Title

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Publication Date

2019

Peer reviewed|Thesis/dissertation

The influence of inflammation on sleep, circadian functioning, and risk among adolescents with an evening circadian preference

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A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Psychology

in the

Graduate Division

of the

University of the California, Berkeley

Committee in Charge:

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Summer 2020

Abstract

The influence of inflammation on sleep, circadian functioning, and risk among adolescents with an evening circadian preference

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Background. Adolescence is associated with a shift toward an evening circadian preference, which is linked to increased risk across health domains. This study examined the influence of inflammation on sleep, circadian functioning, and health at pretreatment and following participation in a psychosocial intervention targeting sleep and health.

Method. Participants were 165 (96 female, average age = 14.7 years) adolescents randomized to receive 6-sessions of a psychosocial intervention targeting sleep and health. Sleep and circadian outcomes included weeknight total sleep time (TST), weeknight bedtime, and the Children's Morningness-Eveningness Preferences Scale. Health domains included emotional, cognitive, behavioral, social, and physical health. Sleep, circadian, and health outcomes were assessed at pretreatment, posttreatment, 6-month follow-up, and 12-month follow-up. Inflammatory markers were soluble tumor necrosis factor receptor 2 (sTNF-R2), interleukin (IL)-6, and C-reactive protein (CRP) measured at pretreatment.

Results. At pretreatment, shorter TST was associated with more emotional domain risk among adolescents with higher CRP. For adolescents with lower IL-6 at pretreatment, a greater evening circadian preference was associated with more behavioral risk. Inflammation also influenced treatment effects. Lower pretreatment sTNF-R2 was related to a decrease in self-reported evening circadian preference following treatment and through follow-up. Lower pretreatment IL-6 was associated with reduced behavioral and physical domain risk following treatment and through follow-up.

Conclusions. These findings support a growing body of literature showing that biological factors such as inflammation may influence sensitivity to positive and negative experiences on outcomes across key domains of health during adolescence.

Keywords: Adolescent; Circadian Rhythm; Health; Inflammation; Sleep.

The influence of inflammation on sleep, circadian functioning, and risk among adolescents with an evening circadian preference

Adolescence is associated with significant change across important domains of life. Although many adolescents thrive, this period is associated with increased emotional, cognitive, behavioral, social, and physical health risk. Hence, there is a need to identify mechanisms that exacerbate these risks. One potential contributor is the shift toward an evening circadian preference, which may be initiated by the onset of puberty (Carskadon, Vieira, & Acebo, 1993). An evening circadian preference is characterized by late bedtimes and waketimes, as well as increased physical and mental activity in the evening compared to the morning. Late weeknight bedtimes often combine with early school start times to lead to insufficient sleep and daytime impairment (Gradisar, Gardner, & Dohnt, 2011). The sleep disturbance resulting from an evening circadian preference contributes to heightened risk observed across emotional (Dagys et al., 2012), cognitive (Preckel, Lipnevich, Schneider, & Roberts, 2011), behavioral (McGlinchey & Harvey, 2015), social (Susman et al., 2007), and physical health domains (Arora & Taheri, 2015). Although existing research has identified that the consequences of an evening circadian preference may be related to risk across these domains, the mechanisms are not well defined.

The immune system has emerged as a biological pathway that contributes to development during adolescence (Brenhouse & Schwarz, 2016). The immune system is responsible for protecting the body from infection via an inflammatory response. Immune system activation is coordinated locally and systemically by immune system mediators following exposure to a pathogen (Hoebe, Janssen, & Beutler, 2004). Cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α are immune system molecules that orchestrate adaptive and innate immune response (Arai et al., 1990). Cytokines are commonly used in research with behavioral and psychological outcomes because cytokines influence both the central and peripheral nervous system (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Szelényi, 2001). C-reactive protein (CRP) is an acute phase protein produced by the liver during immune system activation, and is also frequently measured in studies assessing inflammation and psychological outcomes (Wium-Andersen, Ørsted, Nielsen, & Nordestgaard, 2013).

A reciprocal relationship has been observed in human and animal models between sleep regulation and immune system functioning (Bryant, Trinder, & Curtis, 2004). Although knowledge of the relationship between sleep disturbance and inflammation in adults is progressing (Irwin, Olmstead, & Carroll, 2015), comparatively less research has focused on adolescence. Of the existing studies conducted during adolescence, there is evidence that short sleep duration is associated with higher CRP (Hall, Lee, & Matthews, 2015; Park et al., 2016). Regarding circadian factors, sleeping in by two hours on weekends was twice as likely to be associated with high-risk CRP levels (Hall et al., 2015). These studies suggest that inflammation is a possible correlate of short sleep duration and a delayed circadian rhythm among adolescents. Additional research is needed to further characterize the association between inflammation and sleep and circadian functioning during adolescence.

Inflammation may also play a role in the increase in risk observed during adolescence (Brenhouse & Schwarz, 2016; Nusslock & Miller, 2016). In the emotional domain, higher CRP has been observed in adolescents with depression compared to controls (Tabatabaeizadeh et al., 2018). In the cognitive domain, higher CRP and IL-6 were associated with lower grades in youth ages 7 to 13 (Esteban-Cornejo et al., 2016). In the behavioral domain, higher CRP was related to increased substance use (Costello, Copeland, Shanahan, Worthman, & Angold, 2013). In the

social domain, interpersonal stress was associated with higher CRP (Miller, Rohleder, & Cole, 2009). In the physical health domain, inflammation has been consistently linked to indicators of metabolic syndrome (Gonzalez-Gil et al., 2017). These studies highlight that inflammation is associated with increased risk across multiple domains relevant to adolescent development. The preceding evidence also raises the possibility that inflammation may be involved in the association between sleep disturbance, a delayed circadian rhythm, and heightened risk observed during adolescence.

