Naturalistic Stress Exposure and the Diurnal Cortisol Profile in Children and Adolescents: A Meta-Analysis

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ABSTRACT

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Stress during childhood has been found to impact health across the lifespan. Research has demonstrated that the development of the diurnal cortisol profile can be altered by early exposure to stress. However, the literature is heterogeneous, with evidence of stress exposure being associated with heightened cortisol, lowered cortisol, blunted cortisol, and with no association. This thesis parses this heterogeneity by conducting a systematic review and meta-analysis of 33 studies that assessed the association between stress exposure during childhood using standardized measures of stress and cortisol. Participant characteristics, study methodology, and conceptual factors were examined as potential moderators of this association. We found that stress and cortisol had a small but significant association (Z' = 0.029). The type of stress measure employed impacted the strength of the association: life-events measures were more strongly associated with cortisol secretion when intensity of the stress exposure was taken into account. Distal vs. proximal measures of stress exposure were differentially associated with cortisol and stress. Child report stress had a stronger association with cortisol than parent report stress. Possible cortisol blunting was found in populations "at risk" for stress exposure, mental health problems, and medical conditions. Possible cortisol blunting was found in populations as they age. Together, this thesis contributes to the extant literature on the association between naturalistic stress exposure and diurnal cortisol secretion in children.

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Glossary

AUC_I	Dynamic increase in amount of cortisol secreted following awakening
AUC_{AG}	Total amount of cortisol secreted during the awakening response
AUC_{TG}	Total amount of cortisol secreted during the day
Slope	Calculated by standard linear regression or rise over run. Can be from calculated from either Wake ($Slope_{Awake}$) or from maximum awakening cortisol ($Slope_{Max}$)
Wake	Saliva sample upon awakening or +0 minutes
+30	Saliva sample taken 30 minutes post-awakening
Morning	Saliva sample collected after +30 to noon
Afternoon	Saliva sample collected after noon to 6pm
Evening	Saliva sample collected after 6pm until bedtime
Bedtime	Saliva sample collected at bedtime
Random	Saliva sample collected at unspecified time

Naturalistic Stress Exposure and the Diurnal Cortisol Profile in Children and Adolescents: A Meta-Analysis

Extant research demonstrates an association between exposure to stress during childhood and the diurnal cortisol profile. However, the literature is inconsistent. Variations in the conceptualization and measurement of stress and cortisol may strongly impact the strength and direction of the association. Additionally, these conceptualization and measurement differences make direct comparisons across studies difficult. The overarching goals of this meta-analysis are to harmonize measurement of stress and cortisol in childhood, to systematically review the extant literature on the association between stress exposure during childhood and diurnal cortisol, and to examine conceptual and methodological factors that may impact this association. This meta-analysis is partly informed by longitudinal findings that highlight the importance of the timing of stress exposure on the development of cortisol trajectories. The following background sections will (i) introduce the intersection of stress and health; (ii) describe the hypothalamic-pituitary-adrenal (HPA) axis; (iii) provide an overview of cortisol and its measurement; (iv) provide an overview of children's stress exposure and its measurement; and, (v) describe the current state of evidence supporting the association between stress and diurnal cortisol during childhood.

Introduction

Stress and Health

Early life adversities, or stressful experiences, have been associated with negative health outcomes across the lifespan. During childhood and adolescence, these health outcomes include depression (Adam et al., 2010; Lupien, McEwen, Gunnar, & Heim, 2009; Van den Bergh & Van Calster, 2009), internalizing and externalizing behaviours (Grant et al., 2003), asthma (Johnson, Riley, Granger, & Riis, 2013), and inflammation (Fuligni et al., 2009; Slopen, Kubzansky, Mclaughlin, & Koenen, 2013). During adulthood, an extensive array of health outcomes include depression (Middlebrooks & Audage, 2008; Nusslock & Miller, 2015), metabolic syndrome (Nusslock & Miller, 2015), stroke (Nusslock & Miller, 2015), addiction (Middlebrooks et al., 2008), heart disease (Nusslock & Miller, 2015), and cancer (Nusslock & Miller, 2015). Felitti and colleagues conducted a series of studies using a retrospective stress questionnaire with 17,000 adults in which they assessed both chronic stress and stressful life events (e.g., familial abuse, household dysfunction, violence, neglect; Anda et al., 2009; Dong et al., 2004; Dube et al., 2009). They found that those who experienced these events during childhood were 1.5 to 2 times more likely to suffer

from cardiovascular disease, autoimmune disorders, premature mortality, and suicide, than those who reported no exposure to events that can be appraised as stressful. Further, a clear dose-response relationship emerged: the greater the number of stressors a child experienced, the higher their risk of morbidity during adulthood. For example, those who experienced four or more adversities had an increased risk of 4- to 12-fold for depression, drug abuse, alcoholism, and suicide attempt. Felitti and colleagues' body of work, combined with others' findings, suggests a robust association between childhood stress exposure and later onset of negative health outcomes. However, it is important to note that retrospective reports of childhood stress can be influenced by mood state, depression, stress level, and recall during adulthood. Thus, retrospective reports of childhood stress may be over-reported in depressed people and underreported in healthy populations (Colman et al., 2015).

Reporter bias aside, possible health consequences of childhood stress exposure are particularly troubling given the prevalence of stressful events. Depending on the stressful event and population, stress exposure varies widely. In samples of European and American adolescents, stress exposure ranged from 0.7%-5.6% for death of parent, 6.8%-59.3% for severe illness of family member, and 5.3%-24.5% for economic adversity (Amone-P'Olak et al., 2009; Benjet et al., 2009; Copeland, Keeler, Angold, & Costello, 2007; Schilling, Jr, & Gore, 2007; Vanaelst, De Vriendt, Huybrechts, Rinaldi, & De Henauw, 2012). Two-thirds (68%) of adolescents report exposure to at least one type of chronic childhood adversity (Benjet et al., 2009). Although the prevalence of stress exposure during childhood varies widely, numerous studies have established an association between stress and health.

Stress exposure influences the functioning of the *stress response system*, and in particular, the secretion of the hormone *cortisol*. However, the childhood trajectory of diurnal cortisol secretion and how stress influences the development of this trajectory is unclear. The literature examining the association between stress and cortisol is heterogeneous: stress has been associated with both lower (e.g., Hagan, Luecken, Sandler, & Tein, 2010) and higher levels of cortisol (e.g., Ly, McGrath, & Gouin, 2015) in children. To add further complexity to the issue, stress may also be associated with *blunted* cortisol levels, in which the cortisol response is at first elevated, but over time becomes adaptive and decreases. Cortisol blunting has been established among adults (c.f., Miller, Chen, & Zhou, 2007). Examining the evolution of the cortisol-stress association across child development offers the possibility to see the emergence of the elevated cortisol response and subsequent blunting.

Observing this pattern of blunting is only evident in longitudinal studies, as blunting must follow an elevated response, otherwise it could not be differentiated from a non-elevated cortisol level (e.g., Trickett, Noll, Susman, Shenk, & Putnam, 2010). Accurate identification of blunting as a physiological adaptation to chronic stress exposure poses measurement challenges. The critical issue of cortisol blunting will be returned to later. To better understand the current state of the literature, the stress response system and the important role of cortisol is discussed in greater detail in the following section.

Stress Response System: Hypothalamic-Pituitary-Adrenal (HPA) Axis

The body adapts to various challenges, or stressors, by stimulating physiological change to maintain homeostasis (i.e., a relatively stable internal environment; Martini, 2006). Exposure to stressors in the environment triggers the stress response system, a cascade of physiological sequelae that allows the body to appropriately respond to the stressful stimulus. The central coordinators of the stress response system are located within the hypothalamus, the medulla, and the pons (Stratakis & Chrousos, 1995). The hypothalamic pituitary adrenal (HPA)-axis and the sympathetic-adreno-medullary (SAM)-axis are the peripheral limbs of the stress response system. Both systems serve to regulate the release of stress hormones that influence nearly all organs of the body (Tsigos & Chrousos, 2002). Immediately after a stressor occurs, the SAM-axis, via activation of the sympathetic nervous system, causes the rapid secretion of epinephrine and norepinephrine by the adrenal medulla. (Glaser & Kiecolt-Glaser, 2005; Martini, 2006; Tsigos & Chrousos, 2002; Ulrich-Lai & Herman, 2009). The SAM-axis should be distinguished from the HPA-axis, which consists of a slow cascade of endocrine events originating in the hypothalamus (McEwen et al., 1997). The HPA-axis is controlled by the periventricular nucleus of the hypothalamus, which is regulated by the suprachiasmatic nucleus and segments of the limbic system (Herman & Cullinan, 1997; Lightman & Conway-Campbell, 2010). Activation of the HPA-axis causes the periventricular nucleus of the hypothalamus to release the neuropeptides corticotrophin-releasing hormone and arginine-vassopressin, which, via a reciprocal positive interaction, stimulate the secretion of each other (Tsigos & Chrousos, 2002). Corticotrophin-releasing hormone and arginine vasopressin work synergistically to stimulate the anterior pituitary to secrete another neuropeptide, adrenocorticotropic hormone (Tsigos & Chrousos, 2002). Adrenocorticotropic hormone then stimulates the adrenal cortex to secrete glucocorticoid, a steroid hormone (Bear, 2007; Charmandari, Tsigos, & Chrousos, 2005).

The variation in the release of glucocorticoid hormones during each 24hour period is known as a circadian rhythm. In humans, peak levels are reached during the day. While asleep, the HPA-axis is dormant for the first few hours of the night, after which cortisol secretion progressively increases through the night peaking roughly 30 minutes after awakening. While awake, cortisol secretion gradually decreases through the remainder of the day, attaining nadir at bedtime (Saxbe, 2008). Exposure to stressors triggers to an additional reaction and activation of the HPA-axis. Cortisol is regulated via negative feedback loops. Glucocorticoids play a key regulatory role on both the normative activity of the HPA-axis, and on the termination of the stress response (Stratakis & Chrousos, 1995; Tsigos & Chrousos, 2002). Glucocorticoids regulate the stress response system via two types of receptors: glucocorticoid and mineralocorticoid. Mineralocorticoid receptors are permissive and are characterized by their high affinity for corticosteroids; they are occupied even when there are low levels of circulating cortisol (Gunnar & Vazquez, 2006; Joels & Baram, 2009). Glucocorticoid receptors, on the other hand, are suppressive, and have a far lower affinity for corticosteroids (10-fold lower), and thus only become occupied when corticosteroid levels increase (Gunnar & Vazquez, 2006; Joels & Baram, 2009). By binding to these receptors, glucocorticoids inhibit their own production through negative feedback, which inhibits the stress response system, and is necessary to maintain homeostasis (Herman, Ostrander, Mueller, & Figueiredo, 2005).

From an evolutionary perspective, after exposure to a stressor (e.g., encountering a bear in the wild), the "fight or flight" response to stress is rapidly activated via the sympathetic nervous system and the SAM-axis. This has a widespread effect on involuntary responses to stress, such as increased respiratory rate, heart rate, blood pressure, catabolism, and dilation of pupils (Glaser & Kiecolt-Glaser, 2005; Martini, 2006; Tsigos & Chrousos, 2002; Ulrich-Lai & Herman, 2009). During this stress response, the secretagogue state of vasopressin and corticotropin-releasing-hormone is magnified, which directly results in increased cortisol secretion (Tsigos & Chrousos, 1994). Glucocorticoids promote mobilization of energy stores and enable multiple sympathetically mediated outcomes, such as increase in blood pressure (Ulrich-Lai & Herman, 2009). Glucocorticoids are catabolic, antireproductive, and immunosuppressive (Tsigos & Chrousos, 2002). When this system is activated infrequently and efficiently, the secretion of cortisol is regulatory and helps maintain homeostasis.

While homeostasis has been described as the body's ability to maintain a stable internal environment (Martini, 2006), allostasis is the body's ability to maintain a stable internal

environment through adaptive changes (McEwen, 2005). The body's allostatic adaptations that begin with the onset of the stress response are meant for short-term adaptations; excessive activation may alter the body's ability to adapt and ultimately be damaging to health (Charmandari et al., 2005; Gunnar & Vazquez, 2006; Sapolsky, Krey, & McEwen, 1986). Overexposure to glucocorticoids has been hypothesized to be the cause of the negative health consequences that are associated with stress exposure (Sapolsky, Krey, & McEwen, 1986).

Excessive activation of the stress response system accumulates, leading to "wear and tear" on bodily systems, also known as allostatic load (McEwen, 1998). Cumulative allostatic load can disrupt the negative feedback system, which can lead to a regulatory disruption of the HPA-axis and the diurnal profile of cortisol. In summary, excessive exposure to stressors can alter the regulation of the stress response system, and therefore, cortisol levels.

Cortisol can be assessed in its bound and unbound (free) state. After secretion, approximately 90% of cortisol is bound to corticosteroid-binding globulin and albumin, while only 5-10% of circulating cortisol remains unbound (Kirschbaum. & Hellhammer, 1989). Though there is some debate (Levine, Zagoory-Sharon, Feldman, Lewis, & Weller, 2007), this unbound fraction was chosen to be the most appropriate measure as it is considered to be biologically available to access target tissues (Edwards, Clow, Evans, & Hucklebridge, 2001). Unbound cortisol is most commonly assayed from saliva (Hellhammer, Wu, & Kudielka, 2009; Levine et al., 2007). Compared to blood cortisol, salivary cortisol is argued to more accurately reflect the activity of the HPA-axis (Edwards et al., 2001). Hair samples are increasingly being assayed, as they permit assessment of cortisol over a longer time frame (saliva: current amount of unbound cortisol, hair: 1 month of cortisol exposure per 1cm segment of hair; Levine et al., 2007; Stalder & Kirschbaum, 2012). The conceptualization and measurement of cortisol is discussed in the following section.

Cortisol Conceptualization and Measurement

Cortisol has been studied predominantly within two theoretical frameworks: cortisol reactivity and diurnal cortisol. *Cortisol reactivity* captures the amount of change in cortisol secretion in response to exposure to a stressor, commonly a laboratory stressor. Reactivity measures (e.g., change scores, difference scores) are used to infer the HPA-axis response to a specific stressor. *Diurnal cortisol* reflects the amount of cortisol secretion throughout the day, which follows a circadian rhythm. Diurnal measures are used to infer typical HPA-axis activity. Recently, a third theoretical framework has emerged: awakening response. *Cortisol awakening response* (CAR) is

posited to be a unique measure that captures the cortisol response to awakening, distinct from diurnal cortisol. These three theoretical frameworks are discussed in greater detail in the following sub-sections.

Cortisol Theoretical Frameworks

Cortisol reactivity in response to laboratory-induced stressors is presumed to reflect the typical response to an encountered real-world stressor, a phenomenon known as the Reactivity Hypothesis (cf., Pickering, 1990). Ecological validity of these laboratory stressors is balanced with the aim to standardize stressors within- and across-individuals. Cortisol secretion increases after exposure to a laboratory-induced stressor, following a typical 15 to 20 minute delay from stressor onset. Increase in cortisol, or reactivity, is attenuated by a gradual decrease and return to baseline, approximately one hour after the termination of the stressor (Dickerson & Kemeny, 2004). Experimental studies demonstrate the causal association between laboratory stress exposure and cortisol secretion. The cortisol reactivity response to laboratory-induced stressors is well documented (e.g., Dickerson & Kemeny, 2004). Unfortunately, the generalizability of these cortisol reactivity findings to real-world contexts has been limited. In contrast to common laboratory stressors lasting 5 to 45 minutes (e.g., cold pressor task, serial subtraction, Trier Social Stress Test), naturalistic stressors in daily environments tend to be chronic and ongoing (e.g., bullying, family conflict; Dickerson & Kemeny, 2004). Further, the ecological validity of laboratory-induced stressors is limited as participants can withdraw from the study at any time, thereby increasing controllability and predictability of the stressor (Dickerson & Kemeny, 2004). As such, a more ecologically valid approach involves the study of the stressors in the real world, and their impact on diurnal cortisol.

The HPA-axis is regulated by a circadian rhythm, which results in a distinct pattern of cortisol secretion over each 24hour period (Weitzman et al., 1971). This 24hour period is characterized by an average of nine secretory pulses of cortisol. The circadian rhythm can be divided into four temporal phases: First, “minimal secretory activity” occurring during the 4 hours before and 2 hours after turning the lights out; second, “preliminary nocturnal secretory episode” occurring during the 3rd to 5th hour of sleep; third, the “main secretory phase” occurring during hours 6 to 8 of sleep and the first hour after awakening; and fourth, the “intermittent waking secretory activity” occurring during the remaining 11 hours of the day (Weitzman et al., 1971). Essentially, during sleep, the HPA-axis is dormant throughout the first few hours of the night. After

this dormant period, cortisol secretion gradually increases, reaching its peak approximately 30 minutes after awakening, and declining throughout the remainder of the day. This declining diurnal slope occurs via the negative feedback loop of the HPA-axis (Sapolsky et al., 1986). Steeper diurnal declines are associated with better health outcomes (Adam & Kumari, 2009). Flattened diurnal slope, due to lack of an awakening response *or* failure to decline throughout the day, has been associated with negative health outcomes, independent of other cortisol measures (Adam, 2006; Matthews, Schwartz, Cohen, & Seeman, 2006; Sephton, Robert, & Kraemer, 2000). Importantly, diurnal cortisol has a developmental course. Infants (age 1-2 months) have two daily cortisol peaks, with cortisol secretion operating on a 6- to 11-hour cycle, as opposed to a 24-hour cycle (M. C. Larson, White, Cochran, Donzella, & Gunnar, 1998). Diurnal slope becomes stable at age 4 years (Gunnar & Donzella, 2002). Over the course of childhood and into adolescence, the total amount of cortisol secreted throughout the day increases steadily (Adam, 2006; Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Lupien, King, Meaney, & McEwen, 2001; Walker, Walder, & Reynolds, 2001). As such, prudence is warranted when comparing the diurnal cortisol profile of youth across a wide age range. Specific measures of diurnal cortisol will be reviewed in a later section.

The cortisol awakening response (CAR) is a dynamic increase in cortisol secretion and is part of cortisol's circadian rhythm, occurring during the main secretory phase within the first hour of awakening. During the first half hour after awakening, cortisol levels rise by 50-60%, independent of the time of awakening, use of an alarm, sleep duration, physical exercise, or sleep quality (Pruessner et al., 1997; Schmidt-Reinwald et al., 1999; Schulz, Kirschbaum, Prubner, & Hellhammer, 1998; Wust, Wolf, Hellhammer, Federenko, & Schommer, Kirschbaum, 2000). Arguably, CAR is a measure of cortisol change that is considered a distinct phenomenon within the diurnal cortisol profile (Fries, Dettenborn, & Kirschbaum, 2009), is a reliable measure of the reactivity of the HPA-axis (Hellhammer et al., 2007), and is a true neuroendocrine response to awakening (Wilhelm, Born, Kudielka, Schlotz, & Wust, 2007). From an evolutionary perspective, this cortisol spike may suggest an increased need for energy to meet the demands of the upcoming day (Powell & Schlotz, 2012). Previous findings indicated that CAR was not associated with cortisol levels during the remainder of the day, however, CAR has been negatively correlated with average cortisol levels during the night, indicating that it is related to the circadian rhythm (Edwards et al., 2001; Maina, Palmas, Bovenzi, & Filon, 2009; Wilhelm et al., 2007). While the CAR is an intrinsic part of cortisol's diurnal rhythm, as evidenced by the increase in cortisol levels several

hours before awakening, the magnitude of the CAR is influenced by anticipation of the coming day, as CAR has been found to be elevated on weekdays (vs. weekends; e.g., Kunz-ebrecht, Kirschbaum, Marmot, & Steptoe, 2004; Schlotz, Hellhammer, Schulz, & Stone, 2004; Thorn, Hucklebridge, Evans, & Clow, 2006); among individuals reporting stress (vs. no stress; e.g., Powell & Schlotz, 2012; Schlotz et al., 2004; Wust, Federenko, Hellhammer, & Kirschbaum, 2000); among individuals with work overload (vs. non-overloaded individuals; e.g., Schulz, Kirschbaum, Prubner, & Hellhammer, 1998); and, among competitive individuals on competition day (vs. non-competition day; Rohleder, Beulen, Chen, Wolf, & Kirschbaum, 2007). Wilhelm and colleagues (2007), attempted to prevent study participants from engaging in worrying thoughts and still elicited a reliable CAR, indicating that while the magnitude of the CAR is strongly influenced by psychological factors, the existence of the CAR in and of itself is part of cortisol's circadian rhythm, and represents a genuine response to awakening. To summarize, in contrast to stress-reactivity studies where the cortisol elevation is due entirely to exposure to the stressor, the CAR will be evident regardless of stress levels.

While CAR may be moderated by its heritability (mean cortisol increase, h^2=0.40, AUC, h^2=0.48; Wust et al., 2000), structural equation model analyses have conversely found that CAR AUC_I and AUC_{AG} are largely attributable to state-dependent factors (e.g., day-to-day stressors), as opposed to trait-dependent factors (e.g., genetic loading; Hellhammer et al., 2007). Therefore despite its heritability, CAR is partially determined by preparation for the upcoming day. Finally, developmental timing is also relevant to CAR. The awakening response only begins to emerge at age 2 to 3 months (Kiess et al., 1995; Larson et al., 1998; Price, Close, & Fielding, 1983). In summary, cortisol can be considered within three theoretical frameworks: cortisol reactivity, diurnal cortisol, and the cortisol awakening response. These measures will be discussed in detail in the following section.

Measures of Diurnal and Awakening Cortisol

Distinct measures of cortisol are used throughout the literature to capture these three theoretical frameworks. The scope of this thesis is limited to diurnal cortisol and CAR measures, as well as other measures commonly reported in the literature. Issues pertinent to saliva sampling protocols will be discussed later in the subsequent study design section.

Five commonly reported measures reflect the level, dynamics, and concentration of cortisol: (1) cortisol awakening response (CAR); (2) total cortisol secretion; (3) diurnal slope; (4) bedtime

sample; and (5) convenience samples (Rotenberg, McGrath, Roy-Gagnon, & Tu, 2012). These measures include aggregate measures, or composites derived from cortisol levels at multiple timepoints, and single measures, reflecting one timepoint.

CAR is traditionally measured by calculating the dynamic increase in the amount of cortisol secreted following wakening (AUC_I, area under the curve with respect to increase), or by calculating the total cortisol secreted during the awakening response (AUC_{AG}, area under the curve of the awakening response relative to ground; for formulae, see Rotenberg & McGrath, 2014). Calculation of AUC_I emphasizes change over time with awakening as the baseline referent; thus, the area reflects the relative increase, ignoring the distance from zero. AUC_{AG} measures cortisol increase with respect to ground (i.e., zero or no cortisol). AUC_{AG} differs from AUC_I in that it provides information on the overall levels of cortisol post-awakening. AUC measures are considered superior to single timepoint measures because they account for intensity (distance of each measure from ground or awakening) and sensitivity (difference between multiple individual samples; Fekedulegn et al., 2007; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

Total cortisol concentration is measured by calculating AUC_{TG}, which reflects the overall secretatory activity of the HPA-axis throughout the day, excluding the awakening response. AUC_{TG} is an aggregate cortisol measure, thought to be representative of the underlying diurnal activity of the HPA-axis, and provides no indication of diurnal change (Adam & Kumari, 2009; Edwards et al., 2001).

Diurnal slope reflects the decline in cortisol throughout the day. Different formulae have been reported in the literature. Diurnal slope can be measured using standard linear regression or by rise over run (i.e., difference between first and final cortisol sample, divided by time between sampling; Adam & Kumari, 2009). Regardless of which formula is used, diurnal slope is then anchored from either awakening (+0 min; $Slope_{awake}$), or maximum cortisol ($Slope_{max}$) to the final timepoint. Thus, $Slope_{max}$ inherently includes awakening peak (usually +30, +45, min post awakening), while $Slope_{awake}$ does not. Researchers differentiate between these two slope conceptualizations (and calculations) because CAR is thought to be regulated by a distinct neurobiological mechanism than the rest of the diurnal curve (i.e., hypothalamic suprachiasmatic nucleus via distinct neural pathways to the adrenal cortex; Clow, Thorn, Evans, & Hucklebridge, 2004). Normally, diurnal cortisol slopes decline across the day, and thus, have negative slope or

values. Flattened slopes have values closer to zero, and may reflect blunted awakening responses, or elevated evening cortisol levels (i.e., failure to decline across day).

Besides diurnal slope and CAR, several other single timepoint measures are reported in the literature. Bedtime cortisol is salient as it provides a final timepoint measure across the diurnal profile. Elevated bedtime cortisol is indicative of a flatter diurnal slope (Adam & Kumari, 2009). Bedtime cortisol is regulated by the negative feedback loop of the HPA-axis, which suppresses cortisol secretion during the day (Sapolsky et al., 1986; Urry et al., 2006). Experimental studies restricting sleep have found that partial sleep deprivation results in dramatic increases in bedtime cortisol the subsequent evening (Leproult, Buxton, & Cauter, 1997; Spiegel, Leproult, Spiegel, Leproult, & Cauter, 1999). Lack of sleep has been characterized as a physiological stressor (McEwen, 2006), and been found to mediate the association between stress exposure and cortisol secretion (Ly et al., 2015). Thus, elevated bedtime cortisol may be attributable to sleep deprivation or a compromised restorative process, leading to failure to decline over the day.

Finally, it is extremely common for researchers to report convenience cortisol measures. Single saliva samples are frequently collected, including awake (+0 min), maximum or peak (samples collected at the diurnal peak), or unstandardized, random times (e.g., morning, time of school or lab visit; El-Sheikh, Buckhalt, Keller, & Granger, 2008; Lupien et al., 2001). The maximum sample is advantageous due to its stability as a general index of cortisol level (Rotenberg et al., 2012). Yet, single samples are problematic because they fail to reflect the natural circadian rhythm, and diurnal profile of cortisol, and they are generally less stable than aggregate cortisol measures (Rotenberg et al., 2012). Additionally, some researchers report anomaly cortisol measures, making comparisons across studies difficult (e.g., Zalewski, Lengua, Kiff, & Fisher, 2012).

The cortisol measures described above capture important and nuanced aspects of the circadian rhythmicity of cortisol secretion. Cortisol measures are not necessarily correlated (Clow et al., 2004; Angela Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010; Fries et al., 2009; Morris, Kouros, Hellman, Rao, & Garber, 2014). A recent principal components analysis found that out of 15 potential cortisol measures, a distinct two-component structure reliably emerged across multiple diverse samples, representing total cortisol output (AUC_{TG}, AUC_{AG}) and cortisol change (AUC_{I}, diurnal slope; Khoury et al., 2015). These findings are reasonably consistent with a separate analysis (Fekedulegn et al., 2007), which also found a two-component structure; the first component was largely identical: total cortisol output. The second component, time-course of salivary cortisol,

largely overlapped with the cortisol change component. However, this second component included an additional cortisol measure (AUC above baseline), which was not examined in the Khoury's analysis (2015). These principal component analyses imply the existence of latent variables underlying individual cortisol measures.

Hair cortisol offers another physiological measure of the HPA-axis. Hair cortisol can be used to assess cumulative stress exposure over prolonged periods of time. Each centimetre of hair collected from the root end represents one month of cortisol exposure (Stalder & Kirschbaum, 2012; Wennig, 2000). The maximum length of time in which cortisol can be extracted from a hair sample is debated (Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013). It is possible to compare cortisol in hair segments before and after a stressful event, therefore enabling researchers to consider cortisol reactions over months without a longitudinal study design. Methodological and study design factors play an important role for all of these cortisol measures derived from saliva and hair samples.

Methodological and Study Design Factors

Methodology critically influences study results in the examination of the association between cortisol and stress. Important study design decisions can be delineated into those related to *saliva sample collection* (number of daily samples, day of week [school day/weekend], number of days, compliance with sampling), *study protocol* (time of awakening, time since last meal; protocol restrictions [eating/drinking/brushing teeth before collecting sample, caffeine use]), *participant characteristics* (e.g., race, socio-economic status, sex, body mass index, pubertal status, health status,), and *salivary assaying* (e.g., location of saliva collection, assaying method, sampling tube, laboratory analyzing samples).

To obtain reliable aggregate cortisol measures in children, it is necessary to sample multiple saliva samples over multiple days. For some measures, three to four days of saliva collection are necessary (e.g. AUC_{TG}), while for other measures, more than two weeks of samples may be required (e.g. diurnal slope; Rotenberg et al., 2012). According to the MacArthur Network guidelines (2000), as well as a recent expert panel on the assessment of CAR (Stalder et al., 2016), saliva samples should be collected at awakening (+0 minutes), +30 minutes post-awakening, +45 minutes post-awakening, 4 to 6 pm, 6 to 9 pm, and 9 to bedtime (MacArthur Network, 2000; Rotenberg et al., 2012). Among children and adolescents, the maximum sample is the most reliable cortisol measure, followed by aggregate measures derived from at least 3 days of saliva sample collection (AUC_{AG}

ICC = .49; Maximum ICC = .54; AUC_{TG} ICC = .58, Rotenberg et al., 2012). Cortisol collected on weekdays as opposed to weekends has been associated with an elevated CAR in adults (Kunz-ebrecht et al., 2004; Schlotz et al., 2004; Thorn et al., 2006), although these findings have not been replicated in children. The time of awakening can affect CAR in two ways. First, compliance with sampling procedures can impact CAR, such that the awakening response can be missed if the samples are not collected at the point of awakening (+0). Children are capable of reliably collecting the awakening saliva sample (Rotenberg & McGrath, 2014). Secondly, when an individual anticipates awakening (i.e., awakening is planned), earlier wake times have been associated with a more pronounced CAR (Federenko et al., 2004; Fries et al., 2009). Study design variables also influence cortisol measures. AUC_{TG} has been demonstrated to increase after a recent meal (Gibson et al., 1999). The length of time suggested to wait between meal or beverage consumption and cortisol collection is between 30-minutes to 1-hour (Hanrahan, McCarthy, Kleiber, Lutgendorf, & Tsalikian, 2006; Michels et al., 2011). Consumption of caffeine is associated with increased morning cortisol levels (Lovallo et al., 2005). Recent tooth brushing also accounts for a small amount of variance in total cortisol secretion (<5%); as such, it is recommended that a 45-minute window between tooth brushing and cortisol sampling be observed (Granger, 2007). When cortisol is collected according to recommended guidelines, and is controlled for study design factors (e.g., weekend or weekday collection, time of awakening, mealtime, caffeine use, tooth brushing), the stability of the measures is upheld, which is essential to maintain environmental validity.

Participant characteristics also significantly influence cortisol secretion. Ethno-racial status is linked to cortisol. For example, individuals identifying as Hispanic and black exhibit lower CAR, flattened diurnal slope, and higher bedtime cortisol, than white individuals (S. Cohen et al., 2006; De Bellis et al., 1999; DeSantis et al., 2007). Increased body mass index has been associated with heightened CAR (Therrien et al., 2007). Adult women in the luteal phase of their menstrual cycle emitted a greater cortisol response to laboratory stressors and a greater diurnal AUC_{TG}, than men (Clemens Kirschbaum, Kudielkas, Gaab, Schommer, & Hellhammer, 1999). In adolescents, pubertal status may play an important role in cortisol secretion. Adolescents at later stages of puberty have increased single sample cortisol levels, lower CAR, and steeper slopes (Adam, 2006), indicating that the dramatic hormonal changes that occur during puberty influence cortisol and alter its diurnal trajectory. Pubertal status (based on adrenarche, not gonadarche) accounts for a small, but significant amount of variance in cortisol (3.7%, Adam, 2006; Rotenberg et al., 2012). Health status

of the participants also impacts cortisol secretion. For example, reduced single sample cortisol levels have been associated with attention-deficit hyperactivity disorder and oppositional defiant disorder (Kariyawasam, Zaw, & Handley, 2002); heightened AUC has been associated with panic disorders (Bandelow et al., 2000); elevated mean cortisol response to stress in individuals with a stutter and with personality disorders (Blood, Blood, Bennett, Simpson, & Susman, 1994; Weinstein, Diforio, Schiffman, Walker, & Bonsall, 1999); and, other physical and mental illnesses as well as medications have been associated with both blunted and heightened AUC_I (Herbert, 2013; Hibel, Granger, Cicchetti, & Rogosch, 2007; Kudielka & Kirschbaum, 2003).

Finally, details related to the saliva collection and assaying may impact cortisol levels. Saliva is generally collected in two ways: 1) with participants spitting or drooling directly into a sterilized tube until adequate volume (~ 2 ml) has been collected; or 2) a cotton swab is inserted into the mouth until it becomes saturated with saliva. Once saliva has been collected, some researchers analyze saliva using cortisol assay kits themselves, while others send their samples to professional laboratories (e.g., Trier University, Salimetrics). There are four common types of assays used to assess cortisol in saliva: enzyme immunoassay, fluorescence, chemiluminescence, and radioimmunoassay. These techniques rely on competitive binding between free cortisol and reagents (chemicals added for analysis; Lupien, 2013). Immunoassays generally overestimate cortisol levels in saliva (Jönsson, Malmberg, Amilon, Garde, & Ørbæk, 2003), and different commercial assay kits can yield strikingly different results (e.g., Salimetrics and DSL are both enzyme immunoassay kits with varying cortisol results; Kirschbaum. & Hellhammer, 1989; Miller, Plessow, Rauh, Gröschl, & Kirschbaum, 2013).

Cortisol is a complex biological mechanism and can be measured via multiple measures, many of which are hypothesized to represent distinct phenomenon. Given the circadian rhythm of cortisol and its dynamic nature, cortisol is challenging to measure. Guidelines regarding the collection and assessment of cortisol have been recommended, which, when followed, increase the stability and reliability of cortisol. Practically, some guidelines are more challenging to implement due to cost and time limitations (e.g., stable measure of diurnal slope requires two weeks of samples), thus, most researchers do not derive all of these cortisol measures, nor do they control for methodological variables in their study design. The question remains whether cortisol measures are robust to these methodological and study design factors, or the extent to which they are influenced by differences.

The following section will shift from cortisol to a review of the conceptualization and measurement of stress.

Stress Measurement

For over a century, the maintenance of homeostasis has been recognized as an integral part of normative physiological functioning (Bernard, 1865; Cannon, 1929). In 1956, Selye coined the term *stress* to represent exposure to stimuli that threaten homeostasis (Selye, 1956). *Stressors* are stimuli that have been appraised as threatening and unmanageable to the organism (Selye, 1956), and the physiological and psychological changes in response to the stressor are called the *stress response* (Cohen, Kessler, & Gordon, 1995; Miller, Chen, & Parker, 2012). However, by focusing on the stimulus, this definition of stress does not describe the aspects of the stressor that elicit a particular response (Lazarus & Folkman, 1984a). Additionally, by defining stress in relation to threats to homeostasis, it becomes difficult to differentiate a state of stress from normative life experiences, except when the threats are severe (Lazarus & Folkman, 1984a). Some studies have attempted to differentiate between stressors by incorporating Elliot and Eisdorfer's (1982) taxonomy to classify the stressors: Acute time-limited stressors (e.g., laboratory challenges), brief naturalistic stressors (e.g., academic examinations), chronic stressors (e.g., stable, pervasive stressors, such as caregiving for a child with a serious disability), and distant stressors (e.g., traumatic experiences; Elliot & Eisendorfer, 1982; Segerstrom & Miller, 2004). While these classifications are helpful and explain variations in associations between stress and health outcomes (Segerstrom & Miller, 2004), they do not take into account the subjective perception of the stressor, which many argue is of critical importance. For example, divorce may be a brief naturalistic stressor for some individuals, but for those desperate to leave an unhappy marriage it may be significantly less stressful. Lazarus and Folkman (1984) argue in their transactional model of stress that the individual's perception of stress is integral to one's response to stress. They propose that stress is relational, and can be defined as a transaction between the individual and the environment that is appraised to be demanding, surpasses the individual's resources, and puts well-being at risk. This model emphasizes one's subjective experience of the stressor: two people can experience the same stressor, but perceive and respond to it differently. This difference in perception leads to differences in outcomes. Two factors mediate the relationship between the person and the environment: one's cognitive appraisal of the stressor (i.e., their perception of the stress), and their ability to cope with the demands of the stressor. The conceptualization and measurement of stress have evolved

dramatically over the last 60 years. This section will address: first, a brief summary of the critical paradigm shifts in stress measurement beginning with Selye's definition of stress and concluding with Lazarus and Folkman's transactional model of stress; and second, distinct methods of stress measurement.

Stress Paradigm Shifts

Beginning with Selye's seminal research on laboratory animals, excessive exposure to stress has been found to have a deleterious impact on health. Selye operationalized stress by depriving the animals of food, electrically shocking them, exposing them to extreme cold, and restraining them physically (Selye, 1936). Comparative studies in humans were not ethically possible. The study of stress in humans has its roots in the assessment of exposure to stressful life events. One of the first and most influential standardized measures of stressful life events is the Social Readjustment Scale (Holmes & Rahe, Richard, 1967), a measure which, instead of tallying the number of events one was exposed to, assigned a standardized, proportional weight to the exposure of each stressful event, and therefore, assessed the objective intensity of stress exposure. However, with the development of stressful life event interviews, and the subsequent discrepancy found between interview and checklist measures (McQuaid, Monroe, Roberts, & Johnson, 1992; Oei, Zwart, 1986), the validity of standardized ratings of stress intensity could be questioned. In a major paradigm shift, Lazarus and colleagues proposed a transactional model of stress: they argued that the transaction between the environmental events and the individual's subjective interpretation is critical to the assessment of stress (Lazarus & Folkman, 1984b). With this theory, the notion of measuring an individual's perception of life stress, regardless of life events, emerged (e.g. the Perceived Stress Scale; Cohen, 1984). Lazarus and colleagues also emphasized the clinical significance of experiencing minor, daily stressful occurrences (e.g., Lazarus & Folkman, 1984). These minor events, termed daily hassles, are defined as frustrating or upsetting daily events, situations, or demands that required varying degrees of adaptation, including disputes, concern about safety, and living in challenging environments (Kanner, Coyne, Schaefer, & Lazarus, 1981). These three stress measurement modalities (life events, perceived stress, and daily hassles) will be discussed further in the following section.

Stress Measures: Life Events, Perceived Stress, and Daily Hassles

Common measures of stress in children can be divided into three broad categories: life events measures, perceived stress measures, and daily hassles measures. Life events measures are

the most widely used measure of stress during childhood (Grant et al., 2003). They are generally formatted as checklists or interviews, in which the child or parent indicates how many stressful events have occurred during the time period assessed. The time period assessed by stressful life events measures during childhood varies, from 3-months (e.g., Coddington's Life Event Measure; Coddington, 1972), to two years (e.g., Life Events Checklist; Amone-P'Olak et al., 2009), with authors occasionally adapting the time frame specified on the questionnaire to include the past week (e.g., Hostinar, Johnson, & Gunnar, 2015) or lifetime exposure (e.g., Cullen et al., 2014). Some checklists have been adapted to account for both the number of stressful events experienced and the perceived intensity of the stressful events (e.g., Adolescent Stress Questionnaire; Hankin & Abramson, 2002).

Stressful life-event questionnaires in children are complicated by the issue of who the informant is. When children are very young, it is logical that parents report on the stress of their child. However, as children age, this approach becomes less valid as parents are not privy to all the stressors of their child's life. Studies investigating the concurrence between parents and children on measures of stressful life events found varied results, with some studies finding high agreement (Johnston, Steele, Herrera, & Phipps, 2003; R. Larson & Ham, 1993; Yamamoto & Mahlios, 2001), and others finding low-to-moderate agreement (Bailey & Garralda, 1990; L. H. Cohen, Burt, & Bjorck, 1987; R. Larson & Ham, 1993; Sandberg et al., 1993), depending on the event. These discrepancies may be due to the fact that parents are not necessarily aware of the stress their child experiences, and depending on the domains assessed by the stress measures, parents will be more or less likely to know of the stress experienced. This is supported by agreement being higher for events that were more likely to be known by both parents and child (e.g., pregnancy [health domain], failing an exam [school domain]), but lower for events that were less apparent to parents (e.g., bullying [friend domain]; relationships with the opposite sex [romantic relationship domain]; Allen, Rapee, & Sandberg, 2012; Sandberg et al., 1993). This evidence implies that parents are more likely to underreport the stress of their child. Additionally, it is intuitive that the child should complete perceived stress measures, as the parent may not be in a position to reflect on their child's perception of stress. To the best of our knowledge, no studies have compared and contrasted the use of parent or child report measures when assessing the association between stress and health outcomes. While parent report of stress may be accurate regarding stress in certain contexts, other events that may be appraised as being stressful (e.g., bullying) may be more accurately assessed via

child self-report. The varying levels of agreement between child-report and parent-report may help explain some of the variation in the strength of the association between childhood stress and health. Parent's knowledge of stress exposure appears to partially depend on the domain of stress assessed by the questionnaire. The issue of the domain will be discussed next.

Stressful life events checklists assess stress across a range of life domains. Domains include family, marriage/romantic relationship, friendship, health, death, reproduction, work, finance, housing, and other (Brown & Harris, 1978). When measuring stress in children, various stress measures assess different combinations of these domains, with 'school' included in most measures of child and adolescent stress. In adults, a greater increase in HPA-activity and allostatic load have been associated with specific domains (caregiving, finance, and work; Gallo, Jiménez, Shivpuri, Espinosa de los Monteros, & Mills, Paul, 2011); while stress in interpersonal domains has been predictive of future depressive occurrences (Sheets & Craighead, 2014). Similar studies assessing the impact of specific domains on paediatric samples have not been conducted. However, general exposure to stressful life events during childhood has been associated with negative health outcomes such as internalizing and externalizing problems, particularly depression (e.g., Amone-P'Olak et al., 2009; Ruttle, Armstrong, Klein, & Essex, 2014), chronic pain (Lampe et al., 2003), and respiratory illnesses (Boyce et al., 1995). Exposure to stressful life events during childhood has also been associated with more general morbidity, including increased report of symptom expression, after controlling for prior symptom levels (c.f., Grant, Compas, Thurm, Mcmahon, & Gipson, 2004).

Lazarus and Folkman (1984) argued that the importance of the individual's perception of stress is critical: the appraisal of one's ability to meet the demands of the stressor will determine if stress impacts health. The standard measure of perceived stress is the Perceived Stress Scale (Cohen, Kamarck, & Mermelstein, 1983), which examines the extent to which individuals find their lives overwhelming, irregular, and overloaded, and is a measure of general, non-specific stress (Cohen et al., 1983). The Perceived Stress Scale was originally designed for use in adult populations; however, it has been used in children as young as 8 years old (e.g., (Ly et al., 2015; Martin, Kazarian, & Breiter, 1994). There is limited evidence regarding the association between the Perceived Stress Scale and health outcomes in children. In adults, the Perceived Stress Scale has been associated with depression (Hewitt, Flett, & Mosher, 1992), respiratory illness (Cobb & Steptoe, 1996), and inflammation and wound healing (Glaser et al., 2016), among many others.

Emerging evidence indicates that children and adolescents who have higher Perceived Stress Scale scores show greater depression symptoms (Martin et al., 1994), and poorer sleep (Ly et al., 2015).

Measures of daily hassles focus on frustrating or upsetting daily events, situations, or demands that required varying degrees of modification, including disputes, concern about safety, being teased, and living in challenging environments (Kanner et al., 1981). Childhood daily hassles questionnaires generally take into account the intensity of the stress exposure, and have been associated with internalizing symptoms (Byrne, Davenport, & Mazanov, 2007; Miller & Townsend, 2005), general psychological distress (Wagner, Compas, & Howell, 1988), and obesity (T De Vriendt, Moreno, & Henauw, 2009).

When comparing these three stress measures, effect sizes for the association between stress and health outcomes were almost doubled when stress was assessed using the Perceived Stress Scale, compared to stressful life event checklists (e.g., life events and depressive symptoms: r = 0.18-0.29; Perceived Stress Scale and depressive symptoms: r = 0.67-0.76; Cohen et al., 1983; Pbert, Leonard, & DeCosimo, 1992). These findings may be explained by how the Perceived Stress Scale measures a distinct time frame (1 month) and a single life domain (general, non-specific), as opposed to stressful life event measures, which generally assess stress over the past year, and examine multiple life domains. Similar to findings with the Perceived Stress Scale, the association between daily hassles and health outcomes were almost doubled when examined in comparison to stressful life events (Kanner et al., 1981). This may be indicative of two things: 1) that, as Lazarus and Folkman posited (1984), daily hassles or perceived stress alone (regardless of stressful life events) may be sufficient to measure life stress; and, 2) measuring recent or current stress exposure is a better method to elucidate the association between stress and health than more distal measures (e.g., lifetime stress exposure). The strength of the association between stress and health may be strongly impacted by the model of stress measurement chosen.

Variability in theoretical conceptualizations of stress, as well as methodological choices regarding the type of questionnaire used, may impact the strength of the association between health outcomes and stress, as well as the association between cortisol and stress. However, many additional conceptual issues regarding the measurement of stress (e.g., chronicity vs. acuity), the developmental relation between cortisol and stress, as well as cortisol blunting, need to be evaluated. These pertinent issues will be addressed in the following section.

Cortisol, Stress, and Health

The complex interaction between cortisol, stress, and health has puzzled researchers for decades. Cortisol is a stress hormone and changes to its developmental trajectory are associated with many poor health outcomes. Cortisol secretion is impacted by acute stress exposure (e.g., laboratory stress reactivity studies), but exposure to chronic stress outside the laboratory does not elicit a consistent cortisol profile. There is evidence to support that this complex relationship may be strongly impacted by: conceptual stress measurement factors (e.g., chronic vs. acute stress); the developmental timing (chronology) of stress exposure; and, cortisol blunting. These three factors (conceptual stress measurement, chronology of stress exposure, cortisol blunting) will be discussed below.

Conceptual Stress Measurement

The conceptualization of the measurement of stress can dramatically impact the cortisol-stress association in children. Possible factors that may be influencing this association include: 1) the stress measure chosen; 2) acute vs. chronic stress exposure; and 3) the timing of stress exposure. First, stressful life events, perceived stress, and daily hassle measures have been associated with distinct cortisol responses. Stressful life events have been associated with: lower cortisol at awakening (Zandstra et al., 2015); lower morning cortisol (Zalewski et al., 2012); heightened morning cortisol (Cutuli, Wiik, Herbers, Gunnar, & Masten, 2010); heightened afternoon cortisol levels (Bevans, Cerbone, & Overstreet, 2008); heightened and blunted cortisol response to laboratory stressors (Calhoun et al., 2014; Mueller et al., 2011); steeper diurnal slope (Doane et al., 2013; Vanaelst, Huybrechts, et al., 2012); lower baseline cortisol levels (Jaffee et al., 2015; Pagliaccio et al., 2014); disrupted diurnal profile (Ly et al., 2015; Michels et al., 2015); or no association (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2011; Donoho, Weigensberg, Emken, Hsu, & Spruijt-Metz, 2011; Dougherty, Klein, Olino, Dyson, & Rose, 2009; Evans, Greaves-Lord, Euser, Franken, & Huizink, 2013; Hostinar, Johnson, & Gunnar, 2015a; Hostinar et al., 2015b; Kuhlman, Geiss, Vargas, & Lopez-Duran, 2015; Quevedo, Johnson, Loman, LaFavor, & Gunnar, 2012; Schechter, Brennan, Cunningham, Foster, & Whitmore, 2012; Simmons et al., 2015; Wolf, Nicholls, & Chen, 2008; Zeiders, Doane, & Roosa, 2012). Additionally, while the number of stressful life events did not significantly affect AUC_I, the intensity of stressful life events was both negatively and positively correlated with AUC_I (Cullen et al., 2014).

The Perceived Stress Scale has been associated with: a disrupted diurnal cortisol profile (Ly et al., 2015; Rotenberg & Mcgrath, 2016); lower wakening cortisol (Maldonado et al., 2008); or, no

association (Cook, Chaplin, & Stroud, 2015; Isaksson, Nilsson, & Lindblad, 2015; Le & Proulx, 2015; Spicer et al., 2013).

Daily hassles measures have been associated with: elevated basal cortisol (Kliewer, Reid-Quiñones, Shields, & Foutz, 2009); elevated wakening cortisol and decreased wakening cortisol (depending on sex; De Vriendt et al., 2011; Osika, Friberg, & Wahrborg, 2007); a heightened cortisol response to laboratory stress (mediated by negative mood; Eck, Berkhof, Nicolson, & Sulon, 1996); elevated morning cortisol (Schechter et al., 2012); elevated AUC_{TG} (Osika et al., 2007); or, no association (Cullen et al., 2014; Giletta et al., 2015). These diverse findings demonstrate that the impact of stress on cortisol varies dramatically depending on the conceptualization of stress.

Chronicity or acuity of stress exposure may also impact the association between stress and health outcomes. For example, in adults, chronic stress was more strongly related to symptoms of depression than acute stress (Mcgonagle & Kessler, 1990), while exposure to chronic stress magnified the influence of acute stress on incidence and symptoms of depression (Hammen, 2005). Chronic stress in adulthood has been associated with both increases and decreases in HPA-activity and cortisol (Miller et al., 2007), while chronic stress in childhood has been associated with flattened diurnal slopes (Wolf et al., 2008) and lower evening cortisol (Laurent, Shaw, & Fisher, 2014). In children, acute and chronic stress is difficult to differentiate outside of the laboratory. Acute stress may be conceptualized as a single intense event, such as the death of a parent, familial divorce, or changing schools. Although each of these stressors can be characterized as one single event, it is highly unlikely that the effects of these stressors are confined to an acute time frame, and measuring these events within a proximal time frame to exposure (e.g., immediately after the death of a parent) raises ethical and practical issues. As such, disentangling the relative impact of chronic vs. acute stress outside of the laboratory is extremely challenging, and the current state of stress measurement in childhood has not fully addressed this issue.

The timing of stress exposure may also impact the association between cortisol outcomes and stress. Will recent stress exposure impact cortisol differently than distal stress exposure? Stress exposure may incubate, showing its negative effects later in life (Bebbington et al., 1993). Lupien and colleagues (2009) hypothesized that incubation occurs due to stress-induced alterations in the development of synaptic pathways, the effects of which emerge after development has finished (Lupien et al., 2009). This hypothesis is supported by the depression literature, in which children

who suffer abuse only show negative health outcomes later in puberty or in adulthood (Halligan, Herbert, Goodyer, & Murray, 2004). However, stress measures that assess the association between recent stress and health (e.g., the previous two weeks) have found stronger effects when compared to more distal measures of stress (Kanner et al., 1981). A meta-analysis of stress and cortisol in adults found that recent stress exposure is associated with heightened cortisol activity, while more distal stress exposure is associated with blunted cortisol activity (Miller et al., 2007). However, few studies have examined the effects of the timing of stress exposure on cortisol. Doom, Cicchetti, and Rogosch (2014) found that distal stress exposure impacted between- and within-individual variability in single sample cortisol secretion in a sample of maltreated children. Differentiating between timing of exposure and duration of stress exposure is an important issue; the timing of onset may matter because if the stressor is more distal, the individual may have been exposed to the stressful scenario for a longer duration. Additionally, if the child is older they may have been exposed to stress for longer. This issue of age and the developmental timing of stress exposure are discussed below.

Stress Chronology: Developmental Timing

The stress response system is responsive to stress exposure early in life, and exposure to stress appears to be enduring over the life course. Animal models have reliably demonstrated that exposure to stressors early in life (e.g., low-nurturing mothers) leads to increased reactivity of the HPA-axis, and therefore, heightened glucocorticoid release (Caldji et al., 1998; Francis, Diorio, Liu, & Meaney, 1999; Liu et al., 1997; Stern, 1997). To test if stress exposure during early development is the cause of increased HPA-axis reactivity, Francis and colleagues (1999) cross-fostered the rat pups of high-nurturing mothers with the pups of low-nurturing mothers. When low-nurturing mothers raised the biological offspring of high-nurturing mothers, HPA-axis reactivity and subsequent glucocorticoid secretion were heightened. Early stressful environments altered the stress response system of genetically typical rats. Stress during early life in rats has been demonstrated to cause stable epigenetic alterations in the glucocorticoid receptor gene promoter in the hippocampus, which reduces the expression of the gene and alters negative feedback circuits; this in turn alters cortisol reactivity (Weaver et al., 2004). These well-controlled animal studies have demonstrated that exposure to stress in the early stages of mammalian life impacts physiological stress responses in later life.

In humans, exposure to stress during childhood has persistent effects on the body, via the activation of the HPA-axis and the subsequent release of glucocorticoids (Lupien et al., 2009; Sapolsky, 1994). However, the nature of the association between stress and cortisol is obscured by heterogeneous findings in the literature, with reports of elevated cortisol (Chen, Cohen, & Miller, 2010), lower cortisol (Peckins, Dockray, Eckenrode, Heaton, & Susman, 2012), blunted cortisol (Bosch et al., 2012), neither (Barry et al., 2015), or both (Blair, Berry, Mills-Koonce, & Granger, 2013). While the impact of the chronology of stress exposure may strongly impact this association, longitudinal studies - an emerging branch of the literature - are required to truly explore the relation between cortisol, stress, and health.

Few studies in humans have explicitly contrasted the impact of stress exposure during different developmental stages on health outcomes, but developmental timing of stress exposure across development explains some variance in animal studies. For example, social deprivation in neonatal rats leads to altered cortisol reactivity, while social deprivation in adolescent rats leads to altered dopamine function (c.f. Hall, 1998). Miller, Chen, and Parker (2011) hypothesized that when stress is experienced during sensitive stages of development in humans, it programs how bodily systems will perform in response to stress in the future. Lupien and colleagues (2009) also argue that the age in which the stressor occurs will impact the physiological effect of the stress exposure. Their theory is based primarily in neurobiology: in humans, the HPA-axis is highly responsive at birth, while distinct neural regions develop at differing speeds throughout the first decades of life (c.f. Lupien, McEwen, Gunnar, & Heim, 2009). Because brain regions mature at different rates, the timing of stress exposure differentially affects neurodevelopment. In humans, hippocampal volume, corpus callosum volume, and frontal cortex volume have been differentially impacted by the developmental timing of stress exposure (Andersen et al., 2008; Teicher et al., 2003). This points to a complicated relation between the timing of stress exposure and HPA-axis function later in life.

Contrasting the impact of exposure to stress during different developmental periods on cortisol has not been extensively examined. Bosch and colleagues (2012) assessed stress exposure in adolescents and found that the timing of stress exposure was critical for cortisol development. Stress exposure during childhood (ages 6-11) was associated with higher mean cortisol levels, while stress exposure during early adolescence (ages 12-15) had lower mean cortisol (Bosch et al., 2012). The change from high to low levels of cortisol coincides with pubertal onset, and may be indicative of cortisol blunting. Laurent and colleagues (2014) longitudinally examined the impact of early

exposure to stress on cortisol secretion and found that previous adversity (between age 9 months and 4.5 years) did not impact morning or evening cortisol levels or their stability; however, increased adversity between age 4.5 and 6 years was associated with heightened morning cortisol, lower evening cortisol, and less cortisol stability (Laurent et al., 2014).

Age of stress exposure does not always significantly explain variability in results. Although timing was not the focus of their study, Blair and colleagues (2013) longitudinally examined the impact of early exposure to poverty on cortisol levels, and found that age of change in poverty status (< 24 vs. > 24 months old) did not impact cortisol secretion (Blair et al., 2013). However, Blair's study did not standardize the time of day of cortisol collection (range 07:52-20:10), and the limited age range within the study makes generalization regarding the impact of the timing of stress exposure difficult. To our knowledge, the sole longitudinal study with the primary goal of assessing the impact of developmental stage on stress exposure found evidence of cortisol blunting (Bosch et al., 2012). Blunting will be discussed in the following section.

Stress Chronology: Cortisol Blunting

The physiological outcomes of the impact of stress exposure can vary over time. In an effect called cortisol *blunting*, the diurnal cortisol profile changes over the lifespan. Soon after stress exposure, one may have higher cortisol levels on awakening and a steeper slope when compared to controls. Over the lifespan this diurnal profile changes; one's awakening cortisol levels become lower and the diurnal slope becomes flatter when compared to controls. This blunting pattern is only evident in longitudinal studies, and may explain much of the heterogeneity regarding the association between cortisol and stress. Blunting may occur because after initial exposure to severe stress in childhood, the stress response system becomes over-activated. However, with time, the body habituates to stress exposure, and glucocorticoids are increasingly down-regulated throughout adolescence and early-adulthood (Trickett et al., 2010). Evidence of cortisol blunting has been found in animal models (Sanchez, 2006), as well as in studies of humans (e.g., Bosch et al., 2012; Bouma et al., 2011; Doom et al., 2014; MacMillan et al., 2009; Trickett et al., 2010). In their meta-analysis of adult stress exposure and cortisol, Miller, Chen, and Zhou (2007) found that cortisol release becomes blunted over time after exposure to stress; thus, if cortisol is collected some time after the stress exposure, the direction of the association may change from positive to neutral or negative. The study of blunting is more complicated in children; younger children may have less cumulative exposure to stress, therefore blunting may not have occurred. Blunting has been found to

occur in the transition from childhood to adolescence (Bosch et al., 2012; Trickett et al., 2010). Longitudinal studies in which participants were assessed across a minimum of 5 years found evidence of cortisol blunting; this may explain much of the heterogeneity in the cross-sectional literature (Bosch et al., 2012; Hagan et al., 2010; Ouellet-Morin et al., 2011; Trickett et al., 2010).

Examining the interrelation between cortisol and stress in children is complicated by the conceptualization of stress, chronology of stress exposure, and blunting. These constructs are difficult to measure, and are frequently left unexamined. The current state of the literature as well as the goals for the current study will be discussed below.

Current State of Knowledge

Extant literature examining the interrelation between stress and cortisol in children is heterogeneous. Experimental studies reliably demonstrate that laboratory-induced stressors activate the stress response system (i.e., HPA axis) and elicit cortisol reactivity in children and adults. In contrast, cross-sectional studies examining the relation between naturalistic stress (e.g., early life adversities, daily hassles, perceived stress) and cortisol find a less consistent association, with reports of elevated cortisol, blunted cortisol, both elevation and blunting, or no association (Barry et al., 2015; Bouma et al., 2011; Cicchetti, Rogosch, Gunnar, & Toth, 2010; Hankin, Badanes, Abela, & Watamura, 2010; Ly et al., 2015). Inconsistent findings in cross-sectional studies obscure the dynamic nature of the association between cortisol and stress.

These inconsistent findings may be explained by multiple factors: variable measurements of stress and cortisol; developmental factors in children and the chronology of stress exposure; and the proximal or distal timing of stress exposure. First, there is wide variability in the measurement of stress as well as the measurement of cortisol. Stress questionnaires can differ by: measuring stressful life events, perceived stress, or daily hassles; assessing topography (e.g., frequency, intensity) of the stress exposure; informant (parent or child); timeframe of stress exposure (from lifetime to two weeks); and, the number of life domains examined. Second, cortisol measurement strategies are also variable across studies. The assessment of cortisol can be influenced by: the cortisol measure used (e.g., AUC_I, diurnal slope); the total number of saliva samples collected; how the saliva is analysed; what sampling restrictions are used (e.g., restricting eating/drinking before providing saliva sample); and, the cortisol assay kit used. Third, specific to children, the chronology of stress exposure may strongly impact the strength and direction of findings. As such, age of the child at stress exposure, age of the child at cortisol collection, and time-frame and time span of the

overlap between cortisol and stress assessment are important factors to examine. Pubertal status is also highly relevant. These influencing factors make interpretation across the cortisol-stress literature challenging. A comprehensive synthesis of the stress and cortisol literature among paediatric samples remains to be completed. Ultimately, there is a need to systematically and quantitatively review the paediatric literature on the association of stress and cortisol to parse these influential factors and gain a better understanding of the interrelation of cortisol and stress in relation to naturalistic stressors.

Study Aims

The overarching objective of this study is to empirically evaluate the extant literature on the association between stress exposure and its relation with diurnal cortisol among children and adolescents. Specific goals of this research include: (1) to examine the methodological, study design, and conceptual factors related to *cortisol* that may be influencing the association between stress and cortisol; (2) to examine the methodological, study design, and conceptual factors related to *stress* that may be influencing the association between stress and cortisol; (3) to examine the impact of developmental timing and age on the association between stress and cortisol. These goals are achieved through a comprehensive meta-analysis of the existing paediatric literature on stress and cortisol.

METHOD

Literature Search, Article Inclusion and Exclusion

An electronic search of literature from January 1, 1970 to August 1, 2015 was performed using PsycInfo and PubMed databases, using the following keyword combinations: (“pediatric” OR “paediatric” OR “child*” OR “boy*” OR “girl*” OR “youth” OR “adolescen*” OR “infan*” OR “toddler*” OR “babies”) AND “cortisol” AND (“trauma” OR "early life adversity" OR “adversit*” OR “stress” OR “stressor*” OR “life event” OR “hassle*” OR “abuse” OR “neglect”). Articles were restricted to human studies. This search yielded 2,046 non-redundant articles (see Figure 1). Method sections were initially screened to ensure they included a measure of stress (i.e., questionnaire, interview), a biomarker of cortisol (e.g., saliva, hair), were in English, and were empirical studies, which yielded 245 articles. These articles were then fully reviewed for possible study inclusion. Inclusion criteria were: (a) standardized questionnaire or interview of childhood stress (i.e., prior to age 18 years; standardized defined as having or being derived from a measure with published psychometric properties); (b) salivary cortisol measures, collected during childhood; and, (c)

statistical analyses on the association between stress and cortisol (i.e., not group mean differences). Exclusion criteria based on *a priori* hierarchy were: (a) sample mean age greater than 18 years (k = 7); (b) salivary cortisol not measured (k = 9); (c) child was not target of stress measure (i.e., stress measures targeted parent or family stress; k = 40); (d) standardized stress measure not used (k = 110); (e) data on cortisol-stress association not provided (k = 50); (f) data redundant with another study already included in meta-analysis (k = 1). Of the 245 potentially relevant articles, 28 met inclusion criteria.

Three approaches were used to identify additional articles. A descendancy approach, consisting of reviewing reference lists of included articles, yielded 96 new articles that were located and fully reviewed; none met inclusion criteria. An ascendancy approach, consisting of a forward citation search of the 28 included articles using Web of Science, yielded 61 non-redundant articles; none met inclusion criteria after full review. Letters of solicitation requesting unpublished data were then sent to authors whose articles were missing data (n = 54) on the cortisol-stress association; 5 articles were retained based on the information provided. Thus, a total of 33 articles met inclusion criteria.

Reliability of Article Selection and Coding

A single rater (LW) coded all articles. Ambiguous articles were discussed with a second rater (JM) to resolve coding decisions. A random sample of articles from the original search (34%, k = 694) were recoded by two additional raters (MJ, AC); excellent inter-rater agreement was found for article selection and inclusion (kappa = 0.94).

Article Coding and Data Extraction

Sample characteristics. Sample size, demographic, and sample information were coded. Demographic variables included *age* (years), *sex* (percent female), *ethnicity* (percent white, percent black), *household income* (converted to $US, $K), *parental education* (mean number of years; education degrees converted to years education using International Standard Classification of Education; maternal education was used as proxy when parental education mean not provided; paternal education alone was not provided), *body mass index* (BMI), *pubertal status* (yes/no); and, *Tanner Stage* (per cent at each stage). Sample information included *recruitment setting* [general population (yes/no), school (yes/no), targeted recruitment (e.g., clinic, jail, shelter, medical records; yes/no), other (yes/no), OR not reported], *sample characteristics* [“healthy sample”, “targeted at risk for mental health” (e.g., recruited for self- or familial-risk of mental illness; schizophrenia,

depression, substance use disorder), "targeted at risk for high stress" (e.g., recruited due to status of jail, prison, teen pregnancy, exposure to violence), "targeted for medical condition" (e.g., asthma, obesity), or "combined" (i.e., combined healthy and targeted sample in data reporting], *participant exclusion criteria* [medication use (yes/no), mental health/developmental delay (yes/no)].

Cortisol. Articles' method and results sections were carefully reviewed to identify the exact formula used to derive each cortisol measure. Because different authors use different formulas for the same cortisol measure, cortisol measures were harmonized using the formulas presented in Rotenberg et al., (2012). Cortisol measures were coded as AUC_I, AUC_{AG}, *difference score* (Δ; +30 min post-wakening minus +0 min), *wake* (+0 min, single sample collected upon awakening), AUC_{TG}, $Slope_{Awake}$ (slope calculated from wake +0), $Slope_{Max}$ (slope calculated from maximum cortisol), *single time points* (Plus30, Morning [8am to 12noon], Afternoon [12noon to before dinner], Dinner/Evening [>1 hour before bedtime], Bedtime [within 30min of going to bed], Random time [time of clinic visit]), and *other* (atypical, unstandardized aggregate cortisol measure). Conceptual cortisol constructs were coded [*waking* (AUC_I, AUC_{AG}, difference score), *diurnal* (AUC_{TG}, $Slope_{Awake}$), *area* (AUC_{TG}), OR *change* (AUC_I, AUC_{AG}, difference score, $Slope_{Awake}$, $Slope_{Max}$)]. For cortisol collection, *number of saliva samples* (number of days, samples per day, total samples), *location of sampling* (home exclusively, at least one school sample, laboratory/clinic, OR other [e.g., shelter, jail]). Sample collection restriction (*food/drink* [yes/no], *tooth brushing* [yes/no], *physical activity* [yes/no]), type of saliva collection [*swab* OR *passive drool*], salivary assay [*enzyme immunoassay, fluorescence, chemiluminescence,* OR *radioimmunoassay*]; and, sent to laboratory for assaying [*yes/sent*, *no*, OR *not reported*] were also coded.

Stress measurement. Standardized stress measures with existing psychometric properties (i.e. published reliability or internal consistency; or measures derived from standardized measures) were categorically coded [*life events* (e.g., Stressful Life Events Schedule), *daily hassles* (e.g., Urban Daily Hassle Index), *perceived stress measures* (e.g., Perceived Stress Scale), OR *composite stress measures* [e.g., measure based predominantly on standardized stress measure]. Life events measures were coded by stress topography [*frequency* OR *frequency*intensity*], and number of life domains encompassed (based on item-level review or author report when items unavailable; domains included: school, family, friends/peers, romantic relationships, health/death, finance, neighbourhood/housing, work, general, OR other). Format [*questionnaire* OR *interview)* was coded. Note, article inclusion criteria required stress measures target the child; studies exclusively

measuring parent stress or household disorder were not included. Further, measures assessing a singular situation or isolated stress construct (e.g., bullying, PTSD) were beyond the scope of this meta-analysis. Reporter, or informant, who completed the questionnaire was coded [*parent* OR *child*). Time frame of stress measure (months; mean age of sample if time frame was "lifetime" or "ever"), and time lapsed between cortisol measurement and stress assessment using a questionnaire was also coded (days).

Statistical Analyses

Effect size calculation. Effect sizes are measures of the strength of the association between independent and dependent variables that can be represented as standardized metrics (e.g., Hedges' *g*, Fisher's *Z*; Rosenthal, 1994; Wilkinson & Task Force on Statistical Inference, 1999). Fisher's *Z* was used as the standardized common effect size for this meta-analysis. Fisher's *Z* ranges from -∞ to +∞ and can be interpreted similar to a correlation. When converting statistics derived from small samples it is necessary to be aware of potential bias (e.g., N < 30; Rosenberg et al., 2000). When multiple cortisol-stress associations were reported, effect sizes were derived using an *a priori* hierarchy: [1] Pearson/zero-order correlation, [2] Spearman rank-order correlations, [3] standardized beta coefficients, [4] *p* values, and [5] "not significant" (set $p = 0.000$). Pearson and Spearman correlations, as well as standardized beta coefficients were converted directly to Fisher's Z (Rosenberg et al., 2000; Rosenthal, 1994). Unstandardized beta coefficients were converted to t-test statistics (Peterson & Brown, 2005). Test statistics, such as *t*-values, were converted to r and then to Fisher's *Z* (Rosenthal, 1994; Rosenberg et al., 2000). Non-significant *p* values were coded as zero.

Effect size management. Effect sizes were coded for all possible, relevant data in each article, which produced multiple, redundant, effect sizes per study. Multiple effect sizes were attributable to different samples in the same study (e.g., healthy, targeted at-risk), reporting multiple cortisol measures of the same sample (e.g., AUC_I, slope, bedtime), reporting multiple stress measures (e.g., life events, daily hassles), sex-stratified results (i.e., effect sizes separate for boys and girls); or multiple time points (e.g., cortisol or stress measured at additional cycles). Of the 33 articles included there were a total of 106 effect sizes, for an average of 3.12 effect sizes per study (range 1 to 12). Two analytical strategies were planned to address multiple effect sizes. First, effect sizes were to be analyzed based on the inclusion of all effect sizes. Second, using a stringent approach, effect sizes were to be averaged to yield a single, non-redundant effect size for each study.

Analytic strategy. Fixed and random-effects meta-analytic models were conducted. Random-effects models assume that variability is due to both random variance and sampling error, and are appropriate when coded data represent only a sample of all plausible values (e.g., recruitment: community, clinic, shelter/jail); the assumptions of random models allow results to be generalized to other values (e.g., school; Rosenthal & DiMatteo, 2001). Fixed-effects models are appropriate when coded data sample all possible categories (e.g., type of saliva collection: passive drool or swab; Hedges & Vevea, 1998).

Cumulative effect sizes were calculated for each model. Heterogeneity of effect sizes was tested using the heterogeneity test statistic (Q_T). A significant Q_T indicates a heterogeneous distribution, which suggests that the variability of effect sizes is more different than one would expect from chance alone. Additional moderator analyses are warranted to explore the variability between effect sizes (Rosenthal & DiMatteo, 2001). *A priori* moderator analyses were conducted using continuous and categorical variables. Continuous moderator analyses generate a slope (β) to test whether the strength of the cortisol-stress association (i.e., effect size) differs based on the continuous moderator (e.g., BMI). A significant β indicates that the slope is significantly different than zero, meaning the moderator variable significantly impacts the cortisol-stress association. Categorical analyses partition variance explained by the model (Q_M) and residual error variance (Q_E; Rosenberg et al., 2000). Analogous to ANOVA, a significant Q_M test statistic indicates significant variability between coding categories. Bootstrap methods (1000 samples) were used to produce robust, non-parametric estimates of confidence intervals for each effect size (Rosenthal & DiMatteo, 2001). Lastly, to address concerns of possible publication bias and the file-drawer problem, Orwin's fail-safe numbers were used to determine the number of missing, unpublished, or non-significant comparisons that may change an effect size from significant to meaningless ($Z' = 0.01$). Analyses were performed using MetaWin 2 (Sinauer Associates, 2000).

Modeling strategy. Five sets of meta-analytic models, including continuous (β) and categorical (Q_M) moderators, were conducted. First, an overall cumulative effect size for all studies was calculated. Second, demographic and sample characteristic variables were tested as moderators, specifically: number of participants; sex; racial ethnicity; age; body mass index; parental education; household income; recruitment strategy; population sampled; and, participant exclusion criteria. Third, cortisol variables were tested as moderators, specifically: cortisol measures; number of saliva samples; location of sampling; saliva sampling collection restrictions; type of saliva collection;

salivary assay; and, sent to laboratory for assaying. Fourth, stress variables were tested as moderators, specifically: type of stress measure; stress topography; reporter; number of stress domains; time frame of stress measure; and, time lag from cortisol to stress assessment. Fifth, across each cortisol measure and conceptual cortisol construct, the developmental timing of stress exposure was examined using moderators, including age, age span (range), sex, and body mass index.

RESULTS

Study and Participant Characteristics

A total of 33 studies (k = 33; N = 6,397 participants) were included in the meta-analysis (see Table 1). There were an average of 140 participants (SD = 97) per study, when not including the single outlier study (N = 1,917). Among studies reporting corresponding demographic information, approximately half of all participants were female *(*55%, k = 33, N = 3,489), and of white racial ethnicity (62%, k = 15, N = 1,956), with mean age of 12.19 years (SD = 3.90). Participants had normal body mass (BMI_{avg} = 21.80), were from households with mean income of 64,992 $US, and whose parental education averaged 15.81 years. Participants were most frequently recruited from schools (48.5%, k = 16), included "healthy" samples (45.5%, k = 15), with studies excluding participants for medication use (33.3%, k = 11) and/or mental health issues (45.5%, k = 15). Values for salivary cortisol were presented in 39% of articles (k = 13; see Table 1). Standardized stress measures were predominantly life events measures (69.7%), which were more frequently used to assess stress than daily hassles (18.2%), perceived stress (15.2%), and composite measures (3.0%).

Summary Analyses

The association between stress and cortisol was reported in 33 studies, with 106 redundant effect sizes. The average cumulative effect size indicated that stress accounts for a small, but significant proportion of the variance in cortisol (Z' = 0.029), with greater stress associated with higher cortisol. This cumulative effect size was not heterogeneous. Several stress and cortisol variables identified *a priori* were tested as continuous and categorical moderator variables. Forest plots depicting cortisol-stress effect sizes are presented in Figures 2-6.

Moderator Analyses

Continuous and categorical moderator analyses examined variables from the following four categories: (1) *demographic and sample characteristics* (number of participants; sex; racial ethnicity; age; BMI; parental education; household income; recruitment; population samples;

exclusion criteria), (2) *cortisol variables* (cortisol measures; number of saliva samples; location of sampling; sample collection restrictions; type of saliva collection; salivary assay; sent for assaying), (3) *stress variables* (type of stress measure; stress topography; number of domains; time frame of stress measure; assessment time lag; reporter), and (4) *cortisol measure* (healthy or targeted population; stress measures; age; age span; sex; body mass index). Results from continuous and categorical moderator analyses are presented in Tables 2-5.

Demographic and sample characteristic moderators

Studies with a larger number of participants were associated with a weaker cortisol-stress association, although the slope was small (β = -0.0001, p = 0.001; see Table 2). The slope remains small but significant when one outlier is removed (β = -0.0003, p = 0.005). Participant sex was not associated with the strength of the cortisol-stress association. For racial ethnicity, studies with more black participants had a greater impact of stress exposure on cortisol (β = 0.002, p = 0.058); no relation was found for white racial ethnicity. Age, BMI, parental education, and household income were not associated with the cortisol-stress association; see Table 2.

Studies that recruited participants from the general community (Z' = 0.0002; Q_T = 47.00, p = 0.208), school (Z' = 0.008; Q_T = 138.95, p <0.001), and/or targeted recruitment (i.e., clinic, shelter, jail, medical records; Z' = -0.027; Q_T = 79.69, p <0.001) did not have cortisol-stress associations that were significantly different than zero, albeit the direction of the associations was different. Given that both school and targeted recruitment had heterogeneous Q_T statistics, further exploration was warranted. On further investigation, children recruited at schools differed by population sampled (Q_M = 8.07, p = 0.045), and children recruited through targeted recruitment differed in Bedtime cortisol (Z' = 0.059; Q_M = 4.56, p = 0.033), indicating that those recruited from clinics, shelters, jails, or medical records have elevated bedtime cortisol after stress exposure. Population sampled was a significant moderator of the cortisol-stress association. Participants sampled from the general population had the largest, significant effect size (Z' = 0.042), and those recruited as being targeted at-risk due to stress exposure had effect sizes significantly different from zero (Z' = 0.030). Those recruited because of risk of mental health problems also had a positive effect size (Z' = 0.021), although not significantly different than zero. Studies that excluded participants due to medication use (Z' = 0.040) and/or mental health diagnosis (Z' = 0.026) yielded effect sizes that were homogenous.

Cortisol Moderators

Cortisol measures moderated the cortisol-stress association. Effect sizes of the cortisol-stress association significantly differed based on the cortisol measure ($Q_M = 41.49$, $p < 0.001$; see Table 3). Two cortisol measures yielded effect sizes significantly different than zero: greater stress was significantly associated with higher difference Δ cortisol ($Z' = 0.048$) and AUC_{TG} ($Z' = 0.066$). While not significant, greater stress was associated with higher AUC_I ($Z' = 0.009$), AUC_{AG} ($Z' = 0.034$), $Slope_{Awake}$ ($Z' = 0.046$), Morning ($Z' = 0.131$), and Afternoon ($Z' = 0.027$). And, greater stress was inversely associated with $Slope_{Max}$ ($Z' = -0.038$), Wake (+0 sample; $Z' = -0.050$), +30 ($Z' = -0.012$), Evening ($Z' = -0.060$), Bedtime ($Z' = -0.038$), and Random measures ($Z' = -0.023$). Of note, single cortisol samples were the most frequently reported cortisol measure.

Although not statistically significant, sampling protocol was linked to the cortisol-stress association such that the greater the total number of samples, the stronger the association between cortisol and stress ($\beta = 0.005$, $p = 0.099$). Studies that included at least one saliva sample collected at school had a significant, homogenous cortisol-stress association ($Z' = 0.079$). The majority of studies exclusively sampled saliva at home ($k = 22$), which yielded an average effect size that did not differ significantly from zero ($Z' = 0.014$). Of note, studies that included saliva samples exclusively from a lab setting yielded a negative cortisol-stress association, which was homogenous ($Z' = -0.012$). Sample collection restrictions were not significantly associated with cortisol-stress relation.

General, "healthy" population samples and targeted, at-risk samples (e.g., populations at risk for mental health problems, stress, or medical conditions) were found to have variable effect sizes. As such, certain sampling design moderator analyses were restricted to healthy populations. When restricted to saliva samples from the healthy, general population samples, there was no difference between saliva collection from passive drool versus oral swabs ($Q_M = 0.36$, $p = .547$; Passive Drool $Z' = 0.047$; Swab $Z' = 0.028$). Notably, the targeted at risk sample accounted for the significant, homogenous cortisol-stress association for passive drool when all effect sizes were included in analyses ($Z' = 0.044$, $Q_T = 37.17$, $p = 0.461$). Four different cortisol assays were reported in the literature. While effect sizes for enzyme immunoassays, chemiluminescence, and radioimmunoassays ranged from 0.021 to 0.026, fluorescence assays had a larger but non - significant effect size ($Z' = 0.044$). When analyses were restricted to saliva samples from the healthy, general population, there was no difference between studies which sent assays to laboratories, or assayed locally ($Z' = 0.032$, $Z' = 0.018$, respectively; $Q_M = 0.06$, $p = 0.957$).

However, when at risk samples were included, there was significant heterogeneity between assay locations (Q_M =26.58, p < 0.001).

Stress Moderators

Type of stress measure yielded significantly different cortisol stress associations (Q_M = 18.69, p <0.001; Table 4). Daily hassles had the strongest, significant association with cortisol (Z' = 0.124) and perceived stress was also positively associated (Z' = 0.012). In contrast, greater life events was associated with lower cortisol (Z'= -0.010). Heterogeneous results indicated further moderator analyses were warranted. Among life event measures, stress topography impacted the cortisol-stress association. Stress measures that weighted the frequency and intensity of events yielded a cortisol-stress association that was significantly different than zero and homogenous (Z' = 0.047). While not significant, the greater number of domains assessed by a life event measure, the weaker the association between cortisol and stress (β = -0.004, p = 0.668). Timeframe of stress measure did not alter the strength of the cortisol-stress association. Yet, timelag between cortisol to stress collection was significantly associated with the cortisol-stress association, in that the larger the lag between biomarker collection and stress assessment, the lower the association between cortisol and stress (β = -0.002, p <0.001). Notably, informant was a significant moderator of the cortisol-stress association (Q_M = 31.53, p <0.001). Greater stress reported by children yielded significantly higher cortisol (Z' = 0.029); parent-report stress was relatively large (Z' = -0.063) did not differ from zero. Informant effect sizes were heterogeneous and merit further exploration.

Conceptual Cortisol Constructs

To consider how the cortisol-stress association varied by population characteristics, stress measure, and developmental age, stratified analyses were tested across each cortisol measure and conceptual construct (see Table 5). Population targeted (i.e., general healthy vs. targeted at-risk) had a differential impact based on the cortisol measure used. Inverse cortisol-stress associations were observed for the general healthy versus targeted at-risk samples for AUC_I, $Slope_{Max}$, +30, and Afternoon. More specifically, among the general healthy samples, greater stress was associated with lower AUC_I and $Slope_{Max}$, and higher +30 and afternoon cortisol; among the targeted at-risk samples, greater stress was associated with higher AUC_I and $Slope_{Max}$, and lower +30 and afternoon cortisol. Based on the conceptual cortisol constructs, the targeted at-risk samples yielded larger cortisol-stress associations for Waking, Diurnal, Area, and Change, compared to the general healthy samples (see Table 5).

When stress measures were nested within cortisol measures, the cortisol-stress association was relatively large ($Z' > .05$) and positive for daily hassles among AUC_{TG}, Wake (+0), and Morning cortisol. The association varied for life events measures, yielding relatively large and positive associations with Difference, Morning, and Afternoon, and negative associations with Wake (+0) and Evening. Perceived stress measures yielded relatively large and positive associations with AUC_{TG} (see Table 5). Curiously, the only cortisol measure that had an inverted cortisol-stress association across the stress measures was Wake (+0), which was relatively large and negative with life events measures ($Z' = -.072$), while relatively large and positive with daily hassles ($Z' = .174$).

To consider developmental changes, age was assessed within individual cortisol measures; the direction of the association changed depending on the cortisol measure examined. Age was only significantly and negatively associated with Afternoon cortisol ($Z' = -0.041$), indicating that as children age, the strength of the association between stress and Afternoon cortisol decreases.

Studies with wider age spans (i.e., larger range of ages) had greater strength of the cortisol-stress association for AUC_{AG} and Evening, and weaker cortisol-stress association for Morning cortisol. Studies with more females had significantly stronger cortisol-stress associations for AUC_{AG} and $Slope_{Awake}$, as well as Diurnal and Change constructs. Larger BMI was not significantly associated with the strength of the cortisol-stress association.

Given the results of the moderator analyses of the meta-analysis, combined with the variability of effect sizes across cortisol measures, populations, stress measures, and ages, only a redundant meta-analytic strategy was employed. A stringent, conservative approach to limit to one effect size per study would have masked both important and nuanced differences when examining the cortisol-stress association.

Discussion

The overarching aim of this comprehensive, systematic review was to examine the strength of the association between childhood stress and cortisol. Of the 33 identified studies, childhood stress was significantly associated with increased cortisol secretion, albeit a small overall effect size was observed ($Z' = 0.029$, .008-.052 *95% Bootstrap CI*). Moderating variables that influenced the strength of the cortisol-stress association included: (1) *demographic and sample characteristics* (racial ethnicity, population sampled, exclusion criteria); (2) *cortisol variables* (cortisol measures, number of saliva samples, location of sampling); (3) *stress variables* (type of stress measure, stress topography, timelag from cortisol to stress, informant); and (4) *conceptual*

cortisol construct (population sampled, stress measure used, age, and sex across individual cortisol measures). These results are discussed in more detail in the following sections. Overall, the results revealed three important findings. First, the strength of the cortisol-stress association was influenced by specific aspects of stress measurement. Second, findings suggest the cortisol profile differs across general healthy samples versus targeted, at-risk samples. Third, evidence of cortisol blunting across development was observed within certain cortisol measures. In meta-analyses, it is important to assess relative differences in effect sizes, as opposed to primarily focusing on significance testing. Effect sizes will be discussed in the context of relative size, as well as statistical significance.

Demographic and Sample Characteristics

Although the slope was small (β = -0.0001), the greater the number of participants included in a study, the smaller the association between cortisol and stress. Ethnoracial status influenced the cortisol-stress association, in that the more black children included in the study, the greater the impact of stress on cortisol. The greater the number of white children in the study did not impact the cortisol-stress association. This is consistent with findings in the paediatric literature, in which, in comparison to white children, black children have lower wakening cortisol and higher bedtime cortisol (Cohen et al., 2006; DeSantis et al., 2007). Less than half of the studies reported racial ethnicity.

Higher stress was associated with lower cortisol among targeted recruitment from clinics, shelters, jail, or medical records, although this association was not significant. Recruitment from the general community or from schools yielded overall effect sizes in the opposite direction, but these did not differ from no cortisol-stress association. Among samples that were generally healthy or targeted at-risk due to stress exposure, greater stress was linked to higher cortisol levels. Follow-up analyses (discussed below) delineated these findings depending on the cortisol measure. Studies that excluded children for medication use and/or mental health reasons (e.g., depression, developmental delay) had relatively stronger cortisol-stress associations, albeit these were not significant. Studies excluding children due to medication use largely targeted use of corticosteroids, which suppress cortisol responses (Wlodarczyk, Gibson, & Caeser, 2008). These groups were also homogenous; by excluding participants for medication use or mental health reasons, homogeneity of the sample may have been increased, as those with mental health problems (cf., depression; Hammen, 2005) have been found to have different associations between cortisol and stress then healthy controls.

Cortisol Measures and Sampling Design

Cortisol-stress relations differed depending on the cortisol measure. There was wide variability of cortisol measurement across studies. Meta-analysis results must be interpreted in light of the small number of studies and effect sizes. The vast majority of studies reported the use of single saliva samples, which are one of the least reliable methods of measuring cortisol. Cortisol measures most strongly associated with stress, included AUC_{AG}, Difference (Δ), AUC_{TG}, and $Slope_{Awake}$, which were positively associated with stress; and, Wake (+0) and Evening, which were negatively associated with stress. AUC_{TG} had the strongest association with stress, indicating that higher stress was associated with increased overall cortisol secretion. AUC_I's effect size was not different than zero. When single cortisol samples were examined, lower Wake (+0) and +30 were associated with higher stress. $Slope_{Awake}$ is positively associated with stress, indicating that a steeper slope is associated with stress; $Slope_{Max}$ is negatively associated with stress, indicating that a less steep slope is associated with stress. These measures have been argued to assess different constructs, with $Slope_{Awake}$ assessing the diurnal slope regardless of the CAR, and $Slope_{Max}$ assessing the diurnal slope from the CAR. A negative Wake (+0) and Bedtime sample may explain how a steeper $Slope_{Awake}$ is associated with higher stress levels in some children; the whole diurnal slope may shift down with stress exposure. Another contributing factor may be that different studies contribute different cortisol measures. The association between $Slope_{Max}$ and stress may be partially driven by the relatively small but negative association between +30 and stress, in that a lower +30 sample is associated with a $Slope_{Max}$ that is less steep. Morning samples are positively associated with stress, in that higher stress exposure is associated with higher morning and evening cortisol.

The meta-analytic results, and the way nearly all studies presented data on the cortisol-stress association, preclude the possibility of a curvilinear relation between cortisol and stress exposure. Indeed, the high majority of studies presented linear test-statistics. Two studies that depicted the association between stress severity and cortisol secretion(Gustafsson, Anckarsäter, Lichtenstein, Nelson, & Gustafsson, 2010; Wolf et al., 2008), suggested a curvilinear relation, in which both limited and high stress exposure yielded low cortisol and moderate stress yielded heightened cortisol.

Study design factors also moderated the cortisol-stress association. The greater the total number of saliva samples collected, the relatively stronger the association between cortisol and stress. This may be because the greater the number of samples, the lower the measurement error and the greater the reliability of the cortisol measure. To obtain reliable cortisol measures, it is necessary

to collect between 3 days to over two weeks of saliva samples (Rotenberg, McGrath, Roy-Gagnon, & Tu, 2012). Studies that included at least one sample collected at school had a relatively strong association between cortisol and stress. This may be because school can be in and of itself a stressor, and by sampling at school one is sampling during a period of stress (e.g., Groeneveld et al., 2013). When examined alone, the sole study that reported saliva collection only at school reported a relatively large effect size ($Z' = 0.169$).

Sampling restrictions had minimal influence on the cortisol-stress association. Further examination of effect sizes revealed that studies that restricted both food/drink *and* physical activity for 1 to 1.5 hours before sampling yielded stronger cortisol-stress associations ($Z' = 0.023$). These findings are consistent with the adult literature (Hill et al., 2008). Restricting food and drink alone, or in combination with toothbrushing, did not strongly impact the cortisol stress association. In saliva samples that were sent to the laboratory for assaying, a stronger cortisol-stress association was found. However, 30% of studies did not report where data were assayed.

Stress variables

Stress measures that assessed daily hassles yielded relatively large effect sizes for the cortisol-stress relation, compared to life events and perceived stress measures. Surprisingly, life events measures had a negative effect size, but this result was better explained when the findings were nested by cortisol measure (discussed below). Life event measures that weighted stress frequency by stress intensity yielded stronger effect sizes than measures of stress frequency alone. The scope of life events measures, as reflected by number of domains, was not associated with the cortisol-stress association. The pertinent difference between hassles and life events measures may be rooted in the adaptive capabilities of the stress response. Examining timelag as a moderator established that as time passes between the measurement of cortisol and stress, the association between cortisol and stress weakens. Daily hassles and perceived stress measures assess more proximal, current levels of stress, while life events measures assess distal stressful occurrences from within the last 3 months to anytime during the lifespan. It is plausible that the stress response system is trying to adjust to current stress levels as measured by daily hassles, thereby leading to an increased cortisol-stress association. This may indicate there is a temporal and proximal relation between cortisol and stress during childhood. Time frame of the stress measure did not impact the cortisol stress-association.

Interestingly, the person who reported the stress was a moderator of the cortisol-stress association. Measures based on child self-report yielded larger, positive effect sizes, compared to effect sizes based on parent report. In other words, children's experience of stress was more congruent with their cortisol levels. This finding is consistent with prior literature, which observed parents may be less aware of the stress their children are experiencing (Allen et al., 2012; Sandberg et al., 1993).

Conceptual Cortisol Constructs

Prominent moderator variables were nested within cortisol measures. Due to limited numbers of effect sizes, the interpretation of these findings must be conservative. General population samples (deemed "healthy" in these analyses) were compared with targeted at-risk samples (i.e., mental health, stress, medical conditions). There were distinct cortisol patterns evident across the two groups. A relative comparison indicates the generally healthy sample had lower AUC_I and $Slope_{Max}$, and higher +30, compared to the targeted at-risk sample. For example, in the targeted group, +30 - a cortisol measure one would expect to be positive (indicating that higher stress leads to higher peak cortisol) - the effect size is negative, indicating that higher stress leads to a lower peak cortisol. This may be indicative of a lower CAR in at-risk populations. Additionally, $Slope_{Max}$ for the targeted group, which was negative in the generally healthy sample, is positive and not different than zero. Together, the pattern of these findings may be indicative of cortisol blunting in the targeted at-risk samples.

Conceptual cortisol constructs had relatively stronger associations between cortisol and stress among the targeted samples, than general healthy samples. Given results from this study, one would anticipate a lower association between Waking and stress in the targeted group; however, this is not the case. By grouping Waking-related constructs together, blunting may be masked. Surprisingly, given the literature on increased CAR in healthy populations after exposure to stress, higher stress levels are predominantly associated with Diurnal and Area constructs, not Waking.

When stress measures were examined by individual cortisol measures, daily hassles were strongly associated with higher cortisol levels, while life events vary depending on cortisol measure. High life events scores are associated with low Wake (+0) cortisol while daily hassles are inversely associated with a high Wake (+0) cortisol. Given the relative importance of timelag between cortisol and stress measurement, these findings indicate that more proximal stress exposure leads to an increase in cortisol secretion, while more distal stressors are associated with a decrease in cortisol

secretion, implying that more distal stressors are associated with a blunted cortisol response. This is consistent with Miller and colleagues (2007) meta-analysis of chronic stress during adulthood and cortisol secretion; they found that that cortisol secretion, which is elevated at the onset of the stressor, reduces as time passes (Miller et al., 2007). Measures of daily hassles consistently elicit strong associations with conceptual cortisol constructs, while life events measures are more strongly associated with Diurnal cortisol. The low association with between life events and Waking conceptual cortisol measures may additionally be indicative of the impact of distal stressors on cortisol secretion.

While there were no longitudinal studies with multiple observations of both cortisol and stress across childhood, the results of cross-sectional studies were examined by age to examine possible developmental changes of the cortisol-stress relation. As children who are exposed to stress get older, and presumably have greater cumulative exposure to stress, cortisol secretion may become blunted. When age is considered as a moderator, only Afternoon cortisol influences the cortisol-stress association, in that as children age, the impact of stress on Afternoon cortisol decreases. Conceptual cortisol constructs were not strongly associated with age.

These age-related findings are not necessarily surprising. While the issue of the developmental stage of stress exposure and the HPA-axis has been extensively studied in prenatal and early life, there is a paucity of literature examining stress exposure during later childhood and adolescence and its impact on HPA-axis regulation. The animal literature on sensitive periods of development in early life is extensive and compelling, with evidence of prenatal stress exposure increasing glucocorticoids in the mother and the foetus, and altering brain development (Dean & Matthews, 1999; Seckl, 2007). Rodents who were exposed to prenatal stress have lower numbers of mineralocorticoid and glucocorticoid receptors, which are integral to glucocorticoids' negative feedback (Weaver et al., 2004). The first few weeks of life in rats are viewed as parallel to early development in humans, and an extensive literature has demonstrated that exposing rat pups to stressors during the first three weeks of life alters the development of the HPA-axis (i.e., increased number of glucocorticoid receptors) into adulthood (c.f., Meaney et al., 1991; Meaney & Aitken, 1985). When examining potential critical periods of stress exposure, Meaney and Aitken (1985) found that exposure to stressors during the first week of life had an equal impact on glucocorticoid receptor development, as exposure to stressors over the first three weeks of life. Exposure to stressors during the second week of life was slightly less effective, while similar exposure between

days 15-21 had no impact on HPA-axis development (Meaney & Aitken, 1985). In primates, stress exposure over the first 3 to 6 months of life have been associated with lower wake cortisol and heightened afternoon cortisol in later development (Sanchez et al., 2005). There is a small literature examining the long-term impact of stress during later childhood and adolescence on the HPA-axis in animals and humans. While adult rats habituate to stress exposure (Girotti et al., 2006), juvenile rats increase their secretion of glucocorticoids (Romeo et al., 2006). Additionally, Avital and Richter (2005) examined two groups of rats, one that was exposed to stress as juveniles and one that was exposed only as adults; they found that those who were exposed as juveniles had greater HPA-axis reactivity to stress as adults then those who were exposed to twice as much stress during adulthood, but not exposed as juveniles (Avital & Richter-Levin, 2005). While it is possible that the impact of stress incubates over time and that the varying speeds of development of different brain structures are associated with different health outcomes (Lupien et al., 2009), only two longitudinal studies of humans found that stress during later stages of childhood impacts the development of the HPA-axis (Bosch et al., 2012; Laurent et al., 2014). This meta-analysis restricted stress measures to standardized measures that assess the stress of the child. This was done in order to be able to make comparisons across stress measures, and to reduce measurement error. However, by electing to include studies that assess the stress of the child, no studies were included which assessed prenatal or early life stressors and cortisol. Thus, our ability to fully investigate the strength of the association between age, stress, and cortisol was limited. Additionally, while the age range of our study was large, the majority of studies involved children in late childhood/early adolescence, therefore the variability across ages was limited. Further primary research is required before firm conclusions can be drawn.

Biological sex composition of the sample was associated with the cortisol-stress association; studies with a greater percentage of females had stronger associations for AUC_{AG}, and $Slope_{Awake}$. Prior findings for biological sex differences among adults are mixed, with some research indicating that females have a stronger CAR (cf., Fries, Dettenborn, & Kirschbaum, 2009) and others finding no effect (Fries, Dettenborn, & Kirschbaum, 2009). When conceptual cortisol constructs were examined, the more females that were included in the study led to stronger association between Diurnal and Change measures, with stress. The impact of the percentage of female's likely changes with age and pubertal stage, as puberty accounts for some of the variance in cortisol secretion (Rotenberg & McGrath, 2014). However, very few studies reported pubertal stages of their sample

(9%). Other variables that may have been relevant to nest within cortisol measures, but for which there was not enough data include: racial ethnicity, socio-economic status, stress topography (frequency, intensity of life event measure); and, time lag between cortisol and stress.

Due to limited data available, the interpretation of these nested analyses is limited. Based on the best evidence to date, populations that may be vulnerable to stress exposure appear to have a blunted cortisol profile. Additionally, the examination of proximal stress exposure in comparison to distal stress exposure elicits differential cortisol-stress associations, with potential blunting of the CAR associated with life events measures.

Summary

This study contributes to the literature in three ways. First, to the best of our knowledge, this is the only meta-analysis of stress and cortisol in childhood. Therefore, this study contributes important new knowledge to the study of childhood stress and stress hormones. Second, stress measurement moderates the strength of the cortisol-stress association. Stress measures are most strongly related to cortisol secretion if children, as opposed to parents, reported the stress exposure. If life events measures were used, a stronger association between cortisol and stress was found in measures that assessed intensity of the stress exposure. The assessment of more proximal stress was associated with a heightened cortisol-stress association, while the assessment of more distal stress was associated with a blunted cortisol-stress association. Third, targeted, at-risk populations appear to have a blunted cortisol profile, and blunting seems to increase with age.

State of the Paediatric Cortisol-Stress Literature

There are limitations to the extant literature that impact our understanding of the association between cortisol and stress. First, a comprehensive literature search revealed over 2,000 studies that referred to both stress (or its equivalents) and cortisol in childhood. However, the overwhelming majority did not truly assess stress, or report an association between cortisol and stress. Second, many of the studies identified and included in this meta-analysis published only limited demographic and sample characteristics. For example, there is a lack of published data on the pubertal status of the sample. Given the important developmental changes that occur hormonally during puberty, this is a significant oversight. Because puberty has been associated with an altered perception of and response to stress (Adam, 2006), as well as a change in cortisol secretion (Kang et al., 2014), using standardized measures to assess pubertal status, such as the Tanner Stages questionnaire, would be an informative addition to the study methodologies on children and stress

exposure. The progression from childhood to adulthood leads to an increase in total cortisol secretion; this change has been found to begin during the later stages of puberty (Kang et al., 2014), as such it is plausible that puberty is a contributing factor to heterogeneity in childhood studies of stress and cortisol. The paucity of longitudinal studies on stress and cortisol where both cortisol and stress measures are assessed at more than one assessment wave are also detrimental to the literature. This hinders the thorough analysis of developmental periods of vulnerability to stress exposure.

Third, the differential impact of chronic vs. acute stress exposure on cortisol is not possible given the current paradigm of stress measurement. While it was possible to examine timing of stress exposure via the time frame of the stress measure, the chronicity of stress exposure was not fully described in the literature. Chronic stress can be defined as experiencing stress for anywhere from 4 weeks to 12-months (cf., Hammen, 2005); duration of stress exposure and its association with cortisol was not measured and/or data was not presented in any of the studies included.

Fourth, over half of the studies included (k =19) did not use recommended or standardized cortisol measures, instead opting to use single salivary collection points at inconsistent or random times across participants (e.g., Morning consisted of any sample between 9am and 12noon). Given the diurnal nature of cortisol secretion, it is difficult to meaningfully compare individual cortisol samples taken at different times. Additionally, the ecological validity of the single sample is questionable due to its instability (two weeks to one-month of samples are necessary to get a stable estimate; Rotenberg & McGrath, 2014). Given that the total number of samples impacted the strength of the cortisol-stress association, studies that used single samples weakened their results.

Thus, the extant literature has four important limitations. First, the literature is far smaller then it first appears. Second, there is a lack of important descriptive data about sample demographics and methodological details reported (e.g., pubertal status). Third, stress measurement is restricted and does not comprehensively convey key distinctions related to chronic and acute exposure. Fourth, the most frequently reported cortisol measure is based on a single saliva sample, which is insufficient for stable assessment of cortisol.

Meta-analysis coding decision limitations

Coding decisions introduced limitations to this meta-analysis. First, although prenatal stress exposure has been associated with various maladaptive health outcomes, including the altered regulation of cortisol (Oberlander et al., 2008), stress measure was restricted to the child as the target of stress. Thus, the association of cortisol with prenatal stress could not be assessed. Second,

stress can be experienced across contexts and is unique to each individual. In the present meta-analysis, isolated or singular stress measures or experiences were deemed beyond the scope of this thesis. Stress is measured in a wide variety of ways, and this meta-analysis does not encompass the entirety of the literature on stress and cortisol. Combining all forms of 'stress' as characterized within the literature, such as exposure to maternal depression, exposure to abuse, being in foster care, being bullied, would not be meaningful. This meta-analysis compared stress restricted to the use of standardized stress measures. Unfortunately, the vast majority of studies examining cortisol and stress did not include a standardized measurement of stress, leading to the exclusion of many studies that have findings that are relevant to the cortisol-stress association. It is unknown if a standardized measure was included in study design, but the results were not reported in publications due to space limitations or non-significant findings. Third, the decision to combine effect sizes from different cortisol measures was an important limitation to this study. This choice was made for two reasons: a) this literature is small and as many diverse cortisol measures are used there was insufficient power to assess all moderators across individual cortisol measures; and b) this method permits greater generalizability across different implementations of cortisol methodologies; although it does increase the risk of heterogeneity (Higgins & Green, 2011). This potential for increased heterogeneity is why specific moderator analyses were nested within cortisol measures. Ideally, all moderators would have been examined within individual cortisol measures. Unfortunately, these analyses were precluded due to a lack of data in the available literature. Fourth, given the state of the literature and the lack of longitudinal studies that may capture the true phenomenon of cortisol blunting, only a cursory examination of development and possible blunting was conducted. Inferences about the flattening of the slope and cortisol blunting are possible within the nested analyses. When examined distinctly within cortisol measures, effect sizes of targeted populations and effect sizes of life events measures were suggestive of cortisol blunting. However, without longitudinal studies and the presentation non-linear data, the confirmation of blunting is precluded. Longitudinal studies, with cortisol and stress, both assessed at multiple time points, are necessary to truly examine this issue.

The goal of this study was to take an evidence-based approach to consider the cortisol-stress relation across early childhood through adolescence, and to consider moderators that influence the developmental course of the relation. This was accomplished by conducting an extensive systematic review and harmonizing data from a small and complex literature. *A priori* moderator variables

were extensively assessed. Heterogeneity within the literature was addressed via the harmonization of specific variables: cortisol measures were harmonized by assessing the formulae authors used to calculate cortisol measures; stress measures were harmonized by categorizing them based on type of measure and examining their stress topography. When possible, moderators were examined within individual cortisol measures.

Implications and Conclusions

The extant literature on the cortisol-stress relation is rife with heterogeneity; studies have found evidence of heightened cortisol, lowered cortisol, blunted cortisol, and no association. This meta-analysis harmonized the literature to examine the cortisol-stress association and analyzed various moderators to explore factors that influence this association. Factors related to study design (e.g., population sampled; age of sample), cortisol measurement (e.g., cortisol measures), and stress measurement (e.g., stress measure; stress topography; informant; time lag between cortisol and stress) were found to impact the association between stress and cortisol. By incorporating child report measures of stress, and including life event measures that are weighted by frequency and intensity, the association between childhood stress and cortisol may be more apparent. Distal vs. proximal measures of stress exposure lead to different associations with cortisol. Populations targeted for being "at risk" may be more vulnerable to a blunted cortisol response. Cortisol blunting may also increase as children age. Longitudinal studies with multiple points of assessment of both cortisol and stress are requisite steps to further expand our understanding of the cortisol-stress association. Additionally, greater use of standardized stress-measures would provide better information for comparison in the future.

Due to the important health outcomes associated with a disrupted diurnal cortisol profile, a better understanding of the association between cortisol and stress during childhood is of importance. The present study generated new knowledge about the relation between cortisol and stress, and plausible methodological and conceptual factors that may drive this association. Importantly, these research findings provide evidence-based insights to inform future research, including the design of longitudinal studies.

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Figure 1: Flow Chart for Article Identification and Inclusion in Meta-Analysis.

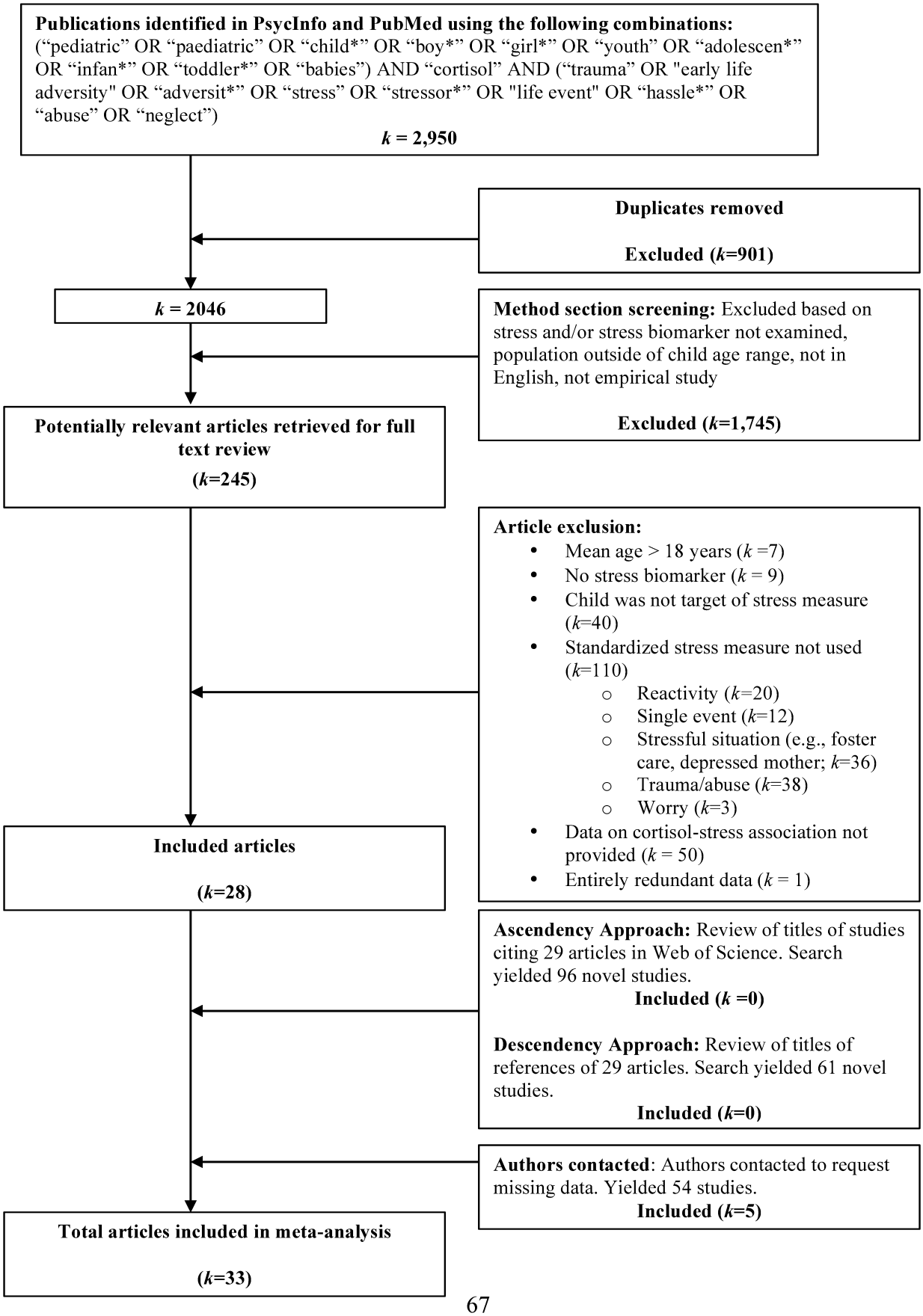

Figure 2: Forest Plot: AUC_I, AUC_{AG}, & Difference, stratified by Healthy and At-Risk.

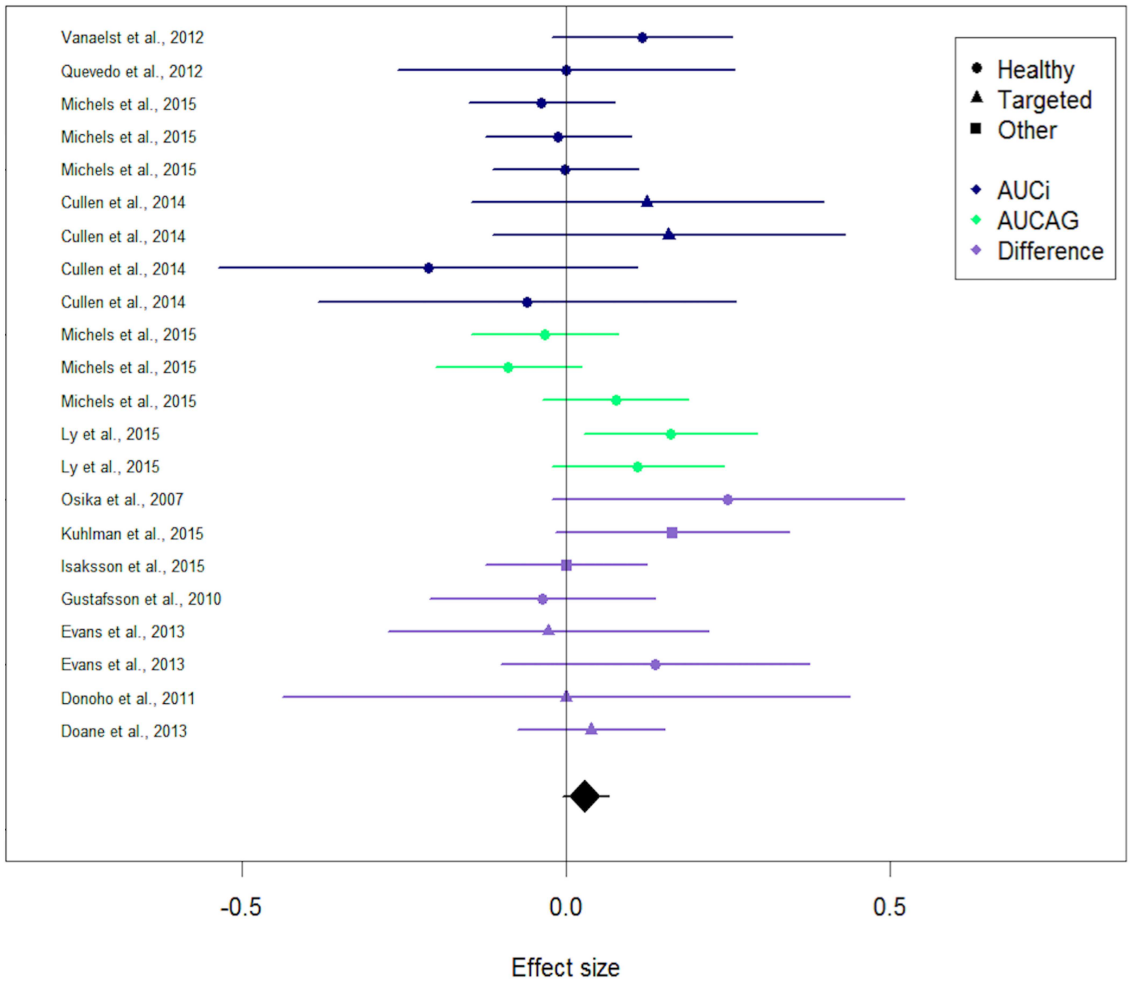

Figure 3: Forest Plot: AUC_{TG}, stratified Healthy and At-Risk.

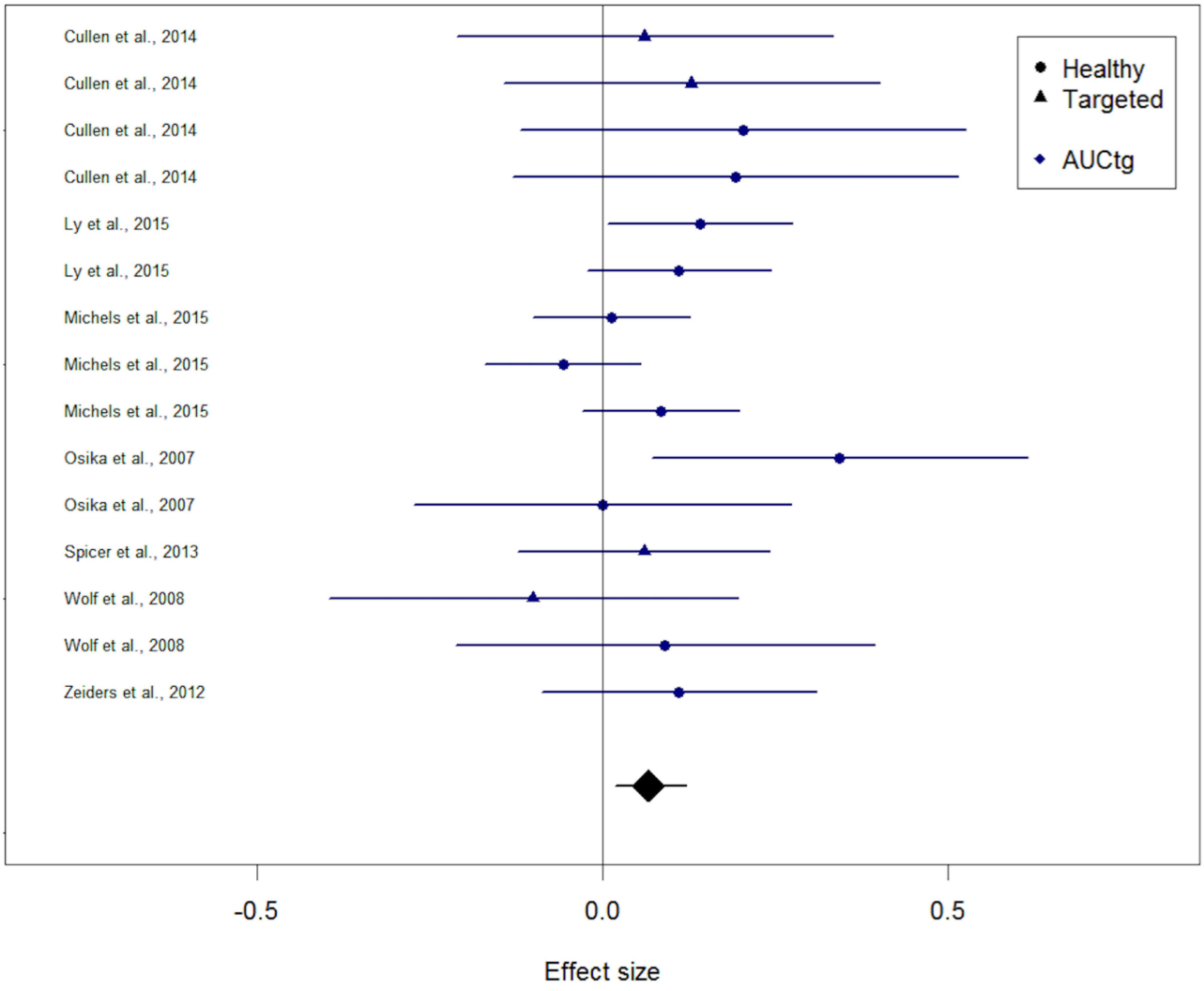

Figure 4: Forest Plot: $Slope_{Awake}$, $Slope_{Max}$, & $Slope_{Other}$, stratified by Healthy and At-Risk.

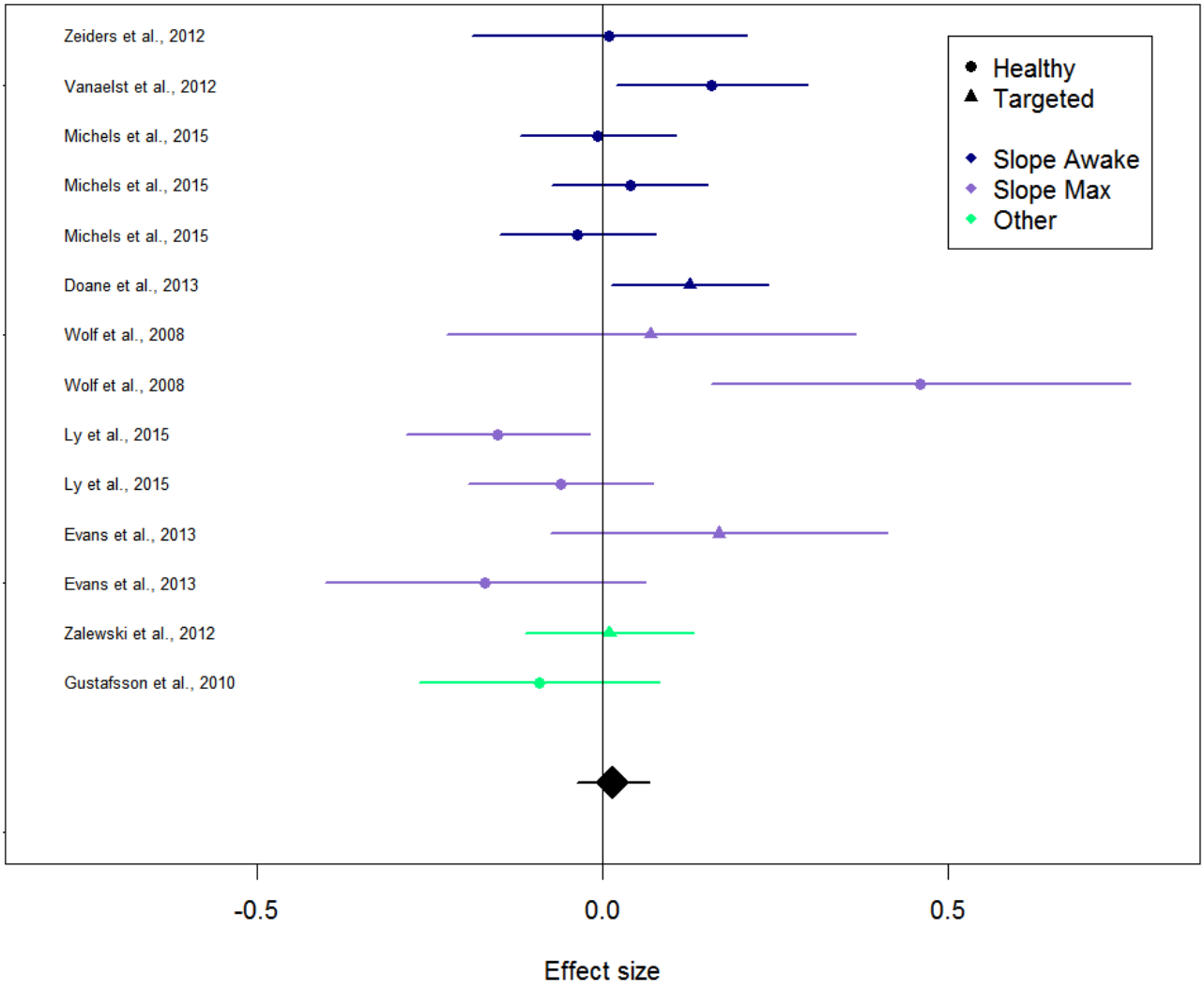

Figure 5: Forest Plot: Wakening, +15, & +30, stratified by Healthy and At-Risk.

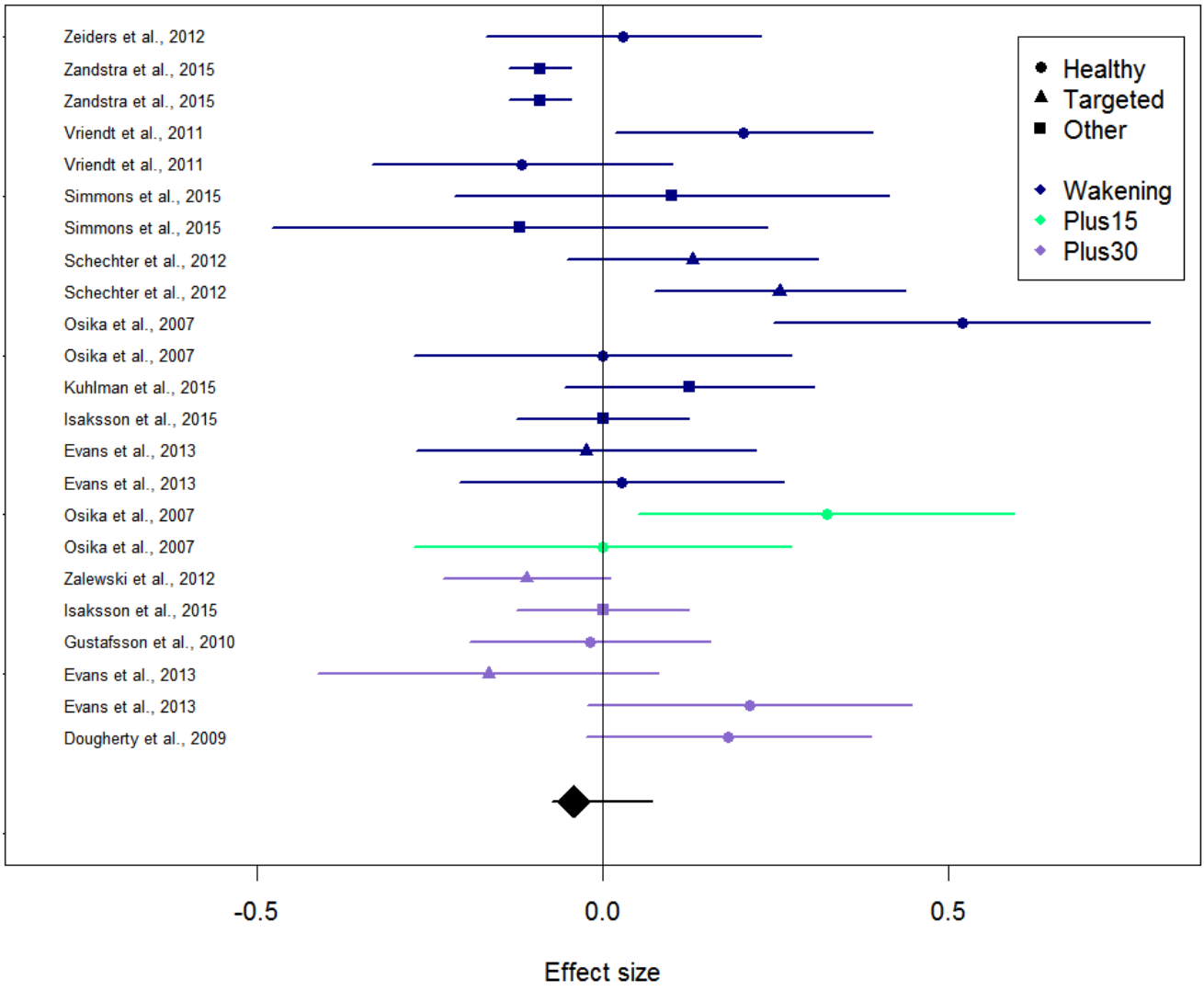

Figure 6: Forest Plot: Morning, Afternoon, Evening, Bedtime, Random, and Other, stratified by Healthy and At-Risk.

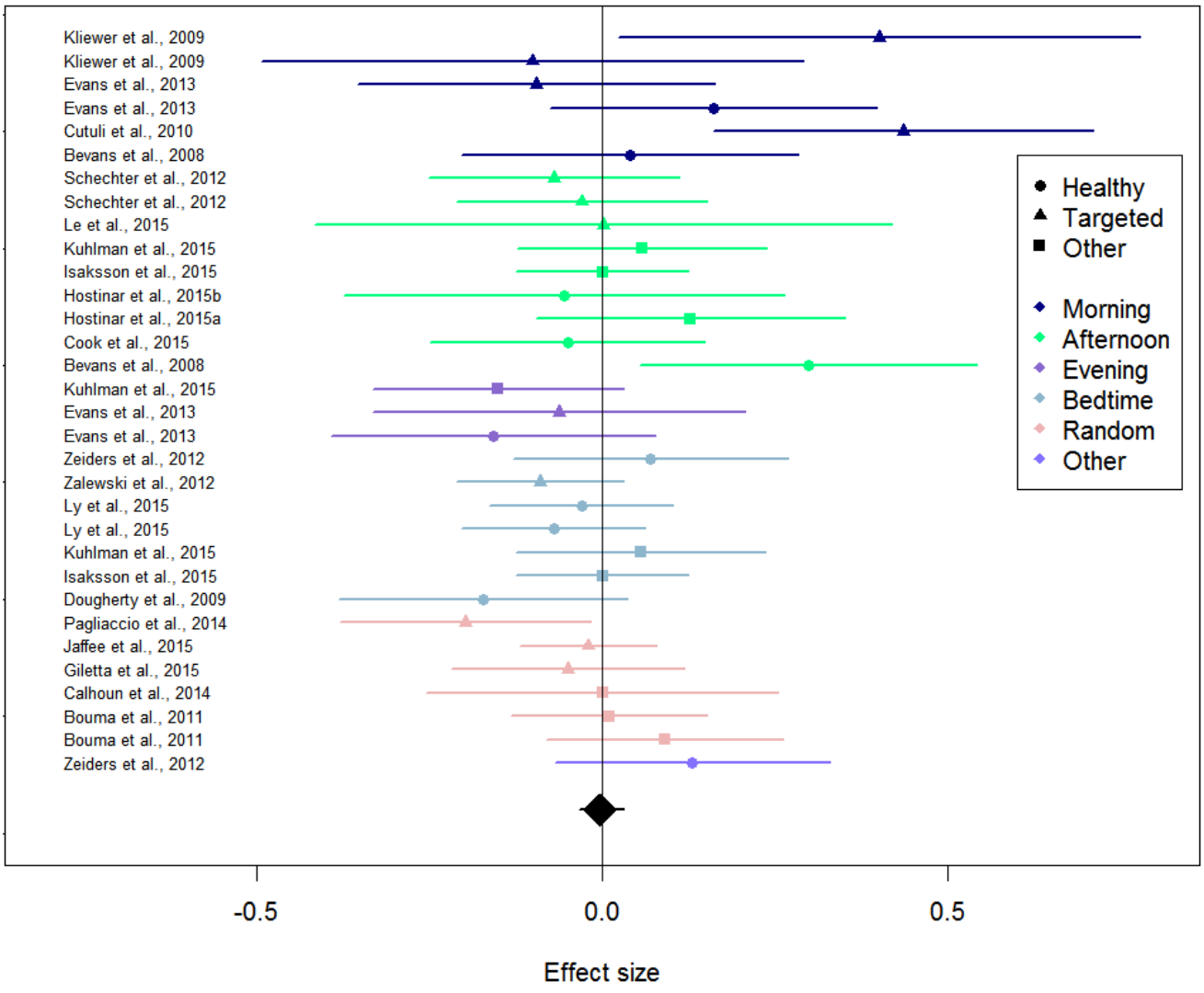

Table 1.

Descriptive Statistics Presented in Coded Articles

Characteristic	*k*	*N*	Minimum	Maximum	*M(SD)*
Effect Size	33	(106)	-0.21	0.52	0.043 (0.14)
Age (years)	33	6,397	3.08	17.88	12.19 (3.90)
Age span (range years)	33	6,397	1	13	6.00 (3.60)
Sex (% female)	33	6,397	0.0	100.0	56.25 (1.70)
Ethnicity					
Percent white (%)	13	1,956	8.0	91.5	61.54% (22.65)
Percent black (%)	14	2,080	2.0	100.0	23.76% (29.69)
SES					
Household Income (K US$)	6	1,002	29.50	117.50	64.992 (35.932)
Parental Education (years)	10	1,621	13	17.35	15.82 (1.11)
BMI (kg/m^2)	7	711	19.71	25.97	21.80 (2.31)
Days of Saliva Collection	33	106	1	7	1.71 (1.02)
Saliva Samples per Day	33	106	1	6	3.80 (1.48)
Total Samples	33	106	2	18	6.17 (3.74)
Salivary Cortisol (nmol/l)					
AUC_I	2	363	22.59	56.33	39.46 (23.86)
AUC_{AG}	1	220	-	-	11.04 (-)
AUC_{TG}	4	616	34.87	119.32	76.35 (35.06)
$Slope_{Aw}$	1	100	-	-	22.60 (-)
$Slope_{Max}$	2	492	-1.03	-0.81	-0.92 (0.16)
Difference Δ	2	76	10.60	23.46	17.03 (9.09)
Wake (+0)	5	877	5.30	12.15	8.57 (3.14)
+30	2	232	11.15	19.80	15.48 (6.12)
Morning	3	242	6.35	17.11	13.25(5.99)
Afternoon	3	290	3.04	10.76	6.53 (3.91)
Bedtime	5	852	1.10	3.75	2.31 (1.01)
Pubertal Status	3	332			
Tanner I (%)			0	61.9	25.97% (32.13)
Tanner II (%)			0	38.1	18.03% (19.13)
Tanner III (%)			0	20	8.33% (10.41)
Tanner IV (%)			0	55	26.00% (27.62)
Tanner V (%)			0	33	18.00% (16.70)

Note. All available demographic statistics presented; many studies did not present values. *k* = number of studies; *N* = number of participants; *M*(*SD*) = mean (standard deviation)

Table 2.

Participant Demographic Characteristics and Study Recruitment

Comparison		***k***	***N*ES**	**Fisher's *Z***	**Bootstrap CI**	**β[a] (*p*)**	***Q*T (*p*)**	**Fail Safe *N***	**Model**
All Studies		33	106	**0.029**	**0.008, 0.052**		104.84 (.486)	202	Random
Number participants		33	106			**-0.0001 (.001)**	124.42 (.095)	160	Random
Sex	Percent Female (%)	33	106			0.001 (.196)	106.40 (.444)	199	Random
Racial Ethnicity	Percent White (%)	13	52			-0.001 (.272)	49.62 (.529)	58	Random
	Percent Black (%)	14	40			**0.002 (.058)**	42.74 (.314)	87	Random
Age, Continuous		33	106			0.004 (.185)	106.76 (.434)	198	Random
Body Mass Index		7	35			-0.020 (.246)	34.34 (.451)	251	Random
Socioeconomic Status	Parent Education (yrs)	10	26			0.032 (.233)	25.00 (.463)	42	Random
	Household Income ($K,US)	6	20			-0.0003 (.863)	16.93 (.594)	20	Random
Recruitment*									
General Community		13	41	0.0002	-0.027, 0.031		47.00 (.208)		Fixed
School		17	42	0.008	-0.037, 0.034		138.95 (<.001)		Fixed
Targeted (clinic/shelter/jail/medical record)		13	42	-0.027	-0.059, 0.036		79.69 (<.001)		Fixed
Population Sampled					Q_M = 0.53 (3, 84) p = 0.912			232	Random
General population		14	58	**0.042**	**0.016, 0.073**		62.98 (.273)		Random
Mental Health Risk		5	14	0.021	-0.048, 0.080		12.48 (.489)		Random
Stress Risk		7	13	**0.030**	**0.037, 0.116**		14.87 (.248)		Random
Medical Condition		2	3	-0.012	-0.100, 0.056		0.64 (.723)		Random
Exclusion criteria*									
Medication		10	30	0.040	-0.002, 0.077		27.98 (.519)		Random
Mental Health		13	45	0.026	-0.007, 0.061		43.98 (.472)		Random

Note. k = Number of studies; N_{ES} = Number of effect sizes; Fisher's Z = Effect size statistic; Boostrap CI = Bootstrap 95% confidence intervals; [a]Slope (β) provided for continuous moderator variables; Q_T = Heterogeneity statistic; Q_M = Between groups variability; * indicated binary coding, therefore no Q_M available.

Table 3.

Cortisol Measures and Sampling Design

Comparison	***k***	N_{ES}	**Fisher's *Z***	**Bootstrap CI**	**β (*p*)**	Q_T **(*p*)**	**Fail Safe *N***	**Model**
Cortisol Measure				Q_M **= 41.49 (14, 90), *p* = (<.001)**			738	Fixed
AUC_I	4	9	0.009	-0.029, 0.069		7.05 (.531)		Fixed
AUC_{AG}	2	5	0.034	-0.045, 0.112		11.29 (.023)		Fixed
Difference (Δ)	7	8	**0.048**	**0.003, 0.121**		6.54 (.479)		Fixed
AUC_{TG}	7	15	**0.066**	**0.019, 0.121**		13.86 (.046)		Fixed
$Slope_{Awake}$	4	6	0.046	-0.007, 0.106		7.45 (.189)		Fixed
$Slope_{Max}$	3	6	-0.038	-0.119, 0.169		17.84 (.003)		Fixed
Single Samples								Fixed
Wake (+0 sample)	9	15	-0.050	-0.077, 0.111		50.51 (<.001)		Fixed
+30	5	6	-0.012	-0.093, 0.098		11.00 (.051)		Fixed
Morning	4	6	0.131	-0.016, 0.296		11.60 (.041)		Fixed
Afternoon	8	9	0.027	-0.017, 0.095		7.63 (.471)		Fixed
Evening	2	3	-0.060	-0.150, 0.032		1.87 (.393)		Fixed
Bedtime	6	7	-0.038	-0.081, 0.009		5.03 (.540)		Fixed
Random	5	6	-0.023	-0.086, 0.028		5.61 (.346)		Fixed
Sampling Protocol								
Days of Saliva Collection	33	106			0.013 (.270)	108.10 (.398)	195	Random
Saliva Samples per Day	33	106			0.010 (.224)	111.69 (.309)	188	Random
Total Samples	33	106			0.005 (0.099)	112.80 (.284)	185	Random
Sampling Location				Q_M **= 5.93 (2, 101), *p* = 0.052**			162	Fixed
Home (Exclusively)	22	74	0.014	-0.007, 0.038		68.82 (.617)		Fixed
At least one school sample	4	22	**0.079**	**0.026, 0.145**		21.88 (.406)		Fixed
Lab	7	8	-0.012	-0.092, 0.057		6.94 (.435)		Fixed
Restriction (food/drink)*								
Food/Drink	22	76	-0.004	-0.033, 0.037		157.75 (<.001)		Fixed
Toothbrushing	10	42	-0.018	-0.048, 0.031		87.10 (<.001)		Fixed
Physical Activity	4	18	0.023	-0.005, 0.054		12.95 (.741)		Fixed
†Saliva collection				Q_M = 0.36 (1, 56), *p* = 0.547			121	Fixed

Comparison	*k*	N_{ES}	Fisher's *Z*	Bootstrap CI	β (*p*)	Q_T (*p*)	Fail Safe *N*	Model
Swab	20	41	0.028	-0.002, 0.063		89.36 (<.001)		Fixed
Passive Drool	11	17	0.047	-0.003, 0.091		12.74 (0.692)		Fixed
Salivary Cortisol Assay				Q_M = 0.65 (3, 99), p = 0.890			205	Random
Enzyme Immunoassay	14	35	0.025	-0.014, 0.069		33.69 (.483)		Random
Fluorescence	9	33	0.044	-0.0001, 0.081		31.76 (.479)		Random
Chemiluminescence	4	19	0.021	-0.014, 0.060		19.54 (.359)		Random
Radioimmunoassay	4	16	0.026	-0.031, 0.110		22.23 (.102)		Random
†Sent to Laboratory for Assaying				Q_M = 0.06 (1, 48), p = 0.957			107	Fixed
Yes	15	44	**0.032**	**0.008, 0.056**		76.34 (0.001)		Fixed
No	5	6	0.018	-0.129, 0.162		10.20 (0.070)		Fixed

Note. *k* = Number of studies; N_{ES} = Number of effect sizes; Fisher's *Z* = Effect size statistic; Boostrap CI = Bootstrap 95% confidence intervals; [a]Slope (β) provided for continuous moderator variables; Q_T = Heterogeneity statistic; Q_M = Between groups variability; * indicated binary coding, therefore no Q_M available. [a]Slope (β) provided for continuous moderator variables. * indicated binary coding, therefore no Q_M available. † Analysis restricted to general population sample.

Table 4.

Stress Measures

Comparison	*k*	N_{ES}	Fisher's *Z*	Bootstrap CI	β (*p*)	Q_T (*p*)	Fail Safe *N*	Model
Type of Stress Measure				Q_M =18.69 (2, 100), *p* = (<.001)			734	Fixed
Life Event	24	73	-0.010	-0.040, 0.033		133.81 (<.001)		Fixed
Hassles	6	18	**0.124**	**0.042, 0.214**		33.33 (.010)		Fixed
Perceived Stress	5	12	0.012	-0.038, 0.062		16.17 (.135)		Fixed
Stress Event Topography								
Frequency	18	55	0.018	-0.008, 0.045		56.45 (.384)		Random
Frequency+Intensity	5	13	**0.047**	**0.003, 0.103**		13.02 (.367)		Random
Domain	18	63		-0.004 (.668)		56.73 (.665)		Random
Time frame of stress measure (mos)	28	94		0.0001 (.654)		94.07 (.421)	123	Random
Timelag from Cortisol to Stress (days)	18	62		**-0.002 (<.001)**		84.22 (.062)	366	Random
Informant				**Q_M = 31.53 (1, 103), *p* = (<.001)**			748	Fixed
Parent	8	16	-0.063	-0.085, 0.043		42.04 (<.001)		Fixed
Child	24	89	**0.029**	**0.007, 0.051**		129.55 (.003)		Fixed

Note. *k* = Number of studies; N_{ES} = Number of effect sizes; Fisher's *Z* = Effect size statistic; Boostrap CI = Bootstrap 95% confidence intervals; [a]Slope (β) provided for continuous moderator variables; Q_T = Heterogeneity statistic; Q_M = Between groups variability; * indicated binary coding, therefore no Q_M available. [a]Slope (β) provided for continuous moderator variables. * indicated binary coding, therefore no Q_M available.

Table 5.

Cortisol Measures and Conceptual Constructs

	Traditionally Reported Cortisol Measures												**Conceptual Cortisol Construct**			
	AUC_I	**AUC_{AG}**	**Difference**	**Δ AUC_{TG}**	**$Slope_{Awake}$**	**$Slope_{Max}$**	**Wake(+0)**	**+30**	**Morning**	**Afternoon**	**Evening**	**Bedtime**	**Waking**	**Diurnal**	**Area**	**Change**
	Fisher's Z^{ES} (Bootstrap CI)															
All Studies	0.009[9] (-.029, .069)	0.034[5] (-.045, .112)	**0.048[8] (.003, .121)**	**0.066[15] (.019, .121)**	0.046[6] (-.007, .106)	-0.038[7] (-.119, .017)	-0.050[15] (-.077, .111)	-0.012[6] (-.093, .098)	0.131[6] (-.016, .296)	0.027[9] (-.017, .095)	-0.060[3] (-.150, .032)	-0.038[7] (-.086, 0.028)	0.032[22] (-.005 .071)	**0.049[23] (.016, .085)**	**0.066[15] (.019, .121)**	0.023[36] (-.006, .056)
Population																
Healthy	-0.002[7] (-.048, .049)	0.034[6] (-.090, .121)	n/a	**0.069[11] (.015, .132)**	0.026[5] (-.020, .094)	-0.071[4] (-.156, .131)	0.097[6] (-.042, .244)	0.101[3] (-.019, .212)	**0.102[2] (.040, .160)**	0.097[3] (-.055, .300)	n/a	-0.048[4] (-.108, .079)	0.023[15] (-.022, .069)	**0.047[17] (.009, .091)**	**0.069[11] (.015, .132)**	0.011[25] (-.030, .050)
Targeted	**0.141[2] (.125, .158)**	n/a	0.034[3] (.000, .038)	0.046[4] (-.032, .101)	n/a	**0.128[2] (.070, .169)**	0.145[3] (-.024, .255)	**-0.123[2] (-.166, -.110)**	0.156[4] (-.097, .430)	-0.045[3] (-.070, .002)	n/a	n/a	**0.063[5] (.033, .129)**	**0.062[6] (.004, .112)**	0.046[4] (-.032, .101)	**0.075[9] (.038, .125)**
Stress Meas.																
Life Ev.	0.005[7] (-.040, .065)	.009[4] (-.061, .092)	**0.059[5] (.015, .126)**	0.041[9] (-.007, .100)	0.046[6] (-.011, .106)	0.018[5] (-.080, .250)	-0.072[9] (-.070, .075)	-0.017[4] (-.126, .200)	0.125[4] (-.025, .321)	0.062[5] (-.033, .179)	-0.060[3] (-.150, .032)	-0.041[5] (-.150, .032)	0.017[15] (-.020, .058)	**0.041[16] (.006, .075)**	0.041[9] (-.007, .100)	0.030[24] (-.001, .064)
Hassles	0.068[2] (-.060, .158)	n/a	n/a	**0.179[4] (.052, .308)**	n/a	n/a	0.174[5] (-.020, .355)	n/a	0.160[2] (-.100, .400)	n/a	n/a	n/a	0.154[3] (-.060, .249)	0.179[4] (.055, .285)	**0.179[4] (.052, .308)**	0.154[3] (-.060, .227)
Perceived	n/a	n/a	n/a	**0.113[2] (.060, .141)**	n/a	n/a	n/a	n/a	n/a	0.006[3] (.000, .021)	n/a	-0.033[2] (-.070, .000)	n/a	n/a	**0.113[2] (.060, .141)**	n/a
	β (*SE*)															
Age	-0.001 (.015)	0.028 (.020)	-0.014 (.014)	0.010 (.008)	0.008 (.009)	-0.001 (-.057)	0.013 (.016)	0.001 (.011)	-0.034 (.022)	**-0.041 (.018)**	0.054 (.039)	0.011 (.006)	0.005[22] (.007)	0.008[23] (.005)	0.010[15] (.008)	0.001[31] (.005)
Age Span (Range)	0.010 (.024)	**0.030 (.014)**	-0.005 (.009)	0.003 (.076)	-0.015 (.029)	-0.015 (.063)	0.001 (.012)	-0.009 (.015)	-0.026 (.021)	0.007 (.018)	0.029 (.022)	0.006 (.012)	0.008[22] (.007)	-0.002[20] (.009)	0.003[15] (.076)	-0.006[28] (.006)
Female (%)	0.002 (.002)	**0.037 (.017)**	0.000 (.003)	0.002 (.001)	**0.004 (.001)**	-0.008 (.010)	-0.001 (.001)	-0.002 (.009)	0.002 (.004)	0.002 (.001)	0.015 (.011)	-0.001 (.007)	0.006[22] (.001)	**0.003[23] (.001)**	0.002[15] (.001)	**0.002[31] (.001)**

BMI	0.001[4] (.001)	n/a	-0.111[4] (.069)	-0.021[8] (.018)	n/a	0.057[4] (.145)	-0.091[6] (.067)	n/a	n/a	n/a	n/a	n/a	-0.089[8] (.066)	-0.021[9] (.018)	-0.021[8] (.018)	0.012[12] (.048)

Note: Bold values are significant (p<.05). k = Number of studies; N_{ES} = Number of effect sizes; Fisher's Z = Effect size statistic; Boostrap CI = Bootstrap 95% confidence intervals; [a]Slope (β) provided for continuous moderator variables; Q_T = Heterogeneity statistic; Q_M = Between groups variability; * indicated binary coding, therefore no Q_M available. For Aggregate Measures: Waking (AUC_I, Difference Δ, AUC_{AG}), Diurnal (AUC_{TG}, $Slope_{Aw}$, $Slope_{Other}$), Area (AUC_{TG}), Change (AUC_I, AUC_{AG}, Δ, $Slope_{Aw}$, $Slope_{Max}$, $Slope_{Other}$), Superscript indicates number of effect sizes. Targeted population is comprised of studies which recruited participants specifically for mental health risk, stress exposure, or medical problems. Stress Meas. = Stress measures; Life Ev. = life events measures; Hassles = daily hassles measures; Perceived = Perceived Stress Measures.