration based on the distribution of TST in the overall sample, which is a distribution similar to that reported for this age range. (Ohayon et al., 2004)

2.4. Insomnia symptoms and other self-reported behaviors

Parent- and self-reported questionnaires were administered to measure behaviors including internalizing and externalizing symptoms, circadian preference, and insomnia symptoms. All participants older than 18 years completed the Adult Behavior Checklist (ABCL), while the parents of participants younger than 18 completed the Child Behavior Checklist (CBCL). (Achenbach and Rescorla, 2001) Internalizing and externalizing scales T scores from the C/ABCL were used to measure the severity of anxiety, depression, and somatic complaints and inattention, hyperactivity, rule-breaking behaviors etc., respectively. Circadian preference was measured using the Morningness-Eveningness Questionnaire (MEQ), which has been validated in both adults and adolescents. (Carskadon et al., 1993) Insomnia symptoms were considered present if participants answered yes to either or both of the following questions:

“do you have difficulty falling asleep” (DFA) or “do you have difficulty staying asleep” (DSA) from a self-report version of the Pediatric Sleep Questionnaire.(Chervin et al., 2000)

2.5. Blood draw and assay procedures

A blood sample was provided by 392 (93.1%) of the 421 participants at 7:00 following the evening PSG recording. Samples were collected in an ethylenediamine teraacetic acid (EDTA)-containing tube then centrifuged for 10 minutes at 3000 RPM. Plasma was aliquoted into cryotubes and stored at -80°C until assayed. Plasma high-sensitivity CRP, IL-6, TNF- α , leptin, and adiponectin were measured via enzyme-linked immunosorbent assay (ELISA; R&D Systems; Minneapolis, MN). The intra- and interassay coefficients of variation were 5.8% and 5.3%, respectively (CRP), 4.7% and 5.1% (IL-6), 4.6% and 4.9% (TNF α), 6.5% and 7.0% (leptin), and 5.6% and 5.6% (adiponectin). The lower detection limits were 0.010 ng/mL (CRP), 0.039 pg/mL (IL-6), 0.106 pg/mL (TNF α), 7.2 pg/mL (leptin), and 0.25 ng/mL (adiponectin). All samples and standards were run in duplicate.

2.6. Statistical analysis

Sociodemographic and physical characteristics of the insomnia symptoms and objective sleep duration groups were calculated using analysis of variance (ANOVA) for continuous variables and chi-square for categorical variables. A 2-way, full-factorial general linear model (GLM) was used to assess the association of insomnia symptoms, PSG sleep duration, and their interaction on the primary outcome, while controlling for the potential confounding effect of sex, age, race, SES, BMI percentile, MEQ, internalizing and externalizing behaviors, AHI, periodic limb movement index (PLMI), alcohol, caffeine, tobacco and drug use, number of chronic health problems, and use of anti-inflammatory medication, with Bonferroni correction for multiple comparisons (unless otherwise stated). Our two independent variables in the GLM were insomnia symptoms (no/yes) and objective sleep duration ($8/7-8/7$ hours), while our primary outcome was CRP levels. Our secondary outcomes included IL-6, TNF- α , leptin and adiponectin levels, for which the same GLM was used. Descriptive data from GLMs are reported as estimated marginal means and their standard error (SEM) after adjustment for all the covariates mentioned above. 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 22.0 (IBM Corp., Armonk, NY).

3. Results

3.1. Demographic and clinical characteristics of the sample

Out of the 392 adolescents who provided a fasting blood draw to assay for inflammation, a final sample of 378 had complete data on insomnia symptoms, objective sleep duration and the primary outcome (i.e., CRP). Overall, participants were 16.9 ± 2.3 years old and 45.8% were female. A total of 38% of the sample identified as fully-developed and 46% identified as late pubescent, while less than 16% of the sample identified as mid-pubescent or earlier stages. In terms of race/ethnicity, 6.5% identified as Hispanic, while 0.2% identified as American Indian, 1.0% Native Hawaiian/Asian, 9.0% Black or African American, and 79.8% White. The average BMI for the sample was at the 65.3 ± 1.4 percentile and 15%

were obese, the average waist circumference was $65.7 \text{ cm} \pm 0.5 \text{ cm}$. The average AHI was 2.7 ± 0.3 and the prevalence of SDB (AHI ≥ 2) was about 38%.

