Poor Sleep as a Pathophysiological Pathway Underlying the Association between Stress Exposure and the Diurnal Cortisol Profile in Children and Adolescents

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ABSTRACT

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Jinshia Ly

There is increasing evidence supporting the potential contribution of poor sleep as a pathophysiological pathway underlying the association between stress exposure and disruption of the diurnal cortisol profile. Specifically, convincing evidence shows that stress exposure leads to poor sleep, which, in part, is responsible for subsequent disruption of the diurnal cortisol profile. The overarching objective of the present study was to examine whether poor sleep mediates the relation between stress exposure and the diurnal cortisol profile in children and adolescents.

Participants included children and adolescents aged 8-18 (N=220, M=12.62 years, 55.90% males). Participants' salivary cortisol was sampled over two days to derive single sample (bedtime cortisol, maximum cortisol) and aggregate cortisol indices (AUC_{AG}, AUC_{I}, AUC_{TG}, $slope_{max}$). Youth-report of stressful life events and daily hassles were obtained. Subjective reports of sleep duration and sleep quality were obtained by youth- and parent-report measures.

Greater stressful life events were associated with elevated AUC_{AG} and AUC_{TG}. Greater daily hassles were associated with elevated maximum cortisol, AUC_{AG}, AUC_{TG}, and steeper $slope_{max}$. Greater stressful life events and daily hassles were associated with poorer sleep quality, which, in turn, was related to higher bedtime cortisol and AUC_{TG}. Mediational analyses revealed that the sleep quality mediated the association between stress exposure and AUC_{TG}.

The present study provided evidence for poor sleep as a potential pathophysiological pathway underlying the effects of stress exposure on the diurnal cortisol profile in children and adolescents. The current research findings have important implications and help reframe prevention efforts and public policies aimed at reducing the physiological sequelae of stress exposure. Future research should replicate current findings using objective measures of sleep to better understand specific sleep parameters that underlie the association between stress exposure and the diurnal cortisol profile.

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INTRODUCTION

Based on evidence in the extant literature, there is strong support for the relation between stress exposure with the disruption of the diurnal cortisol profile. Although the pathophysiological mechanism mediating this association remains unknown, there is emerging evidence to support the contribution of poor sleep as a potential mediating pathway in this association. Specifically, it is thought that stress exposure leads to poor sleep, which, in part, is responsible for subsequent disruption of the diurnal cortisol profile. In the following sections, background literature related to the diurnal cortisol profile and its association with stress exposure will be reviewed to provide context for the current research question. Next, an overview of research findings related to sleep will be described. Finally, evidence supporting its interrelation with stress exposure and the diurnal cortisol profile will be discussed.

Hypothalamic-Pituitary-Adrenal Axis, Cortisol, and Diurnal Cortisol Profile

The hypothalamic-pituitary-adrenal (HPA) axis is chiefly responsible for the regulation of hormones involved in the stress response system. The HPA axis is predominantly regulated by the excitatory and inhibitory effects of the paraventricular nuclei of the hypothalamus, which controls the secretion of corticotropin-releasing-hormone and vasopressin, which are simultaneously released. Together, corticotropin-releasing-hormone and vasopressin act synergistically to stimulate the secretion of adrenocorticotropic hormone from the anterior pituitary into the bloodstream (Charmandari, Tsigos, & Chrousos, 2005). Subsequently, the circulation of adrenocorticotropic hormone to the adrenal cortex via the portal system initiates the synthesis and secretion of cortisol, the final effector of the HPA axis. Cortisol, in turn,

has a negative feedback loop on the anterior pituitary, hypothalamus, and hippocampus, which suppresses the release of corticotropin-releasing-hormone and adrenocorticotropic hormone, resulting in the reduction of cortisol production (Sapolsky, Krey, & McEwen, 1984).

Cortisol secretion typically follows a distinct circadian pattern, known as the diurnal cortisol profile. Cortisol secretion peaks shortly after morning awakening, with cortisol showing a 50-75% increase in concentration within the first hour of morning awakening (Fries, Dettenborn, & Kirschbaum, 2009). This phenomenon is identified as the cortisol awakening response, which is thought to represent a stress response of the HPA axis to morning awakening (Fries, Dettenborn, & Kirschbaum, 2009; Wust, Wolf, Hellhammer, Federenko, Schommer, & Kirschbaum, 2000). Cortisol secretion then gradually declines throughout the day (i.e. diurnal slope), reaching nadir at bedtime (Saxbe, 2008). During nocturnal sleep, the HPA axis remains quiescent within the first few hours of the night. Thereafter, cortisol secretion gradually increases throughout the night until morning awakening.

During the body's "fight-or-flight" response to stress, secretagogue activity of corticotropin-releasing-hormone and vasopressin is amplified, which has the direct effect of increasing cortisol secretion (Tsigos & Chrousos, 1994). This stress response is meant to be short-lived. When the HPA axis is activated efficiently and infrequently, cortisol secretion is adaptive and helps restore homeostasis within the body. The primary function of increased cortisol secretion is to enhance the body's ability to cope with stress by increasing carbohydrate, fat, and protein metabolism, as well as temporarily suppressing reproductive and immune functions (Sapolsky, 1994). However, prolonged cortisol

secretion due to chronic stress exposure causes an ongoing "wear-and-tear" on the body to meet the physiological demands of the stress. This can ultimately disrupt the negative-feedback loop of cortisol on the anterior pituitary, hypothalamus, and hippocampus, resulting in disruption of the HPA axis and diurnal cortisol profile.

Disruption of the diurnal cortisol profile, characterized by heightened cortisol awakening response, higher evening cortisol level, and flatter diurnal slope, is associated with many deleterious health outcomes. For instance, disruption of the diurnal cortisol profile has been implicated in many physical conditions, such as hypertension (Whitworth, Brown, Kelly, & Williamson, 1995), decreased sensitivity to insulin (Misra, Bredella, Tsai, Mendes, Miller, & Klibanski, 2008), asthma (Wolf, Nicholls, & Chen, 2008), obesity (Rosmond, Bouchard, & Bjorntorp, 2002), and metabolic syndrome (Rosmond, 2005). In children and adolescents, disrupted diurnal cortisol profiles have also been involved in a host of psychological problems, specifically bipolar disorder (Ellenbogen, Hodgins, Walker, Couture, & Adam, 2006), depression (Adam, Doane, Zinbarg, Mineka, Craske, & Griffith, 2010; Van den Bergh & Van Calster, 2009), externalizing behaviour problems (Schechter, Brennan, Cunningham, Foster, & Whitmore, 2012), and posttraumatic stress disorder (Carrion, Weems, Ray, Glaster, Hessl, & Reiss, 2002). Given the significance of the diurnal cortisol profile on health outcomes, there is a need to uncover pathophysiological pathways and determinants associated with the disruption of the diurnal cortisol profile.

Stress Exposure and Disruption of Diurnal Cortisol Profile

In adults, there is a robust effect of stress exposure on the diurnal cortisol profile. Exposure to laboratory-induced stressors produces an acute increase in cortisol secretion.

This cortisol response is gradually attenuated and returns to baseline levels approximately one hour after the offset of the stressor (Dickerson & Kemeny, 2004). The adverse effect of stress exposure is well-documented across many studies. For example, in a meta-analysis of 208 studies investigating the effects of acute experimental stressors on cortisol in adults, public speaking and mental arithmetic tasks induced cortisol response, with effect sizes varying from 0.20 to 0.87 standard deviation units above baseline cortisol levels (Dickerson & Kemeny, 2004). Of note, Dickerson and Kemeny (2004) found that stressors that were uncontrollable, unpredictable, and/or highly socially threatening produced the highest cortisol response and took the longest time to return to baseline cortisol levels. Taken together, there is clear evidence supporting the adverse effect of laboratory-induced stressors on cortisol.

While experimental studies are a requisite to establish a causal association between stress exposure and cortisol, several characteristics of laboratory-induced stressors limit the generalizability of these findings to the naturalistic environment. Laboratory-induced stressors are short in duration, ranging from 5 to 45 minutes (Dickerson & Demeny, 2004). However, typical, naturalistic stressors (e.g. academic problems, family conflicts) are chronic and ongoing, and the consequences can be more profound and enduring. Moreover, laboratory-induced stressors are only of minimal to moderate intensity and never fully uncontrollable as participants can choose to withdraw from the study, thereby limiting the ecological validity of laboratory-induced stressors (Dickerson & Demeny, 2004). In this regard, it is more ecologically valid to investigate the consequences of naturalistic stressors, typically uncontrollable and unpredictable, on the diurnal cortisol profile.

Naturalistic stressors, including both stressful life events and regular daily hassles, are known to disrupt the diurnal cortisol profile in adults. For instance, stressful life events, such as unemployment, divorce, and loss of a close family member or friend, are associated with higher morning and evening cortisol levels and a flatter diurnal slope (see Miller, Chen, & Zhou, 2007 for a review). Individuals who experience greater daily stress and hassles, due to chronic work overload and other occupational stress, have a higher cortisol awakening response compared to less stressed individuals (Schulz, Kirschbaum, Pruessner, & Hellhammer, 1998). Similarly, Pruessner and colleagues (1999) found that public school teachers who perceive greater daily hassles and experience more burnout have a higher cortisol awakening response. While several studies observe cortisol *blunting* due to chronic stress exposure (e.g., Miller, Cohen, & Ritchey, 2002; Vedhara et al., 2002; Yehuda, Halligan, Grossman, Golier, & Wong, 2002), in a recent meta-analysis, Miller and colleagues articulate that this counterintuitive discrepancy is attributable to the timing of stressors, type of stressors, and individual characteristics (Ouellet-Morin et al., 2011; Miller, Chen, & Zhou, 2007). Miller and colleagues (2007) proposed that shortly after stress exposure, the HPA axis will be activated, resulting in elevated cortisol secretion. However, with the passage of time, the chronic "wear-and-tear" on the HPA axis can disrupt the negative feedback loop, resulting in cortisol levels rebounding below baseline levels. Further, Miller and colleagues explain that stressors that are uncontrollable, are perceived as stressful, and threaten physical integrity elicit higher afternoon and evening cortisol levels as well as a flatter diurnal slope. These findings provide strong support for the disruption of the diurnal cortisol profile following stress exposure in adults.

While the relation between stress exposure and cortisol is well-established in adults, findings in children and adolescents are far less consistent. The variability in the measurement of childhood stress may in part account for the inconsistent findings in the pediatric literature. Studies have included a wide range of stress measures, differing in the number of items, life domains (e.g., family, friendship, school), reference periods (e.g., six months, one year), and informants (e.g., youth-report, parent-report, interviewer-rated). Inconsistent findings may be partly explained by the use of stress measures that tap at different constructs and different perceptions of stress. Studies based on parent-report stressful life events within one life domain and/or using a reference period of six months (versus one year) tend to yield null findings for morning cortisol, cortisol awakening response, and diurnal slope (Sieh, Visser-Meily, Oort, & Meijer, 2012; Slatcher & Robles, 2011; Donoho, Weigensberg, Emken, Hsu, & Spruijt-Metz, 2011; Carney, Hazlerm, Oh, Hibel, & Granger, 2010). In contrast, greater youth-report or interviewer-rated stressful life events across multiple life domains over one year are associated with higher cortisol awakening response and elevated afternoon cortisol (Vanaelst et al., 2012; Gustafsson, Anckarsater, Lichtenstein, Nelson, & Gustafsson, 2010; Wolf, Nicholls, & Chen, 2008). While other studies based on youth-report measures reported inconsistent findings for morning cortisol, total cortisol concentration, and diurnal slope (Marceau, Dorn, & Susman, 2012; Slatcher & Robles, 2011; Hagan, Luecken, Sandler, & Tein, 2010; Bevans, Cerbone, & Overstreet, 2008; Maldonaldo et al., 2008), these seem to be better explained by their salivary cortisol sampling procedures (see below). Altogether, the results of these studies allude to the importance

of using youth-report or interviewer-rated stressful life events across multiple life domains over the past year.

To date, few studies have considered the association between daily hassles and cortisol in adolescents and no study has examined this relation in children. Among the two studies that have examined this association in adolescents, cortisol sampling on multiple days at both recommended and convenience time points have been investigated, and these results were only limited to single-sex samples. Elevated daily hassles are associated with a flatter diurnal slope (Lovell, Moss, & Wetherell, 2011), but inconsistently related to morning cortisol (Schechter, Brenman, Cunningham, Foster, & Whitmore, 2012), which seems to be associated with sampling procedure based on convenience time points. Studies available in the literature are too limited to draw conclusions about the relation of daily hassles to cortisol in adolescents, and the question remains whether this association is present in children.

Inconsistent findings may also be partly attributable to the wide variation in saliva sampling procedures used across studies involving children and adolescents. According to the MacArthur Network (2000) guidelines, sampling over three to six days is required to obtain reliable aggregate cortisol indices (e.g., AUC_{AG}, AUC_{I}, AUC_{TG}, diurnal slope). Others have made similar recommendations specific to sampling in youth (Rotenberg, McGrath, Roy-Gagnon, & Tu, 2012). The MacArthur Network (2000) further recommends the use of sampling time points anchored at 1, 4, 9, and 11 hours after morning awakening, or at awake, 45 minutes post-awakening, 4PM-6PM, 6PM-9PM, and bedtime. Despite these recommendations, many studies collect saliva samples on one day

and/or at unconventional time points, which yield greater potential for measurement error.

Findings for the stress and cortisol association greatly vary across different saliva sampling procedures. Saliva sampling over one day taken at convenience time points (e.g. school start time and time of lab visit) is the most commonly used procedure among children and adolescents, but yields findings inconsistent to the adult literature. Studies based on this sampling procedure revealed that greater stressful life events are associated with higher afternoon cortisol (Bevans, Cerborne, & Overstreet, 2008) and steeper slope (Marceau, Dorn, & Susman, 2012; Kelly, Young, Sweeting, Fischer, & West, 2008), but inconsistent findings are observed with cortisol awakening response (Donoho, Weigensberg, Emken, Hsu, & Spruijt-Metz, 2011; Carney, Hazlerm, Oh, Hibel, & Granger, 2010) and total cortisol concentration (Hagan, Luecken, Sandler, & Tein, 2010; Kelly, Young, Sweeting, Fischer, & West, 2008). Among studies sampling over only one day at recommended time points, greater stressful life events are associated with both lower and higher morning cortisol levels as well as flatter and steeper diurnal slope (Sieh, Visser-Meily, Oort, & Meijer, 2012; Slatcher & Robles, 2011; Maldonaldo et al., 2008). In contrast, studies that sample cortisol over multiple days at recommended time points show the most consistent findings, with greater exposure to stressful life events, based on youth-report or interviewer-rated measures, associated with higher cortisol awakening response and a flatter diurnal slope (Vanaelst et al., 2012; Gustafsson, Anckarsater, Lichtenstein, Nelson, & Gustafsson, 2010; Wolf, Nicholls, & Chen, 2008). These findings underscore the need to use recommended cortisol sampling procedures in establishing a reliable association between stressful life events and cortisol.

Taken together, while there is clear evidence supporting the association between stress exposure and disruption of the diurnal cortisol profile in adults, findings are inconsistent in the pediatric literature. Importantly, a causal relation is evidenced based on experimental studies, demonstrating the adverse effect of stress exposure on the diurnal cortisol profile. Given these findings, there is a need to better understand the pathophysiological pathway underlying this association and to consider whether this association emerges during childhood and adolescence.

Extant literature has largely focused on the nonspecific effect of stress exposure on the diurnal cortisol profile. However, it is likely that a pathophysiological mechanism associated with both stress exposure and diurnal cortisol profile is involved. There is increasing evidence suggesting that poor sleep is a mechanism involved. Emerging findings suggest that stress exposure leads to poor sleep, which, in turn, is responsible for the disruption of the diurnal cortisol profile. In the subsequent sections, background literature related to sleep and its association with stress exposure and the diurnal cortisol profile will be discussed.

Overview of Sleep

Polysomnography is a multichannel physiological recording system that comprehensively measures the electrophysiological changes during sleep. Polysomnography records brainwave activity (electroencephalography; EEG), eye movements (electrooccular graphy; EOG), chin and limb muscle movements (electromyography; EMG), and heart rate (electrocardiography; ECG). A typical polysomnography recording consists of a minimum of three EEG, two EOG, two EMG, and one ECG recording channels, but the number of channels may vary to adapt to the

requirements of different studies. Polysomnography data are scored and interpreted by a registered sleep technician or trained professional to derive sleep architecture data. Frequency and amplitude of the EEG waveforms are scored to determine sleep stages. The common EEG frequency bands detected are alpha waves (8-13 Hz), theta waves, (4-7 Hz), delta waves (< 4 Hz), sleep spindles (12-14 Hz; bursts of rhythmic brain waves), and K-complexes (0.5-0.7 Hz; high-voltage negative peaks followed by positive waves; Iber, Ancoli-Israel, Chesson, & Quan, 2007; Rechtschaffen & Kales, 1968). Specific patterns of EEG, EOG, EMG and ECG activities are used to identify sleep architecture.

Normal human sleep is conventionally divided into five stages that are associated with distinct brain activities and behavioural features (Carskadon & Dement, 2011). Stage 1 is the transitional phase between wakefulness and sleep. Stage 1 is associated with low arousal threshold; sleep during this stage can be easily disrupted by noises and other environmental stimuli. Physiologically, Stage 1 is marked by a gradual decline of alpha waves, which are predominant during wakefulness. Emergence of theta waves in Stage 1 is also typically observed in children and adolescents (Iber, Ancoli-Israel, Chesson, & Quan, 2007). The transient occurrence of sleep spindles and K-complexes are physiological markers of Stage 2 sleep (Iber, Ancoli-Israel, Chesson, & Quan, 2007). Jointly, these waveforms are thought to suppress cortical arousal in response to external stimuli and to enhance memory consolidation (Cash et al., 2009). Stages 3 and 4 are marked by high arousal thresholds and the presence of high amplitude delta waves (Iber, Ancoli-Israel, Chesson, & Quan, 2007). Stages 3 and 4 are known as slow-wave sleep and have important restorative functions. Collectively, Stages 1 to 4 are called non-rapid eye movement (NREM) sleep, which is often referred to as “quiet sleep.” Heart rate and

breathing during NREM are slow and steady; blood pressure and brain activities are also at the lowest point of the twenty-four hour period (Kreinin, 2012). Rapid eye movement (REM) sleep is the fifth stage of the sleep cycle. Waveforms in REM sleep are similar in frequency, but lower in amplitude, compared to Stage 1 of NREM sleep. In contrast to NREM sleep, the brain becomes highly active in REM state. Body temperature and blood pressure rise while heart rate and respiration elevate and become increasingly irregular. Most importantly, REM sleep is mainly distinguished from NREM sleep by the complete absence of chin muscle activity and the presence of rapid bursts and symmetrical eye movements. The density of eye movements varies across the time of the night, with increasing ocular activity during REM sleep at later cycles of the night (Goldsmith, Casola, & Varenbut, 2006).

NREM and REM sleep, which cyclically alternate four to six times a night until morning awakening, are characterized by distinct architecture. The distribution of sleep stages in each cycle follows a recurring pattern, with each cycle lasting approximately 90 to 110 minutes (Rechtschaffen & Kales, 1968; Dement & Kleitman, 1957). Under normal conditions, individuals fall directly into NREM sleep, beginning with Stage 1 sleep, which accounts for 3-8% of total sleep time. Stage 2 sleep begins after 10 to 12 minutes of Stage 1 sleep and accounts for 45-55% of total sleep time (Rechtschaffen & Kales, 1968). Stage 2 is then followed by Stages 3 and 4 (slow-wave sleep). The duration of slow-wave sleep is usually longer during early sleep cycles and gradually shortens as the night progresses, occupying 15-20% of total sleep time (Rechtschaffen & Kales, 1968). The first onset of REM sleep occurs approximately 60 to 90 minutes after sleep onset, and recurs approximately every 60 minutes thereafter (Rechtschaffen & Kales, 1968).

Rapid eye movement sleep accounts for 20-25% of a sleep cycle. In contrast to slow-wave sleep, REM sleep lengthens over the course of a night, lasting a few minutes in the first episode to 30 minutes when approaching morning awakening.

In addition to the identification of sleep stages, other important sleep parameters can be derived from polysomnography. Sleep onset and final awakening are required parameters to accurately calculate sleep duration. Although there are many ways to delineate sleep onset, it has been generally defined as the first epoch that is distinct from wakefulness state (Rechtschaffen & Kales, 1968). The beginning of the first NREM sleep episode and emergence of slow eye movements traditionally mark the transition to sleep. Other sleep variables calculated include sleep onset latency (duration of the transition from wakefulness to sleep onset), total sleep time (total time scored as sleeping on a given night), and sleep efficiency (ratio of total sleep time over total time spent in bed). Sleep efficiency is optimal in the 85 to 90% range (Akerstedt, Hume, Minors, & Waterhouse, 1994). Sleep fragmentation refers to sleep disruptions or awakenings caused by frequent bursts of alpha wave activity and sudden increase in muscle tone (Roth, Hartse, Zorick, & Conway, 1980). Together, sleep onset, time of awakening, sleep onset latency, total sleep time, sleep efficiency and sleep fragmentations are parameters used to objectively quantify sleep.

Wrist actigraphy is another method used to derived estimates of sleep. It is considered a viable alternative to the more invasive polysomnography. Actigraphy uses a technique similar to accelerometry, based on a dual or tri-axle detection of motion. Because actigraphy is based solely on movement, this measurement precludes derivation of fundamental parameters about sleep architecture. There is also greater potential for

measurement error. Specifically, actigraphy lacks precision in deriving sleep-to-wake and wake-to-sleep transitions compared to polysomnography. Hence, total sleep time and sleep efficiency tend to be overestimated in actigraphy compared to polysomnography (Kushida, Chang, Gadkary, Guillemault, Carrillo, & Dement, 2001). To increase precision of sleep measures, it is recommended to use actigraphy recording over one week in conjunction with subjective sleep measures (Cole, Kripke, Gruen, Mullaney, & Gillin, 1992). Although actigraphy assessment is less precise than polysomnography-derived sleep, actigraphy is still used extensively in the pediatric literature and is considered a valid and reliable source of data for objective sleep.

The use of self-report questionnaires is a common method to subjectively assess sleep. The Pittsburgh Sleep Quality Index (Buysse, Reynolds III, Monk, Berman, & Kupfer, 1989) and the Epworth Sleepiness Scale (Johns, 1992) are predominantly used in the adult and adolescent literature to assess overall perceived sleep quality, sleep duration, daytime sleepiness, and sleep disturbances. For children, parent-report of sleep quality is typically used (e.g. Child's Sleep Habits Questionnaire; Owens, Spirito, & McGuinn, 2000). Though subjective sleep assessment does not provide information about sleep architecture, it is important in that it accounts for individual differences in the subjective experience and evaluation of sleep. Multiple adult studies have established that higher objectively measured total sleep time, greater sleep efficiency, and lower sleep fragmentation correspond to greater self-report subjective sleep quality (e.g., Akerstedt, Hume, Minors, & Waterhouse, 1994). These findings suggest comparability between subjective report and objective assessment of sleep.

Developmental Changes in Sleep

Many aspects of sleep undergo dramatic developmental changes across late childhood and adolescence. Starting from late childhood, youth show a pattern of decreasing total sleep time and later bed times (Carskadon, Vieira, & Acebo, 1993; Carskadon & Davis, 1989). Delta wave activity is substantially decreased during slow-wave sleep starting from the age of 10 (Feinberg, Higgins, Khaw, & Campbell, 2006; Gaudreau, Carrier, & Montplaisir, 2001; Carskadon, 1982). Slow-wave sleep, REM sleep duration, and REM sleep latency are significantly shortened even when total sleep time remains constant (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004; Carskadon, 1982). Sleep duration decreases from childhood to adolescence. In a meta-analysis of twenty countries, Olds et al. (2010a) found that sleep duration declines by 6.6 minutes each year across adolescence, and the decline is greater during school nights compared to non-school nights, at a rate of 14 minutes per year. Additionally, sleep onset is delayed with increasing age, with greater preference for evening activities among adolescents (Sadeh, Dahl, Shahar, & Rosenblat-Stein, 2009). Older adolescents have later bed and wake-up times when they are unconstrained by early school start-times (Crowley, Acebo, & Carskadon, 2007), and show a significantly slower build-up of sleep debt, which is the effect resulting from cumulative sleep deprivation. In fact, this phase delay evident across late childhood and adolescence is often incompatible with the constraints of early school start time in many countries, which can have significant impact on children and adolescents' daytime functioning (Carskadon, Wolfson , Acebo, Tzischinsky, & Seifer, 1998).

Epidemiology of Sleep Duration

There are considerable inter-individual differences for sleep requirements, or the amount of sleep needed to feel rested. In general, it is recommended that children and adolescents sleep 10 and 9 hours, respectively, each night for optimal functioning (National Sleep Foundation, 2000). However, many children and adolescents do not meet this recommended guideline and there are emerging findings evidencing population-level declines in sleep duration over time, largely due to increasingly later bed times but unchanged wake-times. Sleep duration decreased from 9 to 8.8 hours among 14-year-olds (Iglowstein, Jenni, Molinari, & Largo, 2003). Children and adolescents in Australia, aged 10 to 15 years, reported a 30 minute decline in time spent in bed from 1985 to 2004 (Olds, Maher, Blunden, & Matricciani, 2010b). Similarly, Wolfson and Carskadon (1998) found that a quarter of American high school students reported six hours of sleep or less per night. These population-level declines in sleep duration are further evidenced in a recent meta-analysis of 641 studies across 20 countries (Matricciani, Olds, & Petkov, 2012). Children and adolescents, aged 8 to 18, showed a decrease of 0.75 minutes nightly per year during the period of 1905 and 2008, with most decrease found among children from Canada, United States, Europe, and Asia. Taken together, sleep duration is on the decline for children and adolescents across nations.