The differential susceptibility framework proposes that neurobiological factors such as inflammation moderate the influence of both negative and positive experiences, contexts, or environments on a variety of outcomes across development (Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van Ijzendoorn, 2011). In one of the foundational studies to inform the differential susceptibility framework, Boyce and colleagues (1995) reported that children with high immune and cardiovascular reactivity had more respiratory illness in high stress environments, but less respiratory illness in low stress environments. Furthermore, there is evidence that inflammation may enhance neural sensitivity to both positive and negative social experiences (Muscatell et al., 2016). Although less research has examined inflammation in positive or supportive contexts, high pretreatment inflammation has been linked to improved treatment response to electroconvulsive treatment (Kruse et al., 2018), exercise (Rethorst et al., 2013), and a TNF-α antagonist (Raison et al., 2013) among adults with depression. There is also evidence that lower inflammation may be related to better treatment response in adults with depression or chronic pain (Eller, Vasar, Shlik, & Maron, 2008; Lanquillon, Krieg, Bening-Abu-Shach, & Vedder, 2000; Lasselin et al., 2016). These studies provide evidence that inflammation may moderate the effect of positive and negative experiences on social and emotional outcomes. Further research is needed to examine how inflammation may influence response to both positive and negative experiences during adolescence, a critical developmental period, and across additional domains relevant to adolescence including emotional, cognitive, behavioral, social, and physical health domains.

The goal of this study was to test hypotheses informed by the differential susceptibility framework regarding the influence of inflammation on sleep, circadian functioning, and health in adolescents with an evening circadian preference. This study is one of the first to examine how inflammation may relate to both negative (e.g., sleep and circadian dysfunction) and positive (e.g., a psychosocial intervention) contexts across multiple domains relevant to adolescent development. The first aim was to evaluate if the interaction between inflammation and sleep or circadian dysfunction was related to risk across five health domains (emotional, cognitive, behavioral, social, and physical health). It was hypothesized that adolescents with high inflammation in the context of indicators of sleep and circadian dysfunction (shorter weeknight total sleep time, later weeknight bedtimes, or a greater self-reported evening circadian preference) would exhibit worse outcomes across these five health domains compared to adolescents with lower inflammation. The second aim was to examine if pretreatment inflammation influences sleep, circadian functioning, or risk across five health domains following a psychosocial intervention targeting sleep and health. It was hypothesized that adolescents with higher pretreatment inflammation would exhibit improved sleep, circadian functioning, and health domain functioning following treatment and through 6-month and 12-month follow-up compared to adolescents with lower pretreatment inflammation. These hypotheses were tested in a high-risk sample of adolescents who report both an evening circadian preference and heightened risk in one or more of these five health domains given that not all

adolescents experience sleep disturbance, an evening circadian preference, or increased risk in these health domains.

Method

Participants

The 165 participants (96 female, average age = 14.7 years) were enrolled in a randomized controlled trial to improve sleep and circadian functioning among adolescents with an evening circadian preference (Harvey et al., 2018). A total of 396 participants were assessed for eligibility, and 220 (55.6%) were excluded for not meeting inclusion criteria (n = 154) or refusing to participate (n = 66). One-hundred and seventy-six participants were enrolled and all provided saliva samples for assay. Eleven (6.3%) participants were not included because inflammatory marker levels were not detectable by the assay. All study procedures were approved by the University of California, Berkeley Institutional Review Board. Parents or guardians of all participants provided informed consent and participants provided informed assent.

Inclusion criteria were: (a) age between 10 and 18 years old, living with a parent or guardian, and attending a class or job by 9am at least 3 days per week; (b) fluent in English; (c) able and willing to give informed assent; (d) self-reported evening circadian preference as demonstrated by scoring within the lowest quartile of the Children's Morningness-Eveningness Preferences Scale (CMEP; 27 or lower; Dagys et al., 2012) and a 7-day sleep diary showing a sleep onset time of 10:40pm or later for 10-13 year olds, 11:00pm or later for 14-16 year olds, and 11:20pm or later for 17-18 year olds at least 3 nights per week for the last 3 months (Gianotti & Cortesi, 2002; Maslowsky & Ozer, 2014); and (e) 'at risk' range on measures of at least one of the five health domains (see Table 1).

Exclusion criteria were: (a) an active, progressive physical illness or neurological degenerative disease directly related to the onset and course of the sleep disturbance; (b) evidence of obstructive sleep apnea, restless legs syndrome, or periodic limb movement disorder (those presented with provisional diagnoses of these disorders were referred for a polysomnography evaluation at the parent's discretion and were enrolled only if the diagnosis was disconfirmed); (c) significantly impairing pervasive developmental disorder; (d) bipolar disorder, schizophrenia, or another current disorder if there was a risk of harm if treatment was delayed. Participants ceased taking medications that alter sleep (e.g., hypnotics) 4 weeks prior to the assessment (2 weeks for melatonin) or were excluded; and e) a history of substance dependence in the past six months or current suicide risk sufficient to preclude treatment on an outpatient basis.

Treatments

Participants were randomized to the Transdiagnostic Sleep and Circadian Intervention for Youth (n = 83) or psychoeducation (n = 82). Both treatments consisted of a total of six 50-minute sessions delivered over six weeks during the school year by doctoral or masters-level therapists. Results from this randomized controlled trial are reported elsewhere (Harvey et al., 2018). Between the 6-month and 12-month follow-ups, adolescents were randomized to receive text messages with treatment information reminders (n = 42), text messages with treatment recall prompts (n = 47), or no text messages (n = 47). Twenty-nine (17.6%) participants did not enroll in this portion of the study. The results pertaining to the text messaging treatment phase are reported elsewhere (Dolsen, Dong, & Harvey, In preparation).

Transdiagnostic Sleep and Circadian Intervention for Youth (TranS-C). As described elsewhere (Harvey & Buysse, 2017), TranS-C was developed based on sleep and circadian principles and was derived from Cognitive Behavior Therapy for Insomnia, Interpersonal and Social Rhythm Therapy, Chronotherapy, and Motivational Interviewing. The first session focused on case formulation, goal setting, motivational interviewing, and sleep and circadian education. Subsequent sessions included behavioral modules (e.g., stimulus control, sleep restriction, and regularizing sleep-wake times) and cognitive modules (e.g., correcting unhelpful sleep-related beliefs and reducing sleep-related worry).

Psychoeducation (PE). PE provided information about sleep, stress, diet, health, exercise, accidents, and mood. Participants also selected mediation, yoga, and/or outdoor appreciation activities. PE did not facilitate or plan for behavior change.

Materials and Procedure

Sleep, circadian preference, and health domain measures described below were collected at pretreatment, posttreatment, 6-month follow-up, and 12-month follow-up assessments and assessors were blind to treatment allocations. Inflammation was collected at pretreatment only.

Sleep and Circadian Outcomes. Sleep diary was collected each morning by phone for one week before the pretreatment and posttreatment assessments. The sleep diary was based on the Expanded Consensus Sleep Diary for Morning (Carney et al., 2012). An a priori decision was made to focus on weeknight TST and bedtime because these measures best address the sleep problems observed during adolescence (Gradisar et al., 2011). The 10-item Children's Morningness-Eveningness Preference Scale (CMEP; range 10-43; lower scores = greater evening circadian preference) is a well-validated measure of circadian preference for children and adolescents with good reliability (Borisenkov, Perminova, & Kosova, 2010; Carskadon, Seifer, & Acebo, 1991; Carskadon et al., 1993; Kim, Dueker, Hasher, & Goldstein, 2002).

Health domain outcomes. Five composite scores, each composed of measures of emotional, cognitive, behavioral, social, and physical health, were used to indicate functioning in five health domains. The composite scores were calculated as the mean of the standardized summary scores for measures within each of the five health domains. Summary scores for select measures were reverse coded when necessary to ensure that all scores within a domain would have the same direction. Specific measures for each domain are described below.

Emotional domain. The emotional domain included the 17-item Children's Depression Rating Scale-Revised (CDRS; range 17-113; higher scores = worse depression; Poznanski et al., 1984) and the 39-item Multidimensional Anxiety Scale for Children (MASC; range 0-117; higher scores = worse anxiety; March, Parker, Sullivan, Stallings, & Conners, 1997). The MASC has demonstrated excellent internal consistency, satisfactory to excellent test-retest reliability, and adequate convergent and divergent validity (March et al., 1997). The CDRS has shown good reliability and validity (Mayes, Bernstein, Haley, Kennard, & Emslie, 2010). The CDRS and MASC were positively correlated in the present study ($r = 0.30$, $p < .001$).

Cognitive domain. The cognitive domain was measured by the 20-item Attentional Control Scale (ACS; range 4-80; Derryberry & Reed, 2002) and the six school-related items from the Youth Social Adjustment Scale—Self Report (YSAS; Weissman, Orvaschel, & Padian, 1980). The ACS was reverse coded such that higher scores meant greater risk on the cognitive composite. The ACS and YSAS have demonstrated adequate reliability and validity (Judah, Grant, Mills, & Lechner, 2014; Weissman et al., 1980). The ACS and YSAS school-related items were positively correlated in this study ($r = 0.23$, $p = .003$).

Behavioral domain. The behavioral domain was assessed with the 8-item Brief Sensation Seeking Scale (BSSS; Hoyle, Stephenson, Palmgreen, Lorch, & Donohew, 2002) and the Alcohol and Substance Use Questionnaire (ASU; Johnston, O'Malley, Bachman, & Schulenberg, 2009) to assess consumption of alcohol and recreational drugs in the past 30 days (1-7 rating scale; higher scores = more frequent use). Caffeine use was also added to the ASU. The BSSS and ASU have demonstrated good validity and reliability in adolescents (Hoyle et al., 2002; Johnston et al., 2009; O'Malley, Bachman, & Johnston, 1983). The BSSS and ASU were positively correlated in the present study ($r = 0.35$, $p < .001$).

Social domain. The social domain included the friends, family, and romantic relationships subscales from the Youth Social Adjustment Scale—Self Report (YSAS; higher scores = worse adjustment; Weissman et al., 1980). The YSAS friends subscale was positively correlated with the family subscale ($r = 0.51$, $p < .001$). The YSAS romantic relationships subscale was weakly correlated with both the friends ($r = -0.05$, $p = .477$) and family ($r = -0.10$, $p = .233$) YSAS subscales in the present study.

Physical domain. The physical domain was assessed with the Modifiable Activity Questionnaire for Adolescents (MAQ; hours per week being active/exercising; Aaron & Kriska, 1997) and the Physical Health Questionnaire-15 (PHQ; range 0-30; higher scores = more somatic complaints; Kroenke, Spitzer, & Williams, 2002). The PHQ has demonstrated excellent internal consistency and high validity with other measures of somatic symptoms (Kroenke et al., 2002). The MAQ has been shown to be a valid and reliable measure of physical activity (Aaron & Kriska, 1997; Kriska et al., 1990). The MAQ was reverse coded such that higher scores meant greater risk on the physical composite. The MAQ and PHQ were not significantly correlated ($r = 0.01$, $p = .880$).

Inflammation. Oral mucosal transudate (OMT) samples were collected upon awakening with OraSure devices (OraSure Technologies, Bethlehem, PA). OMT has been validated for assessing cytokines and is correlated with plasma (Nishanian, Aziz, Chung, Detels, & Fahey, 1998). Oral fluids were centrifuged at 800g for 15 minutes and then stored at -80ºC until processed. Biochemical analyses were conducted by ProNovus Bioscience (Mountain View, CA) with IL-6 and soluble TNF receptor 2 (sTNF-R2) Quantikine ELISA kits (R&D Systems, Minneapolis, MN). sTNF-R2 reflects TNF activity and is more stable than measuring TNF directly (Diez-Ruiz et al., 1995). Assay sensitivities were 0.11 pg/mL and 2.3 pg/mL for IL-6 and sTNF-R2, respectively. Intra- and inter-assay coefficients of variation (CV) for the IL-6 ELISA were 3.6% and 5.2%, respectively. Intra- and inter-assay CV for the sTNF-R2 ELISA were 0.6% and 1.02%, respectively. All assay kits were validated and all samples were assayed on the same lot. A secondary assay for CRP was performed on saliva samples collected using untreated Sarstedt Salivettes (Sarstedt, Germany). CRP levels collected with salivettes are comparable to levels obtained with passive drool (Topkas, Keith, Dimeski, Cooper-White, & Punyadeera, 2012). The CRP assays were conducted by Salimetrics using ELISA kits for CRP (Carlsbad, California). Assay sensitivity was 10.0 pg/ml, the intra-assay CV was 1.9-5.9%, and inter-assay CV was 3.7-11.2%. CRP, IL-6, sTNF-R2 were non-normally distributed and were log transformed to better approximate a normal distribution (see Table 2). CRP, sTNF-R2, and IL-6 were selected because previous research indicates that these inflammatory markers are associated with sleep as well as the health domains included in this study.

Statistical analysis

Data analysis was conducted in R (R Development Core Team, 2017). The first aim tested five separate linear models for each of the health domains (emotional, cognitive,

behavioral, social, or physical health). Each model included the main effect of covariates (age, gender, and BMI), the main effect of sleep or circadian variable at pretreatment (weeknight TST, weeknight bedtime, and CMEP), the main effect of inflammation at pretreatment (IL-6, sTNF-R2, and CRP), and nine interaction terms for each combination of inflammation by sleep or circadian variable. The outcome for aim one was the health domain composite measured at pretreatment.

Aim two used hierarchical linear modeling (HLM) with restricted maximum likelihood estimation. All HLM analyses included a random intercept for participant. Five separate HLMs were tested for each health domain. Each model included the following fixed effects: pretreatment inflammation (IL-6, sTNF-R2, and CRP), time (0 = pretreatment, 1 = posttreatment, 2 = 6-month follow-up, and 3 = 12-month follow-up), and three interaction terms for each combination of inflammation by time. The fixed part of these models also included covariates (age, gender, and BMI), an indicator for text messaging condition (0 = no text messages, 1 = repetition text messages, 2 = recall prompt text messages), and an indicator for treatment condition (0 = PE, 1 = TranS-C). Analyses were conducted across treatment condition because PE is an active control and both TranS-C and PE improve sleep and health (Harvey et al., 2018). The outcome for aim two was the health domain composite measured at pretreatment, posttreatment, 6-month follow-up, and 12-month follow-up.

The significance of interaction terms were tested with an omnibus ANOVA. An alpha level of $p < .05$ was used for all analyses. Simple slopes were used to investigate significant interactions and were determined by one standard deviation (SD) above and below the mean for inflammation variables (+1 SD: 'higher inflammation'; -1 SD: 'lower inflammation'). To reduce the number of comparisons, we first examined effects for the health domain composites and conducted subsequent analyses for the component measures following a significant effect for the composite.

Results

Pretreatment associations between study variables

Pretreatment demographics, sleep and circadian outcomes, health domains, and inflammation descriptive statistics are displayed in Table 2. Intercorrelations for inflammation, sleep and circadian outcomes, and the five health domains composites at pretreatment are reported in Table 3. Correlations indicated that sTNF-R2 was associated with IL-6 with a medium-large effect size ($r = 0.53$, $p < .001$). Small, non-significant correlations were observed between CRP and sTNF-R2 ($r = -0.08$, $p = .370$) as well as CRP and IL-6 ($r = -0.15$, $p = .111$). IL-6 was negatively correlated with weeknight bedtime with a small-medium effect size ($r = -0.18$, $p = .026$). CRP and sTNF-R2 were correlated with sleep, circadian preference, and the five health domains with non-significant, small effect sizes (Table 3).

A greater evening circadian preference as measured by the CMEP was associated with greater risk on the cognitive domain composite ($r = -0.17$, $p = .030$) and the behavioral domain composite ($r = -0.19$, $p = .013$) with small-medium effect sizes. Weeknight TST was negatively associated with weeknight bedtime with a medium-large effect size ($r = -0.67$, $p < .001$) and negatively correlated with the social domain composite with a small-medium effect size ($r = -0.16$, $p = .049$). Circadian preference, weeknight TST, and weeknight bedtime were correlated with sleep, circadian preference, and the other health domains with non-significant, small effect sizes (Table 3).

The emotional domain was positively correlated with the social domain composite (r = 0.42, p < .001) and the physical domain composite (r = 0.27, p < .001) with medium effect sizes. The cognitive domain composite was positively correlated with the behavioral domain composite with a small-medium effect size (r = 0.17, p = .032). All other intercorrelations between health domains were non-significant with small effect sizes (Table 3).

Pretreatment inflammation, sleep, circadian preference, and health domains

We tested the interaction between sTNF-R2, IL-6, and CRP with sleep or circadian variables on the five health domains at pretreatment (Table 4). In the emotional domain, the interaction between CRP and weeknight TST was significantly associated with the emotional domain composite and the Children's Depression Rating Scale (CDRS; Table 4). Simple slopes revealed a medium effect size association between shorter TST and higher risk on the emotional domain composite and greater depression severity for adolescents with higher CRP (composite: b = -0.012, p = .002, d = -0.59; CDRS: b = -0.057, p = .010, d = -0.47), but small effect size associations for lower CRP (composite: b = 0.001, p = .835, d = 0.04; CDRS: b = 0.014, p = .473, d = 0.13).

In the behavioral domain, the interaction between IL-6 and CMEP was associated with the behavioral domain composite and the Alcohol and Substance Use Questionnaire (ASU; Table 4). For adolescents with higher IL-6, the CMEP was associated with the behavioral domain composite (b = 0.002, p = .967, d = 0.01) or the ASU (b = -0.006, p = .978, d = 0.00) with small effect sizes. For adolescents with lower IL-6, however, a greater evening circadian preference was associated with higher risk on the behavioral domain composite (b = -0.128, p = .003, d = -0.48) and more alcohol and substance use (b = -0.624, p = .001, d = -0.56) with medium effect sizes.

The interaction between inflammation and sleep or circadian preference was not significantly associated with the cognitive, social, or physical domain composites (Table 4).

Pretreatment inflammation and change following treatment

We next examined if pretreatment inflammation was associated with sleep, circadian preference, or health domain change following treatment and through 6-month and 12-month follow-up (Table 5). There was a significant sTNF-R2 by time interaction for CMEP, but not weeknight TST and bedtime (Table 5). For adolescents with lower pretreatment sTNF-R2 compared to higher pretreatment sTNF-R2, there was a small-medium effect size reduction in self-reported evening circadian preference from pretreatment to posttreatment **(**b = -1.260, p = .008, d = -0.31) and pretreatment compared to 6-month follow-up (b = -1.218, p = .020, d = -0.27).

In the behavioral domain, there was a significant IL-6 by time interaction for the behavioral domain composite and the Brief Sensation Seeking Scale (BSSS; Table 5). For adolescents with lower pretreatment IL-6 compared to higher pretreatment IL-6, there was a small-medium effect size decrease in risk on the behavioral domain composite and the BSSS from pretreatment to 12-month follow-up (composite: b = 0.140, p = .021, d = 0.28; BSSS: b = 0.775, p = .008, d = 0.32), posttreatment to 12-month follow-up (composite: b = 0.176, p = .006, d = 0.34; BSSS: b = 0.651, p = .032, d = 0.26), and 6-month to 12-month follow-up (composite: b = 0.159, p = .012, d = 0.31; BSSS: b = 0.868, p = .005, d = 0.34).

In the physical domain, the interaction between CRP and time was associated with the physical domain composite and the Physical Health Questionnaire (PHQ; Table 5). For adolescents with lower pretreatment IL-6 compared to higher pretreatment IL-6, there was a decrease in risk on the physical domain composite and the PHQ from pretreatment to 12-month

follow-up (composite: $b = 0.188$, $p = .059$, $d = 0.23$; PHQ: $b = 0.744$, $p = .012$, $d = 0.30$), posttreatment to 12-month follow-up (composite: $b = 0.278$, $p = .007$, $d = 0.33$; PHQ: $b = 0.755$, $p = .013$, $d = 0.30$), and 6-month to 12-month follow-up (composite: $b = 0.236$, $p = .020$, $d = 0.28$; PHQ: $b = 0.842$, $p = .005$, $d = 0.34$).

Pretreatment inflammation was not significantly associated with emotional, cognitive, or social domain change following treatment (Table 5).

Discussion

The present study tested hypotheses informed by the differential susceptibility framework regarding associations between inflammation and sleep or circadian preference on emotional, cognitive, behavioral, social, and physical health outcomes in adolescents. The first aim tested the hypothesis that greater sleep disturbance or evening circadian preference would be associated with more risk in the five health domains for adolescents with higher inflammation compared to lower inflammation. In the *emotional domain,* shorter sleep duration was associated with higher risk on the emotional domain composite and greater depression severity for adolescents with higher CRP. Both short sleep duration and higher CRP have been previously linked to emotional problems during adolescence (Dagys et al., 2012; Tabatabaeizadeh et al., 2018). The present findings add to this literature by providing evidence that shorter sleep duration in the context of higher inflammation may contribute to worse depression for adolescents with an evening circadian preference.

Regarding the *behavioral domain,* a greater evening circadian preference was related to higher risk on the behavioral domain composite and more alcohol and substance use for adolescents with lower IL-6. Prior studies have reported that sleep behaviors characteristic of an evening circadian preference are associated with more substance use (McGlinchey & Harvey, 2015). In the current study, it was surprising that this association was only observed for adolescents with lower IL-6. One possible explanation was that our measure of substance use included caffeine use, which has been linked to downregulation of inflammatory pathways (Iris, Tsou, & Sawalha, 2018). Furthermore, IL-6 has both pro-inflammatory and anti-inflammatory properties depending on the signaling pathway (Scheller, Chalaris, Schmidt-Arras, & Rose-John, 2011). Additional research is needed to better understand the potential role of inflammation in the relationship between an evening circadian preference and alcohol or substance use during adolescence.

We did not find evidence that pretreatment inflammation moderated the influence of sleep or circadian preference on the *cognitive*, *social*, or *physical domains*. Although previous research indicates that sleep disturbance, a delayed circadian rhythm, and inflammation may be associated with the cognitive (Esteban-Cornejo et al., 2016; Preckel et al., 2011), social (Miller et al., 2009; Susman et al., 2007), and physical domains (Arora & Taheri, 2015; Gonzalez-Gil et al., 2017), there is comparatively less research in these domains relative to the emotional domain. Indeed, there is growing evidence that sleep disturbance and inflammation are putative mechanisms associated with the onset and course of depression across the lifespan (Armitage, 2007; Dagys et al., 2012; Raison, Capuron, & Miller, 2006; Tabatabaeizadeh et al., 2018). Furthermore, we recruited adolescents with an evening circadian preference. Future research would benefit from including a morning circadian preference comparison group.

The second aim was to examine the influence of pretreatment inflammation on sleep, circadian preference, and health domain change following a psychosocial intervention. Lower levels of pretreatment sTNF-R2 were associated with decreased self-reported evening circadian

preference from pretreatment to posttreatment and pretreatment compared to 6-month follow-up. Pretreatment inflammation was also associated with change in the *behavioral* and *physical* health domains. Lower levels of pretreatment IL-6 and CRP were associated with reduced sensation seeking and physical health problems, respectively, following treatment and through 6- and 12-month follow-up. These findings for inflammation in the context of treatment do not appear to be explained by the differential susceptibility framework. Higher rather than lower levels of inflammation would be hypothesized to confer greater sensitivity to a psychological intervention (de Villiers, Lionetti, & Pluess, 2018; Pluess & Belsky, 2013). Instead, the present study indicated that lower levels of inflammation were associated with improved treatment response, which is consistent with prior studies demonstrating that lower inflammation is associated with better response to psychosocial and pharmacological treatments (Eller et al., 2008; Lanquillon et al., 2000; Lasselin et al., 2016). Although we hypothesized that higher levels of inflammation would enhance sensitivity to a psychosocial intervention based on prior research (Boyce et al., 1995; Muscatell et al., 2016), it is possible that higher levels of inflammation may make it more challenging to engage with treatment by negatively impacting emotional, cognitive, and social processes (Lasselin et al., 2016; Lopresti, 2017). These findings may also suggest that individuals with higher levels of pretreatment inflammation could benefit from additional or different types of treatment. However, caution is warranted given that this study was not designed to establish clinical cut-offs for treatment selection.

We did not find evidence that pretreatment inflammation influenced weeknight TST or bedtime change. Both short and long TST are associated with worse outcomes (Buysse, 2014), and our calculation of TST may not reflect improvement for short sleepers (i.e., a lengthening of TST) and long sleepers (i.e., a shortening of TST). Additionally, bedtime was calculated as the time when adolescents got into bed, which may not represent the time that adolescents disengage from technology and try to initiate sleep (Exelmans & Van den Bulck, 2015, 2017). Future studies should consider measures of sleep that address the complexity of adolescent sleep problems. We also did not observe that pretreatment inflammation was related to change in the *emotional*, *cognitive*, or *social domains* following treatment. The present study measured basal levels of inflammation rather than reactivity following an experimental stressor. Although basal variation of biological processes is consistent with the differential susceptibility framework (Boyce, 2016), much of the empirical support for this theory has focused on physiological and biological reactivity to experimental tasks. Further, a meta-analysis indicated that basal levels of cortisol were not associated with psychosocial treatment response among patients with anxiety disorders (Fischer & Cleare, 2017). Future research may benefit from examining immune system reactivity following an experimental manipulation. Given the mixed findings regarding pretreatment inflammation on sleep and health domain change, additional research is needed to precisely define the role of inflammation on treatment response among at-risk adolescents.

Although this study provides promising support for the hypothesis that inflammation may influence sleep, circadian functioning, and select health domains during adolescence, there are several limitations that should be noted. First, analyses examining sleep, circadian preference, and inflammation at pretreatment were cross-sectional and it is not possible to determine the directionality of the effects. For instance, depression may lead to inadequate sleep, which may increase inflammation. Second, although a strength of the present study was the use of an objective measure of inflammation, this study utilized self-reported measures of sleep and the health domains. Although these measures are well-validated, including objective measures of sleep or multiple informant reports of the health domains should be a priority in future research.

Third, this study was conducted within the context of a randomized controlled trial (Harvey et al., 2018), and may be underpowered. Although it is not advised to conduct a post-hoc power analysis (Levine & Ensom, 2001), if we were to replicate this study using the observed effect sizes, a sample size of approximately 300 participants would be required to achieve 80% power (Faul, Erdfelder, Lang, & Buchner, 2007). Thus caution is warranted regarding the interpretation and generalizability of the effects described in this study. Finally, multiple comparisons were used. Procedures that correct for multiple comparisons tend to reduce power and increase the likelihood of type II error (Nakagawa & Cuthill, 2007). Additionally, given that the health domain composites were correlated, correcting for multiplicity would likely be too conservative. Rather than correct for multiple comparisons, our interpretation of results focused on effect sizes in addition to traditional significance criteria. Future studies should also be conducted to examine whether findings are replicated.

In sum, adolescence is a developmental period associated with significant risk as well as opportunities to thrive. The current study provides evidence that the combination of higher inflammation and shorter weeknight sleep duration may confer risk for depression symptoms whereas a greater evening circadian preference and lower inflammation may be related to more alcohol and substance use. In the context of a psychosocial intervention, lower pretreatment inflammation was related to a shift away from an evening circadian preference as well as reduced behavioral and physical domain risk after treatment and through 6- and 12-month follow-up. The present study represents an important initial step towards identifying biological factors that may contribute to heightened sensitivity to the effects of positive and negative experiences on outcomes across key domains of health during adolescence.

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Table 1. Inclusion criteria for risk in the emotional, behavioral, cognitive, social, and physical domains.

Health Domain	Criteria for Inclusion
Emotional	• Greater than four on Difficulty Having Fun, Social Withdrawal, Irritability, Depressed Feelings, Excessive Weeping on the CDRS, *or* • T-score of 61 or above on the MASC, based on age group (10-11 years, 12-15 year, 16-19 years) using the MASC Profile.
Behavioral	• A BSSS score greater than 3.93 for males aged 10-13, greater than 3.19 for females aged 10-13, greater than 4.07 for males aged 14-18, or greater than 3.19 for females aged 14-18, *or* • Taking medication prescribed for attention-deficit/hyperactivity disorder (ADHD), *or* • A diagnosis of ADHD from the KSADS, which was administered by a doctoral-level graduate student, *or* • Current alcohol or substance abuse assessed by the KSADS.
Social	• A CBCL parent rating of their child as "worse" than peers on one or more of the social behavior items (Section VI).
Cognitive	• A CBCL parent rating of their child as “failing” in one or more academic classes (Section VII).
Physical	• Greater than four on the PHQ, *or* • Six or more days of school absences, *or* • BMI above the 85th percentile for the participant's sex and age.

CDRS: Children's Depression Rating Scale; MASC: Multidimensional Anxiety Scale for Children; BSSS: Brief Sensation Seeking Scale; KSADS: Kiddie Schedule for Affective Disorders and Schizophrenia; CBCL: Child Behavior Checklist; PHQ: Physical Health Questionnaire; BMI: Body Mass Index.

Table 2. Pretreatment demographics, sleep and circadian outcomes, health domains, and inflammation characteristics (N = 165).

Characteristic	*M*	*SD*	Skew	Kurtosis
Age (Years)	14.7	1.9	-0.27	-0.50
Body Mass Index	22.3	4.8	1.28	2.37
Inflammation				
sTNF-R2 (unadjusted; pg/ml)	35.02	25.26	1.27	1.76
sTNF-R2 (Log transformed; pg/ml)	3.25	0.90	-0.91	0.46
IL-6 (unadjusted; pg/ml)	5.67	11.54	3.39	11.57
IL-6 (Log transformed; pg/ml)	0.36	1.74	0.07	-0.69
CRP (unadjusted; pg/ml)	6796.80	16359.48	6.70	56.74
CRP (Log transformed; pg/ml)	7.87	1.21	0.90	0.37
Sleep and circadian variables				
CMEP	21.26	3.78	-0.43	-0.61
TST (Weeknight; Minutes)	456.31	63.85	-0.18	-0.32
Bedtime (Weeknight; Decimal hours)	22.96	1.06	0.42	0.04
Emotional domain				
CDRS	32.93	9.00	0.61	1.37
MASC	46.33	16.84	0.30	-0.35
Composite	-0.04	1.57	0.52	0.28
Cognitive domain				
ACS	51.15	7.73	-0.33	1.52
YSAS (School/Cognitive items)	11.80	2.82	0.47	0.02
Composite	0.03	1.23	0.29	0.96
Behavioral domain				
Alcohol and Substance Use	-0.05	1.61	0.95	1.88
BSSS	18.62	4.70	0.53	0.25
Composite	12.12	3.62	0.60	0.40
Social domain				
YSAS: Friends	18.62	4.70	0.53	0.25
YSAS: Family	12.12	3.62	0.60	0.40
YSAS: Romantic	7.54	1.78	-0.74	0.63
Composite	0.06	1.89	0.45	0.00
Physical domain				
PHQ	8.94	4.91	0.71	0.50
MAQ (Hours per week)	3.18	4.93	2.81	10.39
Composite	0.03	1.43	1.27	2.35

sTNF-R2: soluble tumor necrosis factor receptor 2; IL-6: interleukin-6; CRP: C-reactive protein; CMEP: Children's Morningness-Eveningness Preferences Scale; TST: Total Sleep Time; CDRS: Children's Depression Rating Scale; MASC: Multidimensional Anxiety Scale for Children; ACS: Attention Control Scale; YSAS: Youth Social Adjustment Scale; BSSS: Brief Sensation Seeking Scale; PHQ: Physical Health Questionnaire; MAQ: Modifiable Activity Questionnaire.

Table 3. Intercorrelations between inflammation, sleep or circadian preference, and the five health domains at pretreatment (N = 165).

	1	2	3	4	5	6	7	8	9	10	11
1. sTNF-R2 (Log-transformed)	-										
2. IL-6 (Log-transformed)	0.53***	-									
3. CRP (Log-transformed)	-0.08	-0.14	-								
4. CMEP	0.14	0.08	0.08	-							
5. Weeknight TST	0.06	0.12	-0.12	0.06	-						
6. Weeknight bedtime	-0.04	-0.18*	0.14	-0.08	-0.67***	-					
7. Emotional composite	0.09	0.11	-0.12	-0.05	-0.14	0.05	-				
8. Cognitive composite	-0.08	-0.09	0.00	-0.17*	0.02	0.08	0.01	-			
9. Behavioral composite	-0.07	-0.08	-0.03	-0.19*	-0.03	-0.04	-0.11	0.17*	-		
10. Social composite	0.12	0.00	-0.02	-0.09	-0.16*	0.11	0.42***	-0.01	-0.08	-	
11. Physical composite	0.05	0.11	-0.08	0.02	-0.02	-0.05	0.27***	0.00	0.15	0.05	-

*** $p < .001$; ** $p < .01$; * $p < .05$

sTNF-R2: soluble tumor necrosis factor receptor 2; IL-6: interleukin-6; CRP: C-reactive protein; CMEP: Children's Morningness-Eveningness Preferences Scale; TST: Total Sleep Time.

Table 4. The interaction between inflammation and sleep or circadian preference on the five health domains at pretreatment (N = 165).

Outcome	CMEP × CRP	CMEP × IL-6	CMEP × sTNF-R2	Bedtime × CRP	Bedtime × IL-6	Bedtime × sTNF-R2	TST × CRP	TST × IL-6	TST × sTNF-R2
Emotional composite	0.673	1.548	0.446	0.175	0.656	0.269	4.356*	0.005	0.229
CDRS	0.942	0.355	0.591	0.029	0.419	3.856	2.817	0.014	0.198
MASC	0.174	2.069	0.140	0.278	0.388	0.949	2.861	0.000	1.284
Cognitive composite	0.508	0.491	2.754	2.559	0.105	0.397	2.035	0.198	0.033
Behavioral composite	0.476	5.308*	0.583	2.832	0.051	0.405	1.541	1.303	2.427
Alcohol and Substance Use	2.045	7.208**	0.022	2.319	0.246	0.340	0.540	0.481	1.156
BSSS	0.006	1.152	0.798	1.539	0.024	0.282	1.478	1.430	2.159
Social composite	1.219	0.005	0.905	0.009	0.113	0.229	0.231	0.019	1.040
Physical composite	0.199	3.582	0.632	0.019	1.699	0.100	0.000	2.710	0.204

Note. The values presented in Table 4 are the F statistic calculated from the ANOVA test of the interaction between sleep or circadian preference (weeknight bedtime, weeknight TST, or CMEP) and inflammation (CRP, IL-6, or sTNF-R2).

*** $p < .001$; ** $p < .01$; * $p < .05$.

sTNF-R2: soluble tumor necrosis factor receptor 2; IL-6: interleukin-6; CRP: C-reactive protein; CMEP: Children's Morningness-Eveningness Preferences Scale; TST: Total Sleep Time; CDRS: Children's Depression Rating Scale; MASC: Multidimensional Anxiety Scale for Children; BSSS: Brief Sensation Seeking Scale.

Table 5. The interaction between inflammation and time on sleep, circadian preference, or health domain change following treatment and through follow-up (N = 165).

	sTNF-R2 × time		IL-6 × time		CRP × time	
Outcome	χ^2	*p*	χ^2	*p*	χ^2	*p*
Bedtime (Weekday)	1.04	0.79	1.15	0.77	0.64	0.89
TST (Weekday)	2.95	0.40	6.67	0.08	1.78	0.62
CMEP	8.66	0.03	5.15	0.16	3.93	0.27
Emotional	3.02	0.39	1.50	0.68	3.57	0.31
Cognitive	0.69	0.88	3.67	0.30	3.76	0.29
Behavioral	3.94	0.27	9.59	0.02	1.63	0.65
Alcohol and Substance Use	-	-	5.74	0.12	-	-
SSS	-	-	9.98	0.02		
Social	1.45	0.69	6.94	0.07	3.28	0.35
Physical	2.48	0.48	0.88	0.83	8.80	0.03
PHQ	-	-			10.34	0.02
MAQ (Hours per week)	-	-			2.25	0.52

Note. The χ^2 is the test statistic for the interaction between time (pretreatment, posttreatment, 6-month follow-up, and 12-month follow-up) and inflammation (CRP, IL-6, or sTNF-R2).

sTNF-R2: soluble tumor necrosis factor receptor 2; IL-6: interleukin-6; CRP: C-reactive protein; CMEP: Children's Morningness-Eveningness Preferences Scale; TST: Total Sleep Time; BSSS: Brief Sensation Seeking Scale. PHQ: Physical Health Questionnaire; MAQ: Modifiable Activity Questionnaire.