More than a third (36%) of the sample self-reported insomnia symptoms and 25% exhibited objective short sleep duration (i.e., ≤ 7 hours). As shown in Table 1, among individuals reporting insomnia symptoms there was a significantly greater proportion of girls (57.7% vs. 37.8%, respectively), evening-types (42.3% vs. 24.2 %, respectively) and adolescents of low SES (73.7% vs. 60.4%, respectively) as compared to controls. Furthermore, internalizing and externalizing scores were significantly higher in the insomnia symptoms group as compared to controls. Otherwise, no other significant differences were observed between the insomnia symptoms and control groups. Similarly, there were only a few statistically significant differences between objective sleep duration groups in terms of the sociodemographic or clinical characteristics (Table 1).

3.2 Sleep Characteristics of the Sample

Sleep characteristics were largely similar between adolescents who reported insomnia symptoms and those who did not. As shown in Table 2, adolescents who reported insomnia symptoms had a slightly longer sleep onset latency ($p=0.064$) as compared to those without insomnia symptoms, which was consistent with the fact that DFA was the most prevalent complaint (92.6%) among adolescents with insomnia symptoms (39.7% for DSA). These non-significant, small differences in PSG parameters further supported the existence of heterogeneity and potential subgroups among adolescents with insomnia symptoms. Furthermore, there were no significant differences in terms of number of apneas/hypopneas or periodic limb movements between the two groups. As would be expected, there were significant differences in sleep characteristics between the objective sleep duration groups in respect to sleep continuity and sleep architecture parameters. Interestingly, there was no significant difference in terms of the proportion of adolescents reporting insomnia symptoms (i.e., DFA/DSA) across the objective sleep duration groups (Table 2); specifically, there were 46, 57, and 34 adolescents with insomnia symptoms and 66, 113, and 62 without insomnia symptoms across the ≥ 8 hours, 7–8 hours and ≤ 7 hours objective sleep duration groups, respectively.

3.3 Association of insomnia symptoms and short sleep duration with inflammation

As shown in Table 3, adolescents who reported insomnia symptoms had significantly higher levels of CRP compared to adolescents without insomnia symptoms ($p < 0.01$) and adolescents who slept objectively ≤ 7 hours had significantly higher levels of CRP compared to those with objective normal sleep duration ($p < 0.05$). Importantly, the interaction between insomnia symptoms and objective sleep duration was statistically significant ($p < 0.001$), indicating a synergistic effect on CRP levels. As shown in Figure 1, elevated CRP levels were significantly higher ($1.79 \pm 0.18 \text{ mg/L}$) in adolescents who reported insomnia symptoms and slept objectively ≤ 7 hours as compared to all other groups but, most importantly, as compared to controls who slept ≤ 7 hours ($0.74 \pm 0.13 \text{ mg/L}$, $P = 0.00005$), to adolescents with insomnia symptoms who slept ≥ 8 hours ($0.98 \pm 0.14 \text{ mg/L}$, $P = 0.005$) or to adolescents with insomnia symptoms who slept 7–8 hours ($0.85 \pm 0.13 \text{ mg/L}$, $P =$

0.0004). Among controls, there were no significant differences in CRP levels across the three objective sleep duration groups (all Ps for least significant difference = 0.374).

There were no statistically significant differences in the secondary outcomes of IL-6, TNF- α , leptin or adiponectin levels associated with insomnia symptoms or objective sleep duration and none of the interactions were statistically significant (Table 3).

4. Discussion

This is the first study to examine the association of insomnia symptoms with objective short sleep duration with inflammation in a population-based sample of adolescents. Consistent with the adult literature, we found that insomnia symptoms and short sleep duration are associated with systemic inflammation in adolescents. Importantly, our study showed that elevated CRP levels are primarily present in adolescents who report insomnia symptoms and slept objectively ≤ 7 hours in the laboratory, and that this association is independent of demographic factors or comorbid factors frequently associated with insomnia symptoms or inflammation such as depression, anxiety, evening circadian preference, substance use or medical conditions, among others. Furthermore, other inflammatory biomarkers followed a similar pattern, but did not reveal statistically significant differences across groups. Our findings provide further evidence that objective measures of sleep duration in insomnia, even as early as adolescence, may be a useful marker of the biological severity and medical impact of the disorder. (Vgontzas et al., 2013)

In our study, adolescents with complaints of insomnia symptoms were associated with significantly elevated CRP levels. When we introduced the criterion of objectively-measured short sleep duration, we showed a strong and significant joint effect on the association of insomnia symptoms with increased CRP levels. Adolescents with insomnia symptoms who slept ≤ 7 hours had higher CRP levels than adolescents who slept > 7 hours and did not complain of insomnia symptoms (1.8 vs. 0.8 mg/L, respectively). In contrast, adolescents with insomnia symptoms who slept > 7 hours did not show increased CRP levels compared with the control group. CRP is a particularly stable biomarker that does not exhibit a circadian secretory pattern and is currently used to estimate risk of cardiovascular diseases. In adults, CRP levels < 1 mg/L are considered optimal, while levels 1–3 mg/L are indicative of average cardiovascular risk and those ≥ 3 mg/L indicative of high cardiovascular risk; (Ridker, 2003) however, no guidelines or cut-offs have been developed yet for adolescents. The average CRP levels observed in this study for the most severe group (adolescents with insomnia symptoms and objective short sleep duration) were 1.8 mg/L and twice as high as the other groups. Given their age (mean 17 years) and the fact that all other study groups had healthy CRP levels on average (< 1 mg/L), the observed differences may be indicative of potential cardiometabolic risk. Our finding on this synergistic effect of insomnia symptoms and objective sleep duration on CRP levels is consistent with previous reports that insomnia with objective short sleep duration is associated with physiologic hyperarousal and adverse health outcomes. (Bathgate et al., 2016; Vgontzas et al., 2013). Indeed, insomnia with objective short sleep duration has been associated with increased 24-hour activity of both limbs of the stress system (i.e., hypothalamic-pituitary-adrenal and sympatho-adrenal-medullary axes) and increased daytime secretion of pro-inflammatory cytokines. (Bonnet

and Arand, 2010; Irwin et al., 2003; Vgontzas et al., 1998, 2001, 2002, 2013; Zhang et al., 2014). It is likely that this 24-hour physiological hyperarousal is responsible for the dissociation beyond short sleep duration observed in adolescents with insomnia symptoms, a hypothesis that should be tested in future studies. Thus, the results of the present study further support the proposal that insomnia symptoms with objective short sleep duration are a more biologically severe phenotype, (Vgontzas et al., 2013) as indicated by increased systemic inflammation, and that this effect may be present as early as adolescence.

With respect to IL-6 and TNF- α levels, molecules tightly related to CRP, we did not find statistically significant differences across insomnia symptoms subgroups. These results are somewhat consistent with a previous meta-analysis in adults in which IL-6 and TNF- α , as compared to CRP, showed a weaker association with either insomnia symptoms or short sleep duration.(Irwin et al., 2015) However, we observed that the pattern of elevated IL-6 and TNF- α levels in adolescents with insomnia symptoms with short sleep duration was similar to that of CRP. A potential explanation could be found in the well-documented circadian secretory pattern of IL-6 and TNF- α (Vgontzas et al., 2005; Keller et al, 2009) as compared to the 24-hour stability of CRP. In fact, Vgontzas et al. (2002) reported no significant differences in mean 24-hour IL-6 or TNF- α secretion between controls and short sleeping insomniacs; however, in that sample of young adults with severe insomnia, there was a significant shift in the circadian secretion of IL-6 and TNF- α from nighttime to daytime peak (e.g., insomniacs had significantly elevated IL-6 levels during the 19:00–21:00 period when compared to controls, but not in the morning). (Vgontzas et al., 2002) It is likely that in the present study, this shift was not captured for IL-6 and TNF- α given that only a single morning fasting blood draw was obtained. Future studies should examine the joint effect of insomnia and short sleep duration on IL-6 and TNF- α levels using continuous 24-hour sampling.

Finally, leptin and adiponectin levels did not show a statistically significant association with insomnia symptoms or short sleep duration, nor did the observed pattern indicate any abnormal levels across objective sleep duration groups in adolescents with insomnia symptoms. Leptin and adiponectin are cytokines secreted by adipocytes (i.e., “adipokines”) and have not been previously hypothesized to be associated with insomnia, although some experimental studies demonstrate altered leptin levels in response to sleep deprivation. (Capers et al., 2015; Pejovic et al., 2010) However, these adipokines have been shown to be strongly associated with SDB, a disorder in which central obesity and visceral adiposity plays a key pathophysiologic role. (Bixler et al 2016; Tsaoussoglou et al, 2010) In fact, we have shown in this population-based sample that adolescent SDB is associated with significantly increased leptin and decreased adiponectin levels,(Gaines et al., 2016) which suggests that the inflammatory pattern found for insomnia symptoms with objective short sleep duration appears to be different from that of SDB in adolescents. Alternatively, it is also plausible to speculate that adolescents with insomnia symptoms and short sleep duration may have increased noradrenergic activity, (Irwin et al., 2003; Vgontzas et al., 1998) which is known to affect the secretion of these inflammatory markers. (Chrousos, 1995) Future studies should examine the association of this insomnia phenotype with sympathetic nervous system activation in adolescents.

Some limitations should be taken into account when interpreting the results of the present study. Despite the high response rate (60%) of the Penn State Child Cohort, the present study is limited in that it may not perfectly represent the adolescent general population. However, this population-based sample overcomes the limitations of clinical samples of adolescents in which multiple comorbidities may affect the relationships examined. Second, the determination of short sleep duration was from a single night of PSG recording and, therefore, may have been susceptible to the first night effect and may not be representative of the PSG sleep duration in the adolescent community. For example, adolescents may obtain insufficient sleep during schooldays and compensate for their sleep debt during weekends, which is something not captured by a single night of PSG. However, a recent study has shown that a single night of PSG for evaluating the relative classification of insomniacs as normal vs. short sleepers is a reliable and stable measure indicative of a subjects' physiologic sleep ability. (Gaines et al., 2015) Third, inflammatory makers were assayed at one single time point and did not include 24-hour sampling, which did not allow examining the circadian secretory pattern of cytokines and may explain the lack of some associations as discussed above. Fourth, insomnia status was assessed by the presence of the nighttime sleep symptoms of insomnia and did not include an assessment of duration, frequency or daytime functioning criteria that would have allowed establishing a diagnosis of an insomnia disorder. Future studies should examine the association of insomnia and objective sleep duration with inflammatory markers using a diagnostic definition of insomnia disorder in population-based, research-volunteer and clinical samples. Fifth, we did not have physical activity available in this study and, therefore, could not control for its' potential confounding effect. Finally, due to the cross-sectional nature of the study, no causal inferences can be made about the direction of the results.

In conclusion, insomnia symptoms with objective short sleep duration is associated with elevated inflammation in adolescents, and this association appears to be of a clinically significant magnitude in this general population sample. In contrast, insomnia symptoms with objective normal sleep duration did not show elevated inflammation as compared to controls. These findings are consistent with adult studies demonstrating that insomnia symptoms with objective short sleep duration is a more biologically severe phenotype of the disorder associated with activation of both limbs of the stress system and cardio-metabolic and neurocognitive morbidity. Future studies should examine the role of systemic inflammation in adolescence in predicting adverse health outcomes into adulthood in this insomnia phenotype.

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HIGHLIGHTS

- Insomnia symptoms with objective short sleep duration is associated with elevated inflammation in adolescents, while insomnia symptoms with objective normal sleep duration is not.
- This association appears to be of a clinically significant magnitude even in a general population sample of adolescents.
- Consistent with adult studies, insomnia symptoms with objective short sleep duration appears to be a more biologically severe phenotype of the disorder as early as adolescence.
- Future studies should examine the role of systemic inflammation in predicting adverse health outcomes in the insomnia with short sleep duration phenotype.

Figure 1. Association of adolescent insomnia symptoms with plasma CRP levels across objective sleep duration groups

Adolescents who reported insomnia symptoms and slept objectively less than 7 hours had increased CRP levels as compared to all other groups. In contrast, adolescents who reported insomnia symptoms and slept objectively more than 7 hours did not have significantly different CRP levels as compared to controls. Data are estimated marginal means after adjustment for sex, age, race, SES, BMI percentile, MEQ, internalizing and externalizing behaviors, AHI, PLMI, alcohol, caffeine, tobacco and drug use, number of chronic health problems, and use of anti-inflammatory medication. Error bars represent the standard error of the mean (SEM).

Table 1
Demographic characteristics of the sample stratified by insomnia symptoms and objective sleep duration

	Insomnia Symptoms			Objective Sleep Duration			
	No	Yes	P	8 hours	7–8 hours	7 hours	P
N	241	137		112	170	96	
Female (%)	37.8	57.7	<0.001	55.4	42.9	36.5	0.019
Ethnic-Minority (%)	20.3	25.5	0.241	23.2	22.9	19.8	0.801
Low SES (%)	60.4	73.7	0.009	71.2	59.4	68.8	0.091
Age (years)	16.9 ± 2.3	17.2 ± 2.3	0.182	16.7 ± 2.2	17.2 ± 2.4	17.1 ± 2.2	0.172
Tanner (%)			0.152				0.908
Prepubertal	0.9	1.5		0.9	1.2	1.1	
Early pubertal	0.9	1.5		1.9	1.2	0	
Mid puberty	16.3	7.6		10.2	13.3	16.3	
Late puberty	46.8	47.0		49.1	45.5	46.7	
Adulthood	35.2	42.4		38.0	38.8	35.9	
BMI% (percentile)	65.6 ± 27.8	68.9 ± 27.9	0.260	67.9 ± 28.6	67.5 ± 25.2	64.4 ± 31.2	0.609
BMI% 85 (%)	33.6	39.4	0.257	38.1	32.8	35.3	0.652
Eveningness (score)	26.7 ± 4.8	24.5 ± 5.2	<0.001	25.7 ± 4.8	26.5 ± 5.0	25.1 ± 5.5	0.126
M-type	38.3	21.9	<0.001	26.8	37.6	29.5	0.258
I-type	37.5	35.8		38.4	36.5	35.8	
E-type	24.2	42.3		34.8	35.8	34.7	
Internalizing (T score)	49.4 ± 10.0	52.9 ± 11.1	0.002	50.8 ± 9.5	50.6 ± 10.9	50.7 ± 11.4	0.987
Externalizing (T score)	47.4 ± 9.3	51.8 ± 11.1	<0.001	48.7 ± 10.1	49.3 ± 10.1	49.0 ± 10.6	0.887

Data are mean ± standard deviation, unless otherwise stated. AHI = apnea hypopnea index. BMI% = body mass index percentile. E-type = evening chronotype. I-type = intermediate chronotype. M-type = morning chronotype. SES = socioeconomic status.

Table 2
Sleep characteristics of the sample stratified by insomnia symptoms and objective sleep duration

	Insomnia Symptoms			Objective Sleep Duration			
	No	Yes	P	8 hours	7–8 hours	7 hours	P
N	241	137		112	170	96	
DFA/DSA (%)			N/A				0.753
None	100.0	0.0		58.9	66.5	64.6	
DFA/DSA	0.0	69.3		29.5	23.0	24.0	
Both	0.0	30.7		11.6	10.6	11.5	
Sleep onset latency	24.0 ± 19.3	28.8 ± 30.7	0.064	12.5 ± 6.4	24.3 ± 14.0	43.9 ± 37.0	<0.001
Awakes	36.7 ± 11.4	37.2 ± 13.9	0.703	33.5 ± 9.2	39.2 ± 12.1	36.5 ± 14.9	0.001
Wake after sleep onset	72.7 ± 45.1	67.2 ± 42.7	0.247	33.8 ± 10.3	63.7 ± 19.4	125.9 ± 47.0	<0.001
Total wake time	94.5 ± 52.1	94.1 ± 60.7	0.952	44.6 ± 11.4	86.2 ± 18.3	167.0 ± 54.8	<0.001
Total sleep time	445.6 ± 52.7	446.7 ± 61.6	0.855	496.6 ± 10.7	454.3 ± 18.0	372.1 ± 55.6	<0.001
Sleep efficiency	82.5 ± 9.7	82.5 ± 11.3	0.936	91.7 ± 2.1	84.0 ± 3.4	68.9 ± 10.2	<0.001
%Stage 1	1.1 ± 1.3	0.98 ± 1.9	0.658	0.5 ± 0.43	0.9 ± 1.1	1.9 ± 2.5	<0.001
%Stage 2	53.9 ± 9.2	53.3 ± 10.4	0.553	53.6 ± 8.6	53.7 ± 9.6	53.9 ± 10.9	0.966
%Stage 3	26.4 ± 8.7	27.1 ± 8.9	0.469	26.0 ± 7.7	26.3 ± 9.0	28.1 ± 9.5	0.191
%Stage R	18.6 ± 4.7	18.57 ± 5.8	0.969	19.9 ± 4.4	19.1 ± 4.5	16.1 ± 5.9	<0.001
PLMI	4.3 ± 6.5	3.2 ± 4.9	0.083	3.1 ± 5.4	3.8 ± 5.9	4.7 ± 6.7	0.175
< 5 events/hour	73.4	80.3	0.134	80.4	76.5	69.8	0.201
5 events/hour	26.6	19.7		19.6	23.5	30.2	
AHI	2.5 ± 3.1	2.8 ± 4.5	0.378	2.4 ± 3.8	2.5 ± 2.6	3.0 ± 5.0	0.420
< 2 events/hour	61.0	58.4	0.620	63.4	57.1	61.5	0.539
2 events/hour	39.0	41.6		36.6	42.9	38.5	

Data are mean ± standard deviation, unless otherwise stated. AHI = apnea hypopnea index. DFA/DSA = difficulty falling or staying asleep. PLMI = periodic leg movements index. Stage R = rapid eye movement sleep.

Table 3
Inflammatory characteristics of the sample stratified by insomnia symptoms and objective sleep duration

	Insomnia Symptoms			Objective Sleep Duration			
	No	Yes	P	8 hours	7-8 hours	7 hours	P
CRP (mg/L)	0.84 ± 0.07	1.21 ± 0.09	0.002	0.94 ± 0.10	0.86 ± 0.08	1.26 ± 0.11	0.011
IL-6 (pg/mL)	1.17 ± 0.07	1.24 ± 0.09	0.554	1.14 ± 0.09	1.16 ± 0.08	1.31 ± 0.10	0.400
TNF-α (pg/mL)	1.97 ± 0.10	1.93 ± 0.13	0.839	1.85 ± 0.14	1.85 ± 0.12	2.14 ± 0.16	0.269
Leptin (ng/mL)	12.55 ± 0.69	13.73 ± 0.93	0.328	13.60 ± 0.98	11.94 ± 0.82	13.90 ± 1.11	0.276
Adiponectin (μg/mL)	7.60 ± 0.35	8.01 ± 0.48	0.496	8.42 ± 0.49	7.59 ± 0.42	7.41 ± 0.56	0.324

Data are means ± standard errors adjusted for sex, age, race, SES, BMI percentile, MEQ, internalizing and externalizing behaviors, AHI, PLMI, alcohol, caffeine, tobacco and drug use, number of chronic health problems, and use of anti-inflammatory medication.

P for interaction between insomnia and objective sleep duration on CRP levels $p = 0.0002$ (see Figure 1); all other Ps for interaction 0.200