Poor Sleep and Disruption of Diurnal Cortisol Profile

The sleep-wake cycle has a close, temporal association with secretatory activity of cortisol. Onset of nocturnal sleep reliably exerts an inhibitory effect on cortisol secretion (Weibel, Follenius, Spiegel, Ehrhart, & Brandenberger, 1995; Van Cauter, Blackman, Roland, Spire, Refetoof, & Polonsky, 1991). Early studies reveal that emergence of slow-

wave sleep, especially in the first sleep cycle, coincides with the lowest cortisol level of the twenty-hour period (Born & Fehm, 1998). This led to the conclusion that the predominance of slow-wave sleep during early sleep cycles is responsible for the decreased cortisol secretion (Weitzman, Zimmerman, Czeisler, & Ronda, 1983) and this idea continues to be upheld today. Cortisol level increases over the course of the night, which parallels the decline of slow-wave sleep and upsurge of REM sleep during later sleep cycles (Somers, Dyken, Mark, & Abboud, 1993). Pulses of cortisol emerge after the first hours of sleep, followed by a gradual increase in cortisol level throughout the night reaching diurnal peak shortly after morning awakening. Given the modulatory effect of sleep on cortisol secretion, it is plausible that sleep may mediate the association between stress exposure and cortisol. In fact, sleep has been posited to be the mechanism by which stress exposure "gets under the skin" to affect the diurnal cortisol profile (Van Reeth, Weibel, Spiegel, Leproult, Dugovic, & Maccari, 2000).

In adults, poor sleep alters secretatory activity of cortisol. Experimentally manipulating the sleep-wake cycle stimulates acute changes in diurnal cortisol secretion in men and women (Balbo, Leproult, & Van Cauter, 2010). Plasma cortisol level resulted in higher evening cortisol level following a total sleep deprivation procedure among healthy young men (Leproult, Copinschi, Buxton, & Van Cauter, 1997). As well, restricting sleep to only 4 hours per night over 6 consecutive nights results in higher evening plasma cortisol level in healthy young men (Spiegel, Leproult, & Van Cauter, 1999; Leproult, Copinschi, Buxton, & Van Cauter, 1997). Schüssler and colleagues (2006) further found that elevation in evening cortisol level is more pronounced during the second half of the evening following a one-night total sleep deprivation. Comparable

results have been reported among females who underwent a partial sleep deprivation procedure over one night. Women restricted to three hours of sleep show higher plasma evening cortisol level, and a flatter diurnal slope the following day (Omisade, Buxton, & Rusak, 2010). Though some studies did not observe these effects (e.g. Zhang et al., 2011) or revealed blunted cortisol level (e.g. Wu et al., 2008; Vgontzas et al., 2003; Vgontzas, Mastorakos, Bixler, Kales, Gold, & Chrousos, 1999), researchers have argued that these conflicting findings may be accounted for by the difference in timing of the sleep deprivation procedure. Taken together, there is a robust temporal association between sleep and cortisol.

While studies examining the association between sleep and cortisol in children and adolescents are scarce, findings are largely consistent with the adult literature. Shorter sleep duration, lower sleep efficiency, and greater sleep fragmentation based on actigraphy-assessment and youth-report measures are associated with higher afternoon cortisol level in school-aged children (El-Sheikh, Buckhalt, Keller & Granger, 2008). As well, in an actigraphy-based study, shorter sleep duration and lower sleep efficiency are related to higher cortisol awakening response, daytime cortisol, and evening cortisol level as well as flatter diurnal slope in a sample of 8-year-old children (Raikkonen et al., 2010). Similar results have been replicated in a sample of 5-year-old preschool children in two polysomnography-based studies. Poor sleepers, due to long sleep onset latency, short sleep duration, low sleep efficiency, or high sleep fragmentation, evidenced higher morning and daytime cortisol levels compared to good sleepers (Hatzinger et al., 2010; Hatzinger et al., 2008). To date, one study has examined the association between polysomnograpy-based sleep and cortisol in adolescents, but no relation was reported

(Voderholzer et al., 2012). However, this study did not control for sex, which may in part explain the null findings as the relation of sleep to cortisol differed between boys and girls. A recent study revealed that boys who experienced greater sleep problems have lower cortisol levels over the day while girls with greater sleep problems show higher cortisol levels over the day (Pesonen et al., 2012). Overall, the relation of sleep to cortisol seems consistent in children and adolescents. The question remains whether findings are generalizable to subjective report of sleep, which can account for individual differences in subjective experience and evaluation of their sleep.

Stress Exposure and Poor Sleep

Stress exposure has an adverse physiological effect on sleep. Findings based on experimental studies show that chronic stress exposure through a "learned helplessness" paradigm results in increased REM sleep in rats (Adrien, Dugovic, & Martin, 1991). Chronic stress exposure in rats also leads to decreased REM sleep onset latency and deep slow-wave sleep (Cheeta, Ruigt, van Proosdij, & Wilner, 1997). These experimental findings evidence that stress exposure results in changes in slow-wave sleep and REM sleep. Because slow-wave sleep and REM sleep are thought to have inhibitory and excitatory effects on cortisol secretion, this suggests that poor sleep may be in part the mechanism by which stress exposure disrupts the diurnal cortisol profile.

In children and adolescents, findings suggest that stressful life events adversely affect sleep, both subjective report and objectively measured. Greater parental marital conflict is related to greater sleep problems and daytime sleepiness in school-aged children (El-Sheikh, Buckhalt, Mize, & Acebo, 2006; Sadeh, Keinan, & Daon, 2004). Elevated family stress and high academic course load predict shorter subjective report

sleep duration, poorer sleep quality, and greater daytime sleepiness in adolescents (Roberts, Roberts, & Xing, 2010). Similarly, studies based on actigraphy measured sleep showed that greater family stress predicts shorter sleep duration and poorer sleep efficiency (El-Sheikh, Buckhalt,, Mize, & Acebo, 2006; Sadeh, Raviv, & Gruber, 2000). Dewald and colleagues (in print) monitored changes in adolescents' sleep across low stress (regular school week) and high stress weeks (pre-exam and exam weeks). Wake-after-sleep-onset was substantially elevated during the pre-exam and exam weeks compared to regular school week. Greater stressful life events are associated with decreased slow-wave-sleep and REM sleep latency and increased REM sleep duration (Williamson, Dahl, Birmaher, Goetz, Nelson, & Ryan, 1995). These findings suggest that greater stressful life events are associated with poorer sleep among children and adolescents.

Research examining the relation between daily hassles and sleep in children and adolescents is lacking. Thus far, the best evidence for the association between daily hassles and sleep is found in the adult literature. Lund and colleagues (2011) showed that greater daily hassles predict poorer sleep efficiency, longer sleep latency, shorter sleep duration, greater daytime sleepiness, and higher fatigue level in young adults. In a cross-sectional analysis, Akerstedt and colleagues (2002) found that greater daily hassles due to high work demands are associated with poorer subjective sleep quality. Kashani and colleagues (2011) found that adults who experience high levels of daily hassles reported substantively poorer subjective sleep quality. Specifically, high levels of daily hassles were adversely related to sleep quality, daytime sleepiness, fatigue, and daytime functioning. Highly stressed participants also slept 20 minutes less over a 24-hour period

compared to their non-stressed counterparts. Although 20 minutes may appear to be a trivial or inconsequential difference, studies have shown that accumulation of small amounts of sleep loss is sufficient to seriously impair cognitive functions, mood, and daytime functioning (Drake, Roehrs, Burduvali, Bonahoom, Rosekind, & Roth, 2001; Dinges et al., 1997).

Taken together, findings from experimental and cross-sectional studies indicate there are adverse effects of stress exposure on sleep. Given the temporal association between the sleep-wake cycle and secretatory activity of cortisol, it is plausible that sleep is a potential pathway linking stress exposure and disruption of the diurnal cortisol profile.

Current State of Knowledge

Based on emerging evidence in the extant literature, there is strong support for the association between stress exposure and cortisol. There is also data suggesting poor sleep may mediate this association. Findings in the extant literature suggest that greater stress exposure is related to poorer sleep, which, in turn, leads to the disruption of the diurnal cortisol profile. Despite emerging adult evidence suggesting poor sleep as a potential mediating mechanism, this hypothesis has not been tested in children and adolescents. In fact, there are many inconsistencies in pediatric findings regarding the associations between stress, sleep, and cortisol. First, findings for the relation of stress exposure to cortisol are inconsistent and largely attributable to variability in the measurement of stress and cortisol. Stress measures are predominantly based on parent-report of children and adolescents' stressful life events within one life domain and using a time frame of six months or less, which fail to capture the subjective nature of stress, its multidimensional

nature, and its chronicity over time. The number of studies that have examined the relation of daily hassles to cortisol among adolescents is scarce and the question remains whether the association between daily hassles and cortisol levels exists in children. As well, cortisol samples are not based on recommended guidelines, which yield greater potential for measurement error. Second, findings for the association between poor sleep and disruption of the diurnal cortisol profile have only been observed among few pediatric studies. It remains unclear whether findings are comparable to those using subjective report (self-report or parent-report) of sleep. The question remains whether this relation also holds for adolescents. Third, pediatric findings for the relation of stress exposure to sleep are only limited to stressful life events; little is known about the link between elevated daily hassles and adverse cortisol levels. In sum, there is a need to assess whether poor sleep is a potential pathophysiological pathway linking stress exposure and cortisol, using psychometrically sound measures of stress and cortisol.

Present Study

The overarching objective of the present study was to examine whether sleep mediates the relation between stress exposure and cortisol levels in youth (the term "youth" will be used for parsimony to capture "children and adolescents"). The first objective was to address knowledge gaps in the extant literature by establishing the associations between stress, sleep, and cortisol in youth, using a methodology that yielded the most consistent findings. This objective was achieved by testing three research hypotheses. First, it was hypothesized that greater stressful life events and elevated daily hassles would be associated with a disrupted diurnal cortisol profile. Second, it was hypothesized that greater stressful life events and elevated daily hassles

would be associated with poorer sleep. Third, it was hypothesized that poorer sleep would be associated with a disrupted diurnal cortisol profile.

The second objective was to determine whether poor sleep is a plausible pathophysiological pathway linking greater stress exposure and adverse cortisol levels. For the fourth research question, it was hypothesized that the relation of stress to cortisol would be mediated by poor sleep. This mediation hypothesis was largely based on Baron and Kenny's original model of mediation (1986), which was further developed by Hayes (2009). Standard procedures require three associations to first be established: (1) the predictor variable needs to be related to the hypothesized mediators, (2) the hypothesized mediators need to be related to the outcome variable when the effects of the predictor variable is controlled (product of these two associations represents an indirect effect), and (3) the predictor variable needs to be related to the outcome variable (total effect). To provide evidence for the direct effects of stress exposure on cortisol levels through poor sleep, the magnitude of the relation of the predictor variable to the outcome variable must be substantially reduced when the hypothesized mediators are statistically controlled. Finally, it is acknowledged that these analyses would not allow causal conclusions to be made due to the cross-sectional nature of current data.

METHOD

Participants

Youth (*N*=245), aged 8 to 18 years, were recruited as part of the larger Healthy Heart Project, which examined early cardiovascular risk factors among youth. The project was conducted at the Pediatric Public Health Psychology Lab of Concordia University, Montreal, Quebec. Participants were recruited using bookmarks distributed in primary and secondary schools approved by the *English Montreal School Board* and flyers posted through the community and local neighbourhood. Youth with serious psychopathology or medication use known to interfere with cardiovascular or endocrine functioning were not eligible to participate. The project was approved by the Concordia University Research Ethics Committee (UH2005-077).

Measures

Demographic Information. Youth and their parents completed brief demographic questionnaires. Information about youth's age, education level, and sex as well as parental education level and household income was obtained.

Cortisol. Youth collected six saliva samples over two days. The six time points included awakening ($awake_0$), 30 minutes post-awakening ($awake_{+30}$), 45 minutes post-awakening ($awake_{+45}$), pre-lunch, pre-dinner, and bedtime. Saliva samples were collected using the Salivette sampling device (Salimetric, Inc.). Youth were instructed to not eat, drink, or brush their teeth ten minutes before each sampling. They were told to place the cotton swab under their tongue for at least 30 seconds until the cotton swab was saturated. Given the importance of precision of timing of saliva sampling to derive valid cortisol levels, participants were asked to record the date and time of each sample in a

sleep diary, which was initialled by their parents or teachers after each sampling as a measure of compliance. Previous research has demonstrated good compliance to saliva sampling protocols in youth (Rotenberg & McGrath, 2011). To ensure their understanding about the saliva sampling procedure, youth were asked to provide one saliva sample in the laboratory. Participants were instructed to store saliva samples in the freezer at home until they returned them to the laboratory. Samples were stored in sub-zero freezers in the laboratory until they were packaged in dry ice and shipped to University of Trier, Germany for assaying. Cortisol levels are robust to environmental conditions associated with the shipping process (Clements & Parker, 1998).

Cortisol Assay. Samples were assayed using a competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection (DELFIA; Dressendörfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992). The intra-assay coefficients of variation were less than 11.0%.

Cortisol Indices. Aggregate cortisol indices were calculated for each day of sampling using previously published formulae (Rotenberg, McGrath, Roy-Gagnon, & Tu, 2012; Fekedulegn et al., 2007, Kraemer et al., 2006; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Two aggregate indices for the cortisol awakening response were derived: area under the curve relative to ground (AUC_{AG}) and area under the curve relative to increase (AUC_I), with the latter representing the dynamic increase in the amount of cortisol secreted following awakening. Area under the curve for total cortisol relative to ground (AUC_{TG}) was derived to represent the total amount of cortisol secreted throughout the day. Diurnal slope anchored at the maximum cortisol value ($slope_{max}$) was used as an aggregate index of the decline of cortisol over the day. Single

sample measures for each day of sampling included bedtime cortisol and maximum cortisol, which is the highest value within the three awakening samples. The aggregate indices and single sample measures (bedtime cortisol, maximum cortisol) were averaged across the two days and used for analyses.

Daily Hassles. The Perceived Stress Scale (PSS) is a 10-item self-report questionnaire that measures the degree to which individuals appraise their stress level related to daily situations over the past month (Cohen, Kamarck, & Mermelstein 1983). Specifically, it assesses levels of uncontrollability, unpredictability, and overload that individuals perceive in their daily life. Participants rate items on a five-point Likert scale (0 *never* to 4 *very often*). Items are summed for a total daily hassles score (negative items reverse coded), with higher scores indicating higher levels of daily hassles. The Perceived Stress Scale has good internal consistency (α=0.84-0.86), good two-day (*r*=0.85), and adequate six-week test-retest reliability (*r*=0.61; Cohen, Kamarck, & Mermelstein, 1983). In the current sample, internal consistency was high (α=0.81). Though originally designed and validated for adults only, this measure has been used and published in youth as young as 8 years of age (e.g., Rotenberg & McGrath, 2011; Martin, Kazarian, & Breiter, 1995).

Stressful Life Events. A questionnaire based on the original Stressful Life Events Schedule (SLES), a semi-structured interview, was used to identify the occurrence of life stressors in nine domains: education, work, money, housing, crime, health, deaths, romantic relationships, and other relationships over the past year (Williamson et al., 2003). There are youth-report (child 8-12 years; adolescent 13-18 years) and parent-report versions of the SLES. In the current study, youth independently endorsed the

number of stressful events they experienced over the past year from a list of 76 events, adapted from the original SLES interview version. Youth also rated the intensity of the stress associated with each event endorsed on a five-point Likert scale (0 *not at all stressful* to 4 *very stressful*). Three total scores were derived: total number of events endorsed (*stress number*), percent of events endorsed (*stress ratio*), and total stress intensity ratings for endorsed events (*stress intensity*). Some events were not applicable to youth below the age of 12 (e.g., events related to work), and as such, they had fewer events they can endorse. The SLES stress ratio was derived to account for the different number of events youth can endorse. The original SLES has good 5- to 15-day test-retest reliability (r=0.68), with somewhat greater reliability for adolescents (r=0.72) than for children (r=0.61; Williamson et al., 2003).

Sleep Duration. Youth recorded their bed- and wake-times on the same days as cortisol sampling in a sleep diary to derive their sleep duration. Wake-time was anchored to the time at which the awakening saliva sample was taken. Average sleep duration across the two nights was used for analysis. Parent- and self-report of youth's typical bed- and wake-times on school days were also obtained to derive two additional sleep duration variables.

Daytime Sleepiness. The Pediatric Daytime Sleepiness Scale (PDSS; Drake, Nickel, Burduvali, Roth, Jefferson, & Badia, 2003) is an 8-item questionnaire used to assess daytime sleepiness in youth. Youth reported the frequency to which they felt sleepy or alert during their daily routine on a five-point Likert scale (1 *never* to 5 *always*). Items are summed to derive a global daytime sleepiness score, ranging from 0 to 32, with higher scores indicating greater daytime sleepiness. The Pediatric Daytime Sleepiness

Scale has high internal consistency (α=0.80; Drake, Nickel, Burduvali, Roth, Jefferson, & Badia, 2003). In the present sample, the measure had good internal consistency (α=0.74). This scale was originally designed and validated for middle school-aged youth, aged 11 to 15 years, but it has also been used with youth as young as 10 years old (e.g., Beebe et al., 2007).

Sleep Disturbance. The Child's Sleep Habits Questionnaire (CSHQ) is a 35-item parent-report questionnaire that assesses different domains of sleep problems in children over the past month (Owens, Spirito, & McGuinn, 2000). This questionnaire includes eight subscales: bedtime resistance, sleep onset latency, sleep duration, sleep anxiety, sleep behaviours and night waking, sleep-disordered breathing (e.g., snores loudly), parasomnias (e.g., talks during sleep), and daytime sleepiness. Parents rated the frequency of their children's sleep behaviours in a typical recent week on a three-point scale (1 *usually* to 3 *rarely*). Items are summed to derive a sum score. The Child's Sleep Habits Questionnaire and its subscales have good psychometric properties (Owens, Spirito, & McGuinn, 2000), with moderate to high internal consistency (α=0.36-0.93) and moderate 2-week test-retest reliability (*r*=0.62-0.79; Owens, Spirito, & McGuinn, 2000). Internal consistency of the measure for the current sample was comparable to those previously established (Owens, Spirito, & McGuinn, 2000; α=0.59). Though the Child's Sleep Habits Questionnaire was originally designed and validated for children aged 4 to 10, this measure has been used with adolescents up to 17 years old (Beebe et al., 2007).

Subjective Sleep Quality. The sleep quality subscale from the Pittsburgh Sleep Quality Index was used to measure youth's subjective sleep quality over the past month (Buysse, Reynolds III, Monk, Berman, & Kupfer, 1989). Youth answered the item

"Overall, I would rate my sleep as __" on a 10-point Likert scale (1 *very bad* to 10 *very good*). The Pittsburgh Sleep Quality Index has good internal consistency (α=0.81-0.83; Grandner, Kripke, Yoon, & Youngstedt, 2006; Buysse, Reynolds III, Monk, Berman, & Kupfer, 1989) and moderate 18-month test-retest reliability (*r*=0.40; Buysse, Reynolds III, Monk, Berman, & Kupfer, 1989). The original measure was designed and validated for adults, but this measure has been used with elementary school-aged children (e.g., Tan, 2004).

Pubertal Development. Schematic drawings were used to assess pubertal development. Sex-appropriate schematic drawings of pubic hair growth corresponding to Tanner Stages I to V of pubertal development were used to assess adrenarche in youth (Golding, Pembray, & Jones, 2001; Tanner, 1962). Youth were asked to select the drawing that best corresponded to their pubertal stage. Although physician examination is the gold standard method to assess pubertal development, illustrations were used due to privacy concerns and the sensitivity of physician examination. Pubertal illustrations have previously demonstrated good validity and reliability, with high parent-child agreement (*r* = .77-.91; Dorn, Susman, Nottelmann, Inoff-Germain, & Chrousos, 1990; Morris & Udry, 1980).

Procedure

Youth and their parents were scheduled for two laboratory sessions. During the initial laboratory session, informed consent was obtained from parents and youth; youth below the age of fourteen provided their assent. Parents and youth completed questionnaires. Next, participants were provided detailed instructions for the cortisol sampling procedure. To ensure their understanding of the instructions, youth were asked

to provide one saliva sample in the laboratory. Then, they were instructed how to complete the sleep diary, in which they would record the date and time of cortisol samples and prior night bed-time. Finally, participants were provided saliva collection kits for home and school. During the second laboratory session, participants returned the saliva samples and sleep diary and were compensated for their involvement in the study.

Data Preparation

Sample Exclusion Criteria. Of the 245 participants who completed the Healthy Heart Project, youth who did not collect or return any saliva samples (n=14) were excluded from data analyses. Previous research has demonstrated significant differences between weekday and weekend cortisol values in youth (Rotenberg, McGrath, Roy-Gagnon, & Tu, 2012). Due to the small sample number of participants with weekend data only, youth who collected both saliva samples only on weekend (n=11) were also excluded from data analyses. Thus, the final sample consisted of 220 participants.

Missing Data. Single samples that were either not returned or did not contain enough saliva for assay were coded as missing data. The cortisol awakening response indices (AUC_{AG}, AUC_I) were coded as missing if the $awake_0$ sample was missing or if the time lapse between the collection of the $awake_0$ and $awake_{+45}$ samples exceeded one hour. Similarly, $slope_{max}$ and AUC_{TG} were defined as missing if the awakening, maximum, or two other samples were missing. The percentage of missing data for all calculated aggregate indices and single sample measures ranged from 3% to 25%. Multiple imputation procedures were performed for the aggregate indices and single samples. Missing values were imputed 20 times with re-sampling techniques in SPSS 20.0. Finally, if youth collected saliva samples on one weekday and one weekend day,

the weekend cortisol values were replaced by their corresponding weekday value. This method has been previously reported in studies using psychophysiological data (e.g., Rotenberg, Quon, Van Becelaere, & McGrath, 2013).

Data Reduction

Stressful Life Events. Principle components analysis, based on Varimax rotation, was used to identify and compute a composite score for the three stressful life events sum scores (stress number, stress ratio, stress intensity). The Kaiser-Meyer-Olkin measure of sampling adequacy was above the recommended value of 0.60 (*KMO*=0.72, $\chi^2(3)=1076.14$, $p<.001$; Meyers, Gamst, & Guarino, 2013). The analysis yielded a 1-component solution, labelled as S*tressful Event Component*, which explained 92.63% of the variance (Table 1). A composite score was computed for the component, based on the loadings of the indicators on the component. A higher score on the Stress Event Component indicates greater reported stress and events on the Stressful Life Events Schedule.

Sleep. The six sleep variables (sleep diary sleep duration, child-report sleep duration, parent-report sleep duration, daytime sleepiness, sleep disturbance, subjective sleep quality) were analyzed using principle components analysis, based on Varimax rotation. The Kaiser-Meyer-Olkin measure of sampling adequacy was above the recommended value (*KMO*=0.66, $\chi^2(15)=314.23$ $p<.001$; Meyers, Gamst, & Guarino, 2013). The analysis yielded a 2-component solution, accounting for 62.13% of the total variance. Component 1, which explained 35.63% of the variance, was labelled as *Sleep Duration Component* as all three sleep duration variables loaded highly on this component (Table 2). Component 2, which accounted for 26.50% of the variance, was

labelled as *Sleep Quality Component* due to the high loadings of daytime sleepiness, sleep disturbance, and subjective sleep quality (Table 2). A composite score was computed for each component, based on the primary loadings of the indicators on each component. A higher Sleep Duration Component indicates longer sleep duration; higher Sleep Quality Component indicates better sleep quality.

Data Analyses

All analyses were conducted using SPSS, version 20.0. All data were screened to verify outliers and normality of distribution in cortisol, stress, and sleep variables. Values that were above five standard deviations from the mean were considered an outlier and were replaced by the next most extreme value within five standard deviations of the mean. Data were considered non-normally distributed if the skewness and/or kurtosis values exceeded an absolute value of three and twenty respectively. Bedtime cortisol was the only non-normally distributed variable, which was addressed using square root transformation.

Hypothesis Testing. The overarching objective of the present study was to examine whether sleep mediates the relation of youth's stress exposure to their cortisol levels. First, the associations between stress (daily hassles, Stressful Event Component), sleep (Sleep Duration Component, Sleep Quality Component), and cortisol (bedtime cortisol, maximum cortisol, AUC_{AG}, AUC_{I}, AUC_{TG}, $slope_{max}$) were examined. The three hypotheses tested in the present study were: (1) greater stress exposure would be related to disrupted diurnal cortisol profile, (2) greater stress exposure would be related to poorer sleep, and (3) poorer sleep would be related to disrupted diurnal cortisol profile, after controlling for relevant covariates (age, sex, adrenarche, time of awakening,

socioeconomic status). Regression analyses (general linear model) were conducted to test these hypotheses.

The fourth hypothesis to test mediation was conducted by estimating the total, indirect, and direct effects of stress exposure on cortisol through sleep (Preacher & Hayes, 2008). Estimates were provided for the total, indirect, and direct effects of the associations between stress, sleep, and cortisol, after controlling for age, sex, adrenarche, time of awakening, and socioeconomic status. Indirect effects were analysed based on 1000 bootstraps and were evaluated as significant if the 95% bias-correct confidence interval of the indirect effect did not include a value of zero (Preacher & Hayes, 2008). Mediation analyses were conducted separately for each pair of stress and cortisol associations; a total of twelve separate mediation analyses were performed.

Exploratory Analyses: Moderation. A moderator distinguishes from a mediator in that it influences the strength of a relation of a predictor variable to an outcome variable. To rule out potential alternative hypotheses, the moderating effects of sleep in the relation of stress to cortisol were examined as part of the post-hoc analyses. General linear model was conducted to test for the potential interaction between stress exposure and sleep (*Sleep Duration x daily hassles, Sleep Duration x Stressful Event, Sleep Quality x daily hassles, Sleep Quality x Stressful Event*) on cortisol. Interaction effects of stress exposure and sleep on cortisol, controlling for relevant covariates known to affect cortisol, were estimated. Moderation analyses were conducted separately for each pair of stress and cortisol associations.

RESULTS

Sample Characteristics

The final sample (*N*=220) included youth aged 8 to 18 years (*M*=12.62, *SD*=2.04) in grade 3 to 13, with the majority of youth in 8th grade (19.1%). Sample descriptive statistics are presented in Table 3. Youth were predominantly Caucasian (59.5%), adolescents (61.4%), and male (55.9%). The sample included youth across the full range of pubertal stages (Tanner Stage I-V), with the majority of youth in Tanner Stage IV (23.2%). Most parents completed a university degree (*M*=16.44 years, *SD*=3.32) and their household income averaged $73,552 yearly (*SD*=51,902.65).

Cortisol

Descriptive statistics for single sample ($awake_0$, $awake_{+30}$, $awake_{+45}$, pre-lunch, pre-dinner, bedtime) and aggregate cortisol values (AUC_{AG}, AUC_I, AUC_{TG}, $slope_{max}$) are summarized in Table 4. Cortisol values are similar to values previously published in the pediatric literature (e.g., Oskis, Loveday, Hucklebridge, Thorn, & Clow, 2009), showing a peak in cortisol secretion shortly after morning awakening, followed by a gradual decline throughout the day.

Intercorrelations among cortisol values are presented in Table 7. Awake single sample ($awake_0$, $awake_{+45}$, maximum cortisol) and aggregate cortisol values (AUC_{AG}, AUC_I) were highly correlated among each other. AUC_{TG} was strongly associated with all cortisol values, except $slope_{max}$.

Stress Exposure

Descriptive statistics for daily hassles and stressful life events (stress number, stress ratio, stress intensity) are presented in Table 5. Youth endorsed 13.70 stressors (*SD*=7.25) on the Stressful Life Events Schedule, which represent 19.79% of the total number of stressors (*SD*=10.26). Youth rated an average total stress intensity rating of 24.31 for endorsed stressors (*SD*=19.03). Youth reported moderate levels of daily hassles (*M*=15.43, *SD*=5.92).

Intercorrelations among stress variables are presented in Table 8. Stress number, stress ratio, and stress intensity were highly correlated among each other and were highly correlated with Stressful Event Component. Daily hassles showed the lowest correlation with other stress exposure variables.

Sleep

Descriptive statistics for sleep duration variables (sleep diary, child-report, parent-report) and sleep quality variables (subjective sleep quality, daytime sleepiness, sleep disturbance) are presented in Table 6. Based on youth-report, youth experienced good sleep quality (*M*=6.75, *SD*=2.05) and moderate daytime sleepiness (*M*=14.03, *SD*=5.40). Based on parent-report, youth had high sleep problems (*M*=41.32, *SD*=5.24). Youth's sleep duration was similar across respondents, with youth reporting an average 8.98 hours (*SD*=1.20) in the sleep diary and 9.12 hours (*SD*=1.04) during typical school nights; and, parents reporting 9.23 hours (*SD*=1.02) during typical school nights over the past month. Youth's sleep duration in the current sample matches National Sleep Foundation's (2000) recommendations of 9 to 10 hours of sleep per night for youth. Average bed-time was 22.45 (*SD*=1.47) and wake-time was 7.53 (*SD*=1.26) on the two cortisol sampling days.

Intercorrelations among sleep variables are presented in Table 9. Sleep duration measures were highly correlated across respondents (sleep duration, child-report, parent-report) and were highly correlated with Sleep Duration Component. Youth's subjective sleep quality and daytime sleepiness were highly correlated among each other and were highly correlated with Sleep Quality Component. Sleep disturbance showed the lowest correlation with other sleep variables.

Correlations between Stress, Sleep, and Cortisol Variables

Zero-order correlations between daily hassles, stressful life events (stress number, stress ratio, stress intensity), sleep duration (sleep diary, child-parent, parent-report), sleep quality (subjective sleep quality, daytime sleepiness, sleep disturbance), and cortisol (bedtime cortisol, maximum cortisol, AUC_{AG}, AUC_{I}, AUC_{TG}, $slope_{max}$) are presented in Tables 10, 11, and 12. All stress variables were correlated with all sleep variables, except sleep disturbance. Sleep variables were mainly associated with AUC_{TG}. Daily hassles were correlated with most cortisol values. Stress number, stress ratio, and stress intensity were mainly associated with AUC_{AG} and AUC_{TG}.

Hypothesis 1: Stress Exposure Related to Cortisol

The first hypothesis that greater stress exposure would be related to adverse cortisol levels in youth was partially supported (see Table 12). Greater daily hassles were associated with elevated maximum cortisol, AUC_{AG}, AUC_{TG}, and steeper $slope_{max}$, but not bedtime cortisol and AUC_{I} when relevant covariates were controlled. Higher Stressful Event Component was associated with elevated AUC_{AG} and AUC_{TG}, but not bedtime cortisol, maximum cortisol, AUC_{I}, or $slope_{max}$, when relevant covariates are controlled.

Thus, greater stress exposure was most consistently associated with higher AUC_{AG} and AUC_{TG}.

Hypothesis 2: Stress Exposure Related to Sleep

The second hypothesis that greater stress exposure would be related to poorer sleep in youth was partially supported (see Table 12). Greater daily hassles were associated with poorer sleep quality on the Sleep Quality Component, but not Sleep Duration Component when relevant covariates are controlled. Greater Stressful Event Component was associated with lower scores on Sleep Quality Component and Sleep Duration Component when relevant covariates are controlled. Taken together, greater stress exposure was more consistently related to lower sleep quality.

Hypothesis 3: Sleep Related to Cortisol

The third hypothesis that poorer sleep would be related to adverse cortisol levels in youth was partially supported (see Table 13). Poorer sleep quality on the Sleep Quality Component was associated with higher bedtime cortisol and AUC_{TG}, but not maximum cortisol, AUC_{AG}, AUC_{I}, or $slope_{max}$. Shorter sleep duration on the Sleep Duration Component was not associated with any single sample or aggregate cortisol values. Thus, poorer sleep quality was associated with higher bedtime cortisol and AUC_{TG}.

Hypothesis 4: Sleep Mediates Association between Stress Exposure and Cortisol

The fourth hypothesis whether the association between youth's stress exposure and their cortisol levels was mediated by poorer sleep was tested (see Table 13). Based on the above results, the direct effects of daily hassles and Stressful Event Component on AUC_{TG}, the effects of daily hassles and Stressful Event Component on Sleep Quality Component, and the effects of Sleep Quality Component on AUC_{TG} were statistically

significant. To this end, mediational analyses were only conducted with Sleep Quality Component as a hypothesized mediator in the relation between stress exposure (daily hassles, Stressful Event Component) and AUC_{TG}.

The hypothesis that poor sleep quality would mediate the link between stress exposure and AUC_{TG} was supported. The direct effects of daily hassles on AUC_{TG} were substantially reduced when Sleep Quality Component was included as a mediator in the analysis. These results were further supported by bootstrapping analyses, which demonstrated that Sleep Quality Component exerted significant indirect effects on daily hassles in predicting AUC_{TG} (R^2=0.10, F(7, 212)=3.55, p=.001; 95% BCI[0.09, 1.15]; see Figure 1). Likewise, the direct effects of Stressful Event Component on AUC_{TG} were also substantially reduced when Sleep Quality Component was included as a mediator in the analysis. Results based on bootstrapping analyses demonstrated that Sleep Quality Component exerted significant indirect effects on Stress Event Component in predicting AUC_{TG} (R^2=0.11, F(7, 212)=3.69, p=.001; 95% BCI[0.40, 3.82]; see Figure 2). Taken together, the mediation hypothesis that sleep quality was a mediator in the relation between stress exposure and AUC_{TG} was supported.

Further exploratory mediation analyses were conducted to examine whether sleep quality mediated the association between stress exposure and AUC_{TG} when sleep duration was included as a covariate in the mediational analyses. Mediation analyses, controlling for Sleep Duration Component and other relevant covariates, were performed.

Consistent with the above results, greater daily hassles were associated with lower scores on the Sleep Quality Component (β=-0.57, p<.001). Lower scores on the Sleep Quality Component were associated with higher AUC_{TG} (β=-0.21, p=.002). Greater daily

hassles were associated with elevated AUC_{TG} (β=0.14, p=.05). The direct effects of daily hassles on AUC_{TG} were substantially reduced when Sleep Quality Component was included as a mediator in the analysis (β=0.03, p=.77). Results based on bootstrapping analyses demonstrated that Sleep Quality Component exerted significant indirect effects on daily hassles in predicting AUC_{TG} (R^2=0.11, F(8, 211)=3.15, p=.002; 95% BCI[0.06, 1.14]).

Consistent with above results, greater Stressful Event Component was associated with lower scores on the Sleep Quality Component (β=-0.23, p=.001). Lower scores on the Sleep Quality Component were associated with higher AUC_{TG} even when Sleep Duration Component was controlled (β=-0.21, p=.002). Although greater Stressful Event Component were no longer associated with elevated AUC_{TG} when Sleep Duration was included as a covariate (β=0.11, p=.12), the direct effects of Stress Event Component on AUC_{TG} were still substantially reduced when Sleep Quality was included as a mediator in the analysis (β=0.06, p=.37). Results based on bootstrapping analyses also demonstrated that Sleep Quality Component exerted significant indirect effects on Stressful Event Component in predicting AUC_{TG} (R^2=0.11, F(8, 211)=3.25, p=.002; 95% BCI[0.38, 4.19]). Taken together, these results suggest that the mediating effects of sleep quality linking stress exposure and AUC_{TG} remains significant even when sleep duration is controlled.

Post-Hoc Analyses: Moderation

To test alternative hypotheses, post-hoc exploratory analyses were conducted to determine whether sleep (Sleep Duration Component, Sleep Quality Component) moderated the relation between stress (daily hassles, Stressful Event Component) and

cortisol (bedtime cortisol, maximum cortisol, AUC_{AG}, AUC_{I}, AUC_{TG}, $slope_{max}$). General linear model, controlling for age, sex, adrenarche, time of awakening, and socioeconomic status was conducted to test this hypothesis (see Table 14). None of the four interaction effects (daily hassles x Sleep Quality, daily hassles x Sleep Duration, Stress Event x Sleep Quality, Stressful Event x Sleep Duration) had a significant effect on cortisol.

DISCUSSION

There is increasing evidence to support the potential contribution of poor sleep as a pathophysiological pathway underlying the association between stress exposure and disruption of the diurnal cortisol profile. Specifically, convincing cross-sectional and experimental findings point to the adverse effects of greater stress exposure on sleep, which in turn, are responsible for subsequent disruption of the diurnal cortisol profile. The overarching objective of the present study was to examine whether poor sleep was a pathophysiological pathway linking stress exposure and the diurnal cortisol profile in youth. Specifically, the first objective of the present study was to establish the associations between stress, sleep, and cortisol. The second objective was to investigate whether the association between stress exposure and cortisol was mediated by poor sleep.

All of the current study's hypotheses were at least partially supported. First, there was partial support for the hypothesis that greater stress exposure would be related to adverse cortisol levels. Greater stressful life events were associated with higher AUC_{AG}. Greater daily hassles were associated with higher maximum cortisol, AUC_{AG}, AUC_{TG}, and a steeper diurnal slope. Second, there was partial support for the hypothesis that greater stress exposure would be related to poorer sleep. Greater stressful life events were associated with shorter sleep duration and poorer sleep quality. Third, there was partial support for the hypothesis that poorer sleep would be related to adverse cortisol levels. Poorer sleep quality was associated with higher bedtime cortisol levels and AUC_{TG}. Fourth, there was partial support for the mediation hypothesis that poor sleep is the mediating pathway linking stress exposure and the diurnal cortisol profile. The association between greater stress exposure, in terms of both daily hassles and stressful

life events, and elevated AUC_{TG} was mediated by poorer sleep quality. Sleep duration did not mediate this association. Finally, alternative moderation hypotheses were not supported.

The association between stress exposure and cortisol in the current study largely replicates the association found in the existing literature. Consistent with pediatric studies based on child-report stressful life events assessed across multiple life domains over a one-year reference period and recommended saliva sampling procedures (e.g., Gunther et al., 2012; Gustafsson et al., 2010; Wolf et al., 2008), greater stressful life events were associated with higher cortisol awakening response. Although no association was found between stressful life events, single sample (bedtime cortisol, maximum cortisol) and dynamic measures (AUC_I, $slope_{max}$), these null findings may be attributable to the current saliva sampling procedure (see limitations section). Consistent with cross-sectional adult findings, greater daily hassles were associated with a higher cortisol awakening response and total cortisol concentration (e.g., Pruessner et al., 1999; Schulz, Kirschbaum, Pruessner, & Hellhammer, 1998). However, inconsistent with previous adult findings, current results suggest that greater daily hassles were associated with a steeper (as opposed to a flatter) diurnal slope in youth. While it appears counterintuitive, this finding is plausible given the association established between daily hassles and maximum sample. Greater daily hassles were highly correlated with maximum, but not bedtime cortisol levels. As such, it is plausible that the relation between daily hassles and the diurnal slope were mainly driven by the influence of maximum cortisol. This finding suggests that, developmentally, the cortisol awakening response may be more susceptible to the adverse effects of stress exposure, but bedtime cortisol levels and the diurnal slope

are affected by stress exposure only later in the developmental life course. Moreover, it is plausible that bedtime cortisol and the diurnal slope are only adversely affected with repeated stress exposure. Previous findings have documented that the effects of stress exposure on cortisol secretion are moderated by early adversities, such that greater stress exposure is related to higher bedtime cortisol and a flatter diurnal slope among individuals who were previously exposed to early adversities (e.g., Hanson & Chen, 2010; Essex, Klein, Cho, & Kalin, 2002). Given that early adversities were not measured in the current study, future research is required to further elucidate this hypothesis. Taken together, there is clear evidence supporting a disrupted diurnal cortisol profile among youth who experienced greater stress exposure.

Previously reported relations for stress exposure and poor sleep have been partially replicated in the current study. Consistent with pediatric studies, based on polysomnography- and actigraphy assessments of sleep (e.g., Dewald et al (in print); El-Sheikh, Buckhalt, & Acebo, 2006; Sadeh, Raviv, & Gruber, 2000), greater stressful life events were associated with poorer sleep quality and shorter sleep duration. Consistent with adult findings, greater daily hassles were related to poorer sleep quality (Lund et al., 2011). However, inconsistent with findings that demonstrate shorter sleep duration among individuals who experience greater daily hassles (Kashani, et. al., 2011), a similar result was not found in the present study. The null findings for sleep duration may be attributed to the prescribed bedtimes for most youth, especially on weekdays (school days), which are often constrained by parent-imposed bed- and wake-times as well as early school start times. This is, in fact, evidenced by the comparability of sleep durations

reported in the current study and sleep duration recommended by the National Sleep Foundation (2000).

Current findings for the relation between poor sleep and adverse cortisol levels partially parallel associations found in other pediatric studies using objective sleep measures. Shorter sleep duration, lower sleep efficiency, and greater sleep fragmentation are associated with higher bedtime cortisol, cortisol awakening response, and AUC_{TG}, as well as a flatter diurnal slope (Hatzinger et al., 2010; Raikkonen et al., 2010; Hatzinger et al., 2008). However, current findings revealed that poorer sleep quality, based on subjective report, was only associated with higher bedtime cortisol and AUC_{TG}; no association was found for cortisol awakening response and diurnal slope. Inconsistent findings between present and previously reported results may be attributable to age differences across study samples, as most pediatric studies test school-aged children. Despite some of the null findings, the current results greatly corroborate experimental evidence from animal and adult studies, which demonstrated the adverse effects of poor sleep on bedtime/evening cortisol levels (Leproult, Copinschi, Buxton, & Van Cauter, 1997). Bedtime/evening cortisol level is thought to be regulated by the negative feedback loop of the HPA axis, which suppresses cortisol secretion throughout the day (Sapolsky et al., 1984). Given that poor sleep was found to relate to higher bedtime cortisol in the current study, this finding suggests that poor sleep affects the diurnal cortisol profile possibly through alterations at the level of the negative-feedback loop of the HPA axis.

The current findings suggest that poor sleep is a plausible pathophysiological pathway by which stress exposure "gets under the skin" to disrupt the diurnal cortisol profile in children and adolescents. Specifically, the adverse effects of greater daily

hassles and stressful life events on AUC_{TG} were mediated by sleep quality. This hypothesis was supported even when sleep duration was included as a covariate. These findings suggest that the quality of sleep is of greater importance than the quantity of sleep in the association between stress exposure and cortisol. Current findings can be clarified by examining alterations of the inhibitory effects of sleep on cortisol secretion resulting from stress exposure. Onset of nocturnal sleep reliably exerts an inhibitory effect on cortisol secretion (Weibel, Follenius, Spiegel, Ehrhart, & Brandenberger, 1995; Van Cauter, Blackman, Roland, Spire, Refetoof, & Polonsky, 1991), with the emergence of slow-wave sleep coinciding with the lowest cortisol levels during the 24-hour period (Weitzman, Zimmerman, Czeisler, & Ronda, 1983). Experimentally-induced stress is known to reduce the amount of time spent in slow-wave sleep (Cheeta, Ruigt, van Proosdij, & Wilner, 1997), a sleep stage thought to have strong regulatory control over cortisol secretion during sleep (Weitzman, Zimmerman, Czeisler, & Ronda, 1983). As such, the inhibitory effects of sleep on cortisol secretion are likely suppressed following stress exposure, leading to a disrupted diurnal cortisol profile. Taken together, it is plausible that stress exposure disrupts the diurnal cortisol profile through alterations of the inhibitory effects of sleep on cortisol secretion.

Strengths, Limitations, and Recommendations

There were several methodological strengths and limitations in the present study that merit discussion. First, the present study was based on cross-sectional data. It is acknowledged that causal inferences about the mediation hypothesis cannot be drawn. Such causal inferences would require experimental manipulation or longitudinal data. Given the longitudinal nature of the Healthy Heart Project, this hypothesis can be retested

with data to be collected at future time points. Despite this limitation, this study provided important ground work for future studies in this line of research inquiry

Second, the current study used salivary cortisol sampling procedures based on the recommendations proposed by the MacArthur network (2000). Saliva samples were collected at recommended time points over two days to determine single sample (bedtime cortisol, maximum cortisol) and aggregate cortisol values (AUC_{AG}, AUC_{I}, AUC_{TG}, diurnal slope). This methodological improvement reduces the potential for measurement error, especially for aggregate cortisol values. However, previous studies with youth have demonstrated that single sample (bedtime cortisol, maximum cortisol) and dynamic cortisol values (AUC_{I}, diurnal slope) require at least three to seven days to establish stability (Rotenberg, McGrath, Roy-Gagnon, & Tu, 2012; Oskis et al., 2009). The two days of sampling used in the present study were perhaps insufficient to detect some of the associations and may have accounted for some of the null findings in the current study.

Third, this study included a multi-modal approach to provide a reliable and valid measure of youth's stress exposure. Multiple sum scores of stressful life events and youth-report of daily hassles were used to best capture youth's stress exposure. Though daily hassles were not highly correlated with other stress variables, this finding was expected because daily hassles and stressful life events are presumably tapping into different constructs of stress. Measures of daily hassles tap into the everyday, chronic stressors while measures of stressful life events tap into specific, life stressors. Despite this multi-modal approach, there were three limitations related to the measurement of stress in the current study. First, the Perceived Stress Scale, used to measure daily hassles, has not yet been validated for children and adolescents. Second, the adapted

version of the original Stressful Life Events Schedule used in the present study requires further validation. Third, findings from a meta-analysis demonstrated that the effects of stress exposure on cortisol depend on the chronicity of the stressor (Miller et al., 2007). Since stress duration was not assessed in this study, it is likely that the null findings may be accounted for by the short nature of some stressors youth may have experienced.

Fourth, multiple sleep measures, based on multiple informants, were used to comprehensively determine youth's sleep duration and sleep quality. The current study has established the consistency of youth's sleep duration and sleep quality across informants. Nonetheless, there is still a need to replicate findings of the current study using objectively measured sleep (e.g., polysomnography) in order to delineate the influence of specific sleep stages and sleep parameters (e.g., sleep efficiency, sleep fragmentation) that are fundamental to the disruption of the diurnal cortisol profile and underlying the link between stress exposure and cortisol.

Fifth, due to a modest sample size, the current study precluded stratified analyses to examine sex, age, and pubertal differences in the associations between stress exposure, sleep, and cortisol. First, there is evidence suggesting that cortisol values are dependent on sex, age, and pubertal development. Morning salivary cortisol values are higher for girls in Tanner Stages II and III of pubertal development compared to boys (Netherton, Goodyer, Tamplin, & Herbert, 2004). The reverse is true for evening salivary cortisol, such that girls in Tanner Stages II and III of pubertal development have lower evening cortisol levels than boys. Second, there is evidence suggesting poor sleep affects cortisol differentially across sex in youth. Previous finding demonstrated that boys who experienced greater sleep problems had lower cortisol levels over the day while girls with

greater sleep problems showed higher cortisol levels over the day (Pesonen et al., 2012). Taken together, these findings allude to the importance of assessing sex, age, and pubertal differences in future studies examining the associations between stress exposure, sleep, and cortisol.

Implications and Conclusions

Given that a disrupted diurnal cortisol profile is a risk factor for many adult chronic illnesses, a better understanding of its pathophysiology and determinants during childhood and adolescence is of importance. The present study generated new knowledge about sleep as a potential pathophysiological pathway underlying the effects of stress exposure on the diurnal cortisol profile. Importantly, these research findings inform the development of prevention programs and public policies. For instance, prevention efforts should aim at cultivating sleep hygiene among parents and youth as part of stress management programs to reduce the physiological sequelae of stress exposure. Taken together, current findings revealed that greater daily hassles and stressful life events were related to poorer sleep quality, which, in turn, was related to adverse total cortisol concentration levels in children and adolescents.

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Table 1

Stressful Event Component Loadings

SLES Sum Scores	Component 1: Stressful Event Component
Stress Number	0.98
Stress Ratio	0.98
Stress Intensity	0.93

KMO=0.72, $\chi^2(3)=1076.14$, $p<.001$

Table 2

Sleep Duration and Sleep Quality Components Loadings

Sleep Measures	Component 1: Sleep Duration Component	Component 2: Sleep Quality Component
Parent-Report Sleep Duration	**0.90**	0.12
Child-Report Sleep Duration	**0.83**	0.07
Sleep Diary Sleep Duration	**0.75**	0.16
Daytime Sleepiness	-0.01	**-0.84**
Subjective Sleep Quality	0.28	**0.72**
Sleep Disturbance	-0.07	**-0.57**

KMO=0.66, $\chi^2(15)=314.23$, $p<.001$

Table 3

Sample Characteristics

	M (*n*)	*SD* (%)
Youth		
Age (8-18 yrs)	12.62	2.04
Grade		
3	(4)	(1.80)
4	(15)	(6.80)
5	(29)	(13.20)
6	(37)	(16.80)
7	(31)	(14.10)
8	(42)	(19.10)
9	(30)	(13.60)
10	(21)	(9.50)
11	(8)	(3.60)
12	(2)	(0.90)
13	(1)	(0.50)
Sex		
Male	(123)	(55.90)
Pubertal Development		
Tanner Stage I	(36)	(16.4)
Tanner Stage II	(36)	(11.4)
Tanner Stage III	(43)	(19.5)
Tanner Stage IV	(51)	(23.2)
Tanner Stage V	(47)	(21.4)
Parent		
Education (8-22 yrs)	16.44	3.32
Household Income ($)	77,769.71	51,902.65

Table 4

Means and Standard Deviations of Cortisol Values (nmol/L)

	Total (*N*=220)		Child (*n*=85)		Adolescent (*n*=135)	
	M	*SD*	*M*	*SD*	*M*	*SD*
Single Sample Values						
Awake	10.23	5.29	10.79	5.88	9.88	4.87
$Awake_{+30}$	15.44	7.48	14.82	7.83	15.84	7.26
$Awake_{+45}$	13.78	7.71	12.32	7.03	14.70	8.00
Lunch	4.67	3.10	4.31	3.18	4.90	3.03
Dinner	2.58	2.93	2.41	3.30	2.70	2.68
Bedtime	1.72	2.19	1.76	2.72	1.69	1.79
Maximum	18.04	7.93	17.56	7.82	18.33	8.02
Aggregate Values						
AUC_{AG}	11.04	7.93	11.32	6.43	10.87	4.64
AUC_{I}	2.24	5.22	1.07	6.73	2.98	3.85
AUC_{TG}	67.87	34.05	66.38	26.28	68.81	32.67
$Slope_{max}$	-1.03	0.50	-1.03	0.50	-1.03	0.50

Table 5

Means and Standard Deviations of Stress Exposure Values

	Total (*N*=220)		Child (*n*=85)		Adolescent (*n*=135)	
	M	*SD*	*M*	*SD*	*M*	*SD*
Daily Hassles (0-40)	15.43	6.92	14.34	5.97	16.11	7.39
Stressful Life Events						
Stress Number (0-76)	13.70	7.25	12.07	6.81	14.73	7.36
Stress Ratio (0-100)	19.79	10.26	19.41	10.74	20.03	9.98
Stress Intensity (0-304)	24.31	19.03	22.12	18.03	25.70	19.57

Table 6

Means and Standard Deviations of Sleep Measures

	Total (*N*=220)		Child (*n*=85)		Adolescent (*n*=135)	
	M	*SD*	*M*	*SD*	*M*	*SD*
Sleep Diary Sleep Times						
Bedtime (24:00)	22.45	1.47	21.62	1.60	22.99	1.09
Waketime (24:00)	7.53	1.26	7.33	0.84	7.65	1.46
Sleep Duration						
Sleep Diary (hrs)	8.98	1.20	9.47	0.93	8.67	1.26
Child-Report (hrs)	9.12	1.04	9.86	0.81	8.65	0.89
Parent-Report (hrs)	9.23	1.02	10.01	0.71	8.74	0.86
Sleep Quality						
Subjective Sleep Quality (0-10)	6.75	2.05	7.31	1.99	6.41	2.02
Daytime Sleepiness (0-32)	14.03	5.40	13.58	5.25	14.31	5.50
Sleep Disturbance (0-105)	41.32	5.24	41.45	5.61	41.23	5.02

Table 7

Intercorrelation among Cortisol Values

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
1. $Awake_0$	-	-	-	-	-	-	-	-	-	-	-
2. $Awake_{+30}$	**0.41*****	-	-	-	-	-	-	-	-	-	-
3. $Awake_{+45}$	**0.15***	**0.80*****	-	-	-	-	-	-	-	-	-
4. Pre-Lunch	**0.33*****	**0.30*****	**0.39*****	-	-	-	-	-	-	-	-
5. Pre-Dinner	**0.21****	**0.16***	**0.24*****	**0.16***	-	-	-	-	-	-	-
6. Bedtime	0.07	0.04	**0.15***	**0.25*****	**0.34*****	-	-	-	-	-	-
7. Maximum	**0.52*****	**0.90*****	**0.85*****	**0.39*****	**0.27*****	**0.13**†	-	-	-	-	-
8. AUC_{AG}	**0.61*****	**0.75*****	**0.67*****	**0.34*****	**0.27*****	**0.21****	**0.82*****	-	-	-	-
9. AUC_I	**-0.38*****	**0.56*****	**0.62*****	0.08	-0.02	-0.07	**0.40*****	0.05	-	-	-
10. AUC_{TG}	**0.65*****	**0.43*****	**0.44*****	**0.75*****	**0.61*****	**0.41*****	**0.58*****	**0.61*****	-0.10	-	-
11. $Slope_{max}$	**-0.50*****	**-0.86*****	**-0.77*****	**-0.41*****	-0.02	**0.16***	**-0.92*****	**-0.75*****	**-0.38*****	**-0.44*****	-

$^{***}p<.001$, $^{**}p < .01$; $^{*}p < .05$; $^{\dagger}p < .10$

Table 8

Intercorrelation among Stress Exposure Variables

	1.	2.	3.	4.	5.
1. Daily Hassles	-	-	-	-	-
2. Stress Number	**0.42*****	-	-	-	-
3. Stress Ratio	**0.41*****	**0.98*****	-	-	-
4. Stress Intensity	**0.53*****	**0.86*****	**0.86*****	-	-
5. Stressful Event Component	**0.47*****	**0.98*****	**0.98*****	**0.94*****	-

$^{***}p<.001$

Table 9

Intercorrelation among Sleep Variables

	1.	2.	3.	4.	5.	6.	7.	8.
1. Sleep Diary Sleep Duration	-	-	-	-	-	-	-	-
2. Child-Report Sleep Duration	**0.40*****	-	-	-	-	-	-	-
3. Parent-Report Sleep Duration	**0.56*****	**0.68*****	-	-	-	-	-	-
4. Sleep Duration Component	**0.75*****	**0.83*****	**0.90*****	-	-	-	-	-
5. Subjective Sleep Quality	**0.31*****	**0.25*****	**0.28*****	**0.28*****	-	-	-	-
6. Daytime Sleepiness	-0.11	**-0.15***	**-0.13**†	-0.01	**-0.45*****	-	-	-
7. Sleep Disturbance	**-0.15***	-0.08	**-0.17***	-0.07	**-0.18****	**0.23****	-	-
8. Sleep Quality Component	**0.16***	0.07	**0.12**†	0.00	**0.72*****	**-0.84*****	**-0.57*****	-

$^{***}p<.001$, $^{**}p < .01$; $^{*}p < .05$; $^{\dagger}p < .10$

Table 10

Zero-Order Correlation between Stress Exposure and Cortisol

	Bedtime Cortisol	Maximum Cortisol	AUC_{AG}	AUC_{I}	AUC_{TG}	$Slope_{max}$
Daily Hassles	0.10	**0.21****	**0.18****	0.11	**0.15***	**-0.17***
Stressful Life Events						
Stress Number	0.04	0.09	**0.14***	-0.02	0.11	-0.08
Stress Ratio	0.04	0.07	**0.13**†	-0.05	0.11	-0.07
Stress Intensity	0.04	0.11	0.09	0.01	**0.12**†	-0.09

$^{**}p < .01$; $^{*}p < .05$; $^{\dagger}p < .10$

Table 11

Zero-Order Correlation between Stress Exposure and Sleep

	Sleep Duration			Sleep Quality		
	Sleep Diary	Child-Report	Parent-Report	Subjective Sleep Quality	Daytime Sleepiness	Sleep Disturbance
Daily Hassles	**-0.19****	**-0.29*****	**-0.23****	**-0.50*****	**0.49*****	**0.19****
Stressful Life Events						
Stress Number	**-0.19****	**-0.21****	**-0.24*****	**-0.23*****	**0.26*****	0.09
Stress Ratio	**-0.14***	**-0.11**†	**-0.14***	**-0.20****	**0.26*****	0.10
Stress Intensity	**-0.12**†	**-0.14***	**-0.17***	**-0.24*****	**0.28*****	0.06

$^{***}p<.001$, $^{**}p < .01$; $^{*}p < .05$; $^{\dagger}p < .10$

Table 12

Zero-Order Correlation Between Sleep and Cortisol

	Bedtime Cortisol	Maximum Cortisol	AUC_{AG}	AUC_{I}	AUC_{TG}	$Slope_{max}$
Sleep Duration						
Sleep Diary	-0.01	**-0.14***	**-0.11**†	**-0.15***	**-0.20****	0.06
Child-Report	-0.09	-0.04	-0.05	-0.04	-0.10	-0.05
Parent-Report	0.03	-0.08	0.01	**-0.13***	-0.08	0.02
Sleep Quality						
Subjective Sleep Quality	-0.11	-0.10	-0.02	-0.04	**-0.16***	0.05
Daytime Sleepiness	0.10	0.08	-0.003	0.10	**0.15***	-0.04
Sleep Disturbance	0.09	0.004	0.06	-0.06	**0.14***	0.04

$^{**}p < .01$; $^{*}p < .05$

Table 13

Hypothesis Testing Models

Univariate Models	*ß*	*B*	*SE*	*t*	*p*	*partial r*
			DV: Sleep Duration Component			
Age	**-0.60**	**-0.29**	**0.03**	**-10.13**	**<.001**	**-0.57**
Sex	**-0.10**	**-0.20**	**0.10**	**-2.08**	**.04**	**-0.14**
Adrenarche	**-0.12**	**-0.07**	**-0.12**	**-1.98**	**.05**	**-0.13**
Time of Awakening	**0.34**	**0.27**	**0.34**	**7.09**	**<.001**	**0.44**
Socioeconomic Status	-0.05	-0.02	-0.05	-1.13	.26	-0.08
Daily Hassles	-0.06	-0.01	0.01	-1.23	.22	-0.08
Stressful Event Component	**-0.09**	**-0.09**	**0.05**	**-1.86**	**.07**	**-0.13**

Note. Presented in the first part of the table are test statistics for the relation between covariates (age, sex, adrenarche, time of awakening, socioeconomic status) and Sleep Duration Component. Presented in the second part of the table are test statistics for the relation between stress exposure (daily hassles, Stressful Event Component) and Sleep Duration Component when relevant covariates were controlled.

Univariate Models	*ß*	*B*	*SE*	*t*	*p*	*partial r*
			DV: Sleep Quality Component			
Age	**0.14**	**0.07**	**0.04**	**1.69**	**.09**	**0.12**
Sex	-0.03	-0.07	0.14	-0.50	.62	-0.03
Adrenarche	**-0.24**	**-0.15**	**0.05**	**-2.85**	**.01**	**-0.19**
Time of Awakening	-0.01	-0.01	0.05	-0.13	.90	-0.01
Socioeconomic Status	**0.12**	**0.04**	**0.02**	**1.83**	**.07**	**0.12**
Daily Hassles	**-0.56**	**-0.08**	**0.01**	**-9.45**	**<.001**	**-0.54**
Stressful Event Component	**-0.22**	**-0.22**	**0.07**	**-3.21**	**.002**	**-0.22**

Note. Presented in the first part of the table are test statistics for the relation between covariates (age, sex, adrenarche, time of awakening, socioeconomic status) and Sleep Quality Component. Presented in the second part of the table are test statistics for the relation between stress exposure (daily hassles, Stressful Event Component) and Sleep Quality Component when relevant covariates were controlled.

Univariate Models	*ß*	*B*	*SE*	*t*	*p*	*partial r*
			DV: Bedtime Cortisol			
Age	-0.04	-0.04	0.09	-0.43	.67	-0.03
Sex	**0.17**	**0.73**	**0.30**	**2.44**	**.02**	**0.17**
Adrenarche	-0.01	-0.12	0.12	-0.14	.89	-0.01
Time of Awakening	-0.05	-0.09	0.12	-0.78	.44	-0.05
Socioeconomic Status	-0.02	-0.02	0.05	-0.33	.74	-0.02
Sleep Duration Component	-0.01	-0.02	0.21	-0.08	.93	-0.01
Sleep Quality Component	**-0.13**	**-0.29**	**0.15**	**-1.89**	**.06**	**-0.13**
Daily Hassles	0.07	0.02	0.02	0.96	.34	0.07
Stressful Event Component	0.03	0.06	0.15	0.40	.69	0.03
(Mediator: Sleep Duration Component)						
Daily Hassles	0.07	0.02	0.02	0.95	.34	0.07
Stressful Event Component	0.03	0.06	0.16	0.39	.70	0.03
(*Mediator: Sleep Quality Component*)						
Daily Hassles	-0.01	-0.002	0.03	-0.08	.94	-0.01
Stressful Event Component	0.00	-0.001	0.16	-0.01	.99	0.00

Note. Presented in the first part of the table are test statistics for the relation between covariates (age, sex, adrenarche, time of awakening, socioeconomic status) and bedtime cortisol. Relevant covariates were controlled in subsequent models.

Univariate Models	*ß*	*B*	*SE*	*t*	*p*	*partial r*
			DV: Maximum Cortisol			
Age	0.09	0.35	0.32	1.10	.27	0.08
Sex	**0.17**	**2.66**	**1.06**	**2.52**	**.01**	**0.17**
Adrenarche	-0.04	-0.18	0.41	-0.46	.65	-0.03
Time of Awakening	**-0.24**	**-1.49**	**0.42**	**-3.57**	**<.001**	**-0.24**
Socioeconomic Status	-0.05	-0.11	0.16	-0.73	.47	-0.05
Sleep Duration Component	0.05	0.37	0.76	0.48	.63	0.03
Sleep Quality Component	-0.08	-0.62	0.54	-1.15	.25	-0.08
Daily Hassles	**0.18**	**0.20**	**0.08**	**2.54**	**.01**	**0.17**
Stressful Event Component	0.06	0.50	0.54	0.91	.36	0.06
(Mediator: Sleep Duration Component)						
Daily Hassles	**0.18**	**0.21**	**0.08**	**2.59**	**.01**	**0.18**
Stressful Event Component	0.07	0.54	0.54	0.98	.33	0.07
(*Mediator: Sleep Quality Component*)						
Daily Hassles	**0.19**	**0.22**	**0.10**	**2.27**	**.02**	**0.15**
Stressful Event Component	0.05	0.38	0.56	0.68	.50	0.05

Note. Presented in the first part of the table are test statistics for the relation between covariates (age, sex, adrenarche, time of awakening, socioeconomic status) and maximum cortisol. Relevant covariates were controlled in subsequent models.

Univariate Models	*ß*	*B*	*SE*	*t*	*p*	*partial r*
			DV: AUC_{AG}			
Age	0.07	0.20	0.22	0.90	.37	0.06
Sex	**0.14**	**1.55**	**0.72**	**2.15**	**.03**	**0.15**
Adrenarche	-0.08	-0.26	0.28	-0.93	.36	-0.06
Time of Awakening	**-0.24**	**-1.04**	**0.28**	**-3.67**	**<.001**	**-0.24**
Socioeconomic Status	-0.03	-0.04	0.11	-0.38	.71	-0.03
Sleep Duration Component	0.08	0.41	0.51	0.79	.43	0.05
Sleep Quality Component	-0.03	-0.16	0.37	-0.42	.67	-0.03
Daily Hassles	**0.16**	**0.12**	**0.05**	**2.29**	**.02**	**0.16**
Stressful Event Component	**0.11**	**0.61**	**0.37**	**1.65**	**.10**	**0.11**
(Mediator: Sleep Duration Component)						
Daily Hassles	**0.17**	**0.13**	**0.05**	**2.36**	**.02**	**0.16**
Stressful Event Component	**0.12**	**0.66**	**0.37**	**1.76**	**.08**	**0.12**
(*Mediator: Sleep Quality Component*)						
Daily Hassles	**0.20**	**0.16**	**0.07**	**2.46**	**.02**	**0.17**
Stressful Event Component	0.11	0.60	0.38	1.59	.11	0.11

Note. Presented in the first part of the table are test statistics for the relation between covariates (age, sex, adrenarche, time of awakening, socioeconomic status) and AUC_{AG}. Relevant covariates were controlled in subsequent models.

Univariate Models	*ß*	*B*	*SE*	*t*	*p*	*partial r*
			DV: AUC_I			
Age	0.08	0.20	0.22	0.91	.36	0.06
Sex	0.06	0.66	0.71	0.93	.35	0.06
Adrenarche	0.12	0.38	0.27	1.41	.16	0.10
Time of Awakening	-0.06	-0.23	0.28	-0.81	.42	-0.06
Socioeconomic Status	0.10	0.15	0.11	1.43	.15	0.10
Sleep Duration Component	0.02	0.12	0.51	0.23	.82	0.02
Sleep Quality Component	-0.03	-0.14	0.36	-0.37	.71	-0.03
Daily Hassles	0.07	0.05	0.05	0.99	.32	0.07
Stressful Event Component	-0.04	-0.19	-0.37	-0.52	.61	-0.04
(Mediator: Sleep Duration Component)						
Daily Hassles	0.07	0.06	0.05	1.01	.31	0.07
Stressful Event Component	-0.04	-0.18	0.37	-0.49	.63	-0.03
(*Mediator: Sleep Quality Component*)						
Daily Hassles	0.08	0.06	0.06	0.93	.35	0.06
Stressful Event Component	-0.04	-0.23	0.38	-0.61	.54	-0.04

Note. Presented in the first part of the table are test statistics for the relation between covariates (age, sex, adrenarche, time of awakening, socioeconomic status) and AUC_I. Relevant covariates were controlled in subsequent models.

Univariate Models	*ß*	*B*	*SE*	*t*	*p*	*partial r*
			DV: AUC_{TG}			
Age	0.08	1.32	1.39	0.95	.34	0.07
Sex	0.08	5.31	4.58	1.16	.25	0.08
Adrenarche	-0.05	-1.07	1.76	-0.61	.54	-0.04
Time of Awakening	**-0.24**	**-6.43**	**1.81**	**-3.55**	**<.001**	**-0.24**
Socioeconomic Status	0.01	0.05	0.69	0.07	.94	0.01
Sleep Duration Component	-0.06	-1.93	3.28	-0.59	.56	-0.04
Sleep Quality Component	**-0.21**	**-7.24**	**2.28**	**-3.18**	**.002**	**-0.21**
Daily Hassles	**0.14**	**0.70**	**0.35**	**2.01**	**.05**	**0.14**
Stressful Event Component	**0.11**	**3.81**	**2.35**	**1.62**	**.10**	**0.11**
(Mediator: Sleep Duration Component)						
Daily Hassles	**0.14**	**0.68**	**0.35**	**1.96**	**.05**	**0.13**
Stressful Event Component	0.11	3.69	2.37	1.56	.12	0.11
(*Mediator: Sleep Quality Component*)						
Daily Hassles	0.03	0.15	0.41	0.37	.71	0.03
Stressful Event Component	0.07	2.34	2.36	0.99	.32	0.07

Note. Presented in the first part of the table are test statistics for the relation between covariates (age, sex, adrenarche, time of awakening, socioeconomic status) and AUC_{TG}. Relevant covariates were controlled in subsequent models.

Univariate Models	*ß*	*B*	*SE*	*t*	*p*	*partial r*
			DV: $Slope_{max}$			
Age	-0.05	-0.01	0.02	-0.56	.58	-0.04
Sex	**-0.12**	**-0.12**	**0.07**	**-1.74**	**.08**	**-0.12**
Adrenarche	0.04	0.01	0.03	0.50	.62	0.03
Time of Awakening	**0.18**	**0.07**	**0.03**	**2.67**	**.01**	**0.18**
Socioeconomic Status	0.06	0.01	0.01	0.90	.37	0.06
Sleep Duration Component	-0.13	-0.07	0.05	-1.38	.17	-0.09
Sleep Quality Component	0.03	0.01	0.04	0.40	.69	0.03
Daily Hassles	**-0.15**	**-0.01**	**-0.01**	**-2.12**	**.04**	**-0.14**
Stressful Event Component	-0.06	-0.03	0.04	-0.86	.39	-0.06
(Mediator: Sleep Duration Component)						
Daily Hassles	**-0.16**	**-0.01**	**0.01**	**-2.25**	**.03**	**-0.15**
Stressful Event Component	-0.07	-0.04	0.04	-1.05	.30	-0.07
(*Mediator: Sleep Quality Component*)						
Daily Hassles	**-0.19**	**-0.01**	**0.01**	**-2.26**	**.03**	**-0.15**
Stressful Event Component	-0.06	-0.03	0.04	-0.79	.43	-0.05

Note. Presented in the first part of the table are test statistics for the relation between covariates (age, sex, adrenarche, time of awakening, socioeconomic status) and $slope_{MAX}$. Relevant covariates were controlled in subsequent models.

Table 14

Exploratory Analyses: Moderation

Univariate Models	*F*	*B*	*SE*	*p*	η^2
		DV: Bedtime Cortisol			
Age	0.22	-0.04	0.09	.67	0.00
Sex	**5.86**	**0.73**	**0.30**	**.02**	**0.03**
Adrenarche	0.03	-0.12	0.12	.89	0.00
Time of Awakening	0.00	-0.09	0.12	.44	0.00
Socioeconomic Status	0.06	-0.02	0.05	.74	0.00
Daily Hassles x Sleep Duration	0.00	0.00	0.02	.95	0.00
Daily Hassles x Sleep Quality	0.29	-0.01	0.02	.59	0.00
Stressful Event x Sleep Duration	0.00	-0.00	0.14	.99	0.00
Stressful Event x Sleep Quality	0.43	-0.94	0.14	.51	0.00
		Maximum Cortisol			
Age	0.79	0.34	0.32	.27	0.00
Sex	**5.88**	**2.66**	**1.06**	**.01**	**0.03**
Adrenarche	0.19	-0.18	0.41	.65	0.00
Time of Awakening	**0.24**	**-1.49**	**0.42**	**<.001**	**0.00**
Socioeconomic Status	0.16	-0.11	0.16	.47	0.00
Daily Hassles x Sleep Duration	0.09	0.03	0.08	0.76	0.00
Daily Hassles x Sleep Quality	0.94	0.07	0.07	0.34	0.00
Stressful Event x Sleep Duration	0.00	-0.01	0.49	0.99	0.00
Stressful Event x Sleep Quality	2.77	0.85	0.51	0.10	0.01

Note. Age, sex, adrenarche, time of awakening, and socioeconomic status included as covariates in all interaction models.

Univariate Models	*F*	*B*	*SE*	*p*	η^2
			AUC_{AG}		
Age	0.64	0.20	0.22	.37	0.00
Sex	4.55	**1.55**	**0.72**	**.03**	0.02
Adrenarche	0.76	-0.26	0.28	.36	0.00
Time of Awakening	0.62	**-1.04**	**0.28**	**<.001**	0.00
Socioeconomic Status	0.00	-0.04	0.11	.71	0.00
Daily Hassles x Sleep Duration	2.41	0.09	0.06	0.12	0.01
Daily Hassles x Sleep Quality	0.31	0.03	0.05	0.58	0.00
Stressful Event x Sleep Duration	0.92	0.32	0.34	0.34	0.00
Stressful Event x Sleep Quality	3.29	0.63	0.35	0.10	0.02
			AUC_{I}		
Age	0.52	0.20	0.22	.36	0.00
Sex	0.86	0.66	0.71	.35	0.00
Adrenarche	1.46	0.38	0.27	.16	0.01
Time of Awakening	0.33	-0.23	0.28	.42	0.00
Socioeconomic Status	2.58	0.15	0.11	.15	0.01
Daily Hassles x Sleep Duration	0.16	-0.02	0.06	0.69	0.001
Daily Hassles x Sleep Quality	0.40	0.03	0.05	0.53	0.00
Stressful Event x Sleep Duration	0.72	-0.27	0.32	0.40	0.00
Stressful Event x Sleep Quality	0.04	0.07	0.34	0.84	0.00

Note. Age, sex, adrenarche, time of awakening, and socioeconomic status included as covariates in all interaction models.

Univariate Models	*F*	*B*	*SE*	*p*	η^2
			AUC_{TG}		
Age	0.17	1.32	1.39	.34	0.00
Sex	1.04	5.31	4.58	.25	0.01
Adrenarche	0.39	-1.07	1.76	.54	0.00
Time of Awakening	**0.11**	**-6.43**	**1.81**	**<.001**	**0.00**
Socioeconomic Status	0.10	0.05	0.69	.94	0.00
Daily Hassles x Sleep Duration	0.37	-0.22	0.36	0.55	0.00
Daily Hassles x Sleep Quality	0.001	0.01	0.30	0.97	0.00
Stressful Event x Sleep Duration	0.59	1.65	2.14	0.44	0.00
Stressful Event x Sleep Quality	0.13	0.79	2.21	0.72	0.00
			$Slope_{max}$		
Age	0.39	-0.01	0.02	.58	0.002
Sex	**3.32**	**-0.12**	**0.07**	**.08**	**0.02**
Adrenarche	0.25	0.01	0.03	.62	0.001
Time of Awakening	**0.69**	**0.07**	**0.03**	**.01**	**0.003**
Socioeconomic Status	0.52	0.01	0.01	.37	0.002
Daily Hassles x Sleep Duration	0.16	-0.002	0.005	0.69	0.001
Daily Hassles x Sleep Quality	0.73	-0.004	0.004	0.39	0.004
Stressful Event x Sleep Duration	0.03	-0.01	0.03	0.86	0.00
Stressful Event x Sleep Quality	3.57	-0.06	0.03	0.10	0.02

Note. Age, sex, adrenarche, time of awakening, and socioeconomic status included as covariates in all interaction models.

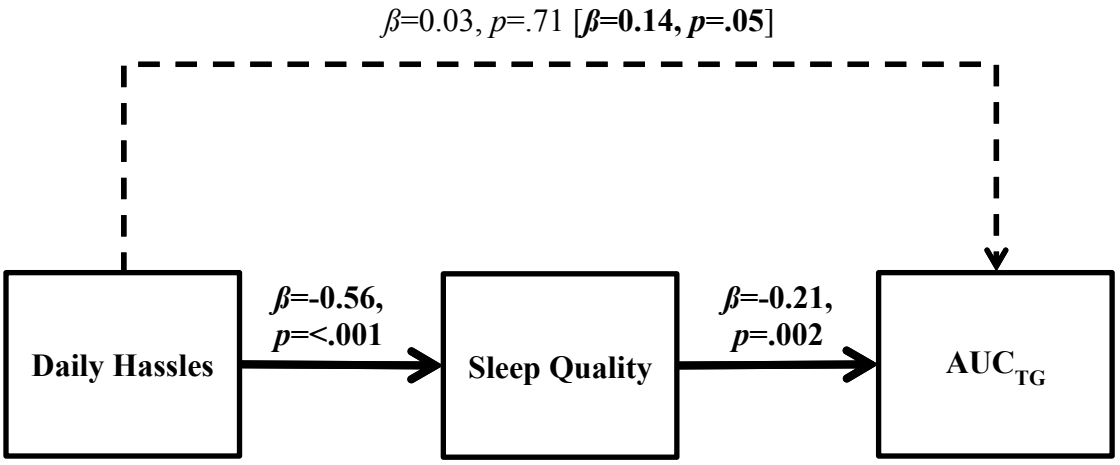

Figure 1. Mediation model 1.

Note. Mediation model testing the indirect effects of Sleep Quality in the Association between daily hassles and AUC_{TG}, controlling for age, sex, adrenarche, time of awakening, and socioeconomic status. Estimates of total effect of daily hassles on AUC_{TG} are presented in brackets above dashed line with adjacent values representing estimates of the total indirect effects of daily hassles on AUC_{TG} through Sleep Quality. (R^2=0.10, F(7, 212)=3.55, p=.001; 95% BCI[0.09, 1.15]).

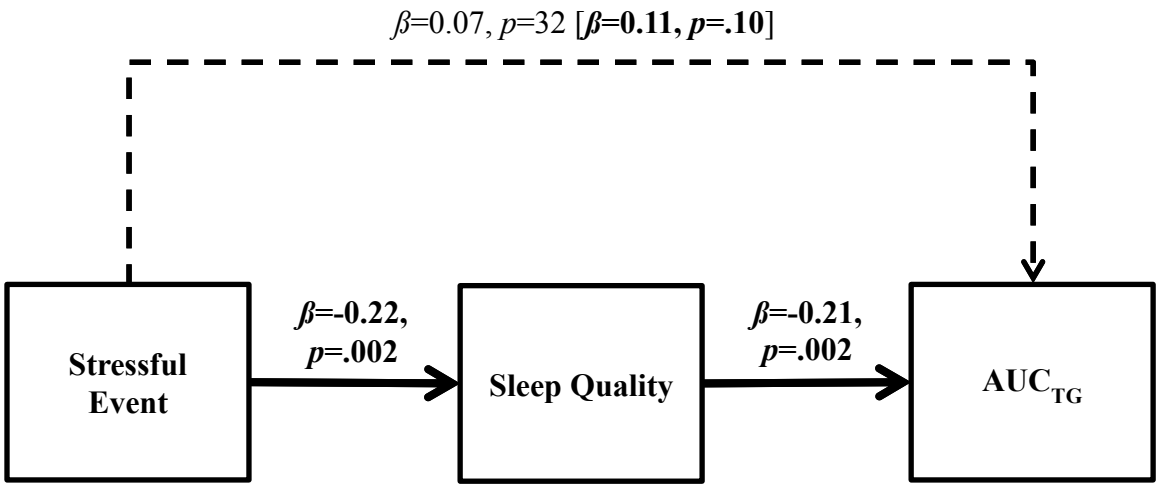

Figure 2. Mediation model 2.

Note. Mediation model testing the indirect effects of Sleep Quality in the Association between Stressful Evens and AUC_{TG}, controlling for age, sex, adrenarche, time of awakening, and socioeconomic status. Estimates of total effect of Stressful Evens on AUC_{TG} are presented in brackets above dashed line with adjacent values representing estimates of the total indirect effects of Stressful Event on AUC_{TG} through Sleep Quality. (R^2=0.11, F(7, 212)=3.69, p=.001; 95% BCI[0.40, 3.82]).