Associations between youth's diurnal cortisol and physical and mental health outcomes: Importance of time since onset, severity and chronicity

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A Thesis

In the Department

of

Psychology

Presented in Partial Fulfilment of the Requirements

For the Degree of Doctor of Philosophy at

Concordia University

Montreal, Quebec, Canada

February 2011

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CONCORDIA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

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and submitted in partial fulfilment of the requirements for the degree of

Doctorate of Philosophy (Psychology)

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General Abstract

Associations between youth's diurnal cortisol and physical and mental health outcomes: Importance of time since onset, severity and chronicity.

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Previous investigations of the association between cortisol and physical and mental health outcomes have yielded inconsistent results. Examining how long the individual has experienced the problem may help clarify these divergent findings. Four questions were examined including (1) Do the associations between diurnal cortisol and internalizing behaviours differ depending on how long youth have experienced the behaviours? (2) Do the associations between diurnal cortisol and externalizing behaviours differ depending on how long youth have experienced the behaviours? (3) Do associations persist if behaviours are examined at a later point in time (i.e. mid-adolescence)? (4) Do the associations between diurnal cortisol and physical health problems in early adolescence differ depending on the chronicity of the health problem?

Data from the Concordia Longitudinal Risk Project were employed to examine these associations. In general, results suggest that the association between cortisol and health outcomes differ depending on how long the problem has been experienced. When examined concurrently in adolescence, youth with more internalizing behaviours had *higher* morning cortisol; however, when examined longitudinally, youth with more internalizing behaviours in childhood had *lower* morning cortisol levels as adolescents. Youth with more externalizing behaviours in childhood had flattened diurnal cortisol rhythms as adolescents, and this finding

persisted when examined in adolescence. Similarly, blunted patterns of adrenocortical activity in early adolescence were also related to more internalizing and externalizing behaviours in mid-adolescence. Furthermore, this notion seems to hold true for physical health problems such that acute health problems (i.e. infections) were associated with elevated levels of cortisol whereas more chronic health problems (i.e. asthma and allergies) were associated with blunted patterns of diurnal cortisol. The findings suggest that both hyper- and hypocortisolism may be evident within the same sample and this is related to how long the problem had been experienced. Future research should consider sampling cortisol at more than one time point to more thoroughly examine the possibility of blunted hypothalamic-pituitary-adrenal axis activity in prospective, longitudinal studies.

Acknowledgements

I would like to thank my supervisor, Dr. Lisa Serbin, for her guidance, patience and understanding. Lisa, your encouragement and confidence in me persuaded me trust my own abilities and judgements, a lesson for which I am eternally grateful. I would also like to thank Dr. Dale Stack and Dr. Alex Schwartzman for their insightful comments and direction on the projects included in this dissertation as well as many others. Many thanks are also extended to my internal committee members Drs. Bill Bukowski and Mark Ellenbogen. Bill, thank you for always having an open door during the many statistical crises I encountered throughout these projects and, Mark, thank you for helping me brainstorm through many tough conceptual problems. This project would never have been completed if it weren't for Claude Senneville's expertise with the data from the Concordia Longitudinal Risk Project and Guang Hui Li's promptness in sending data my way as well as the countless volunteers and who work so hard to keep the Project running. This process would have been much less enjoyable had I not had Donna Craven and Pippa Ross to rely on for their endless knowledge and debriefing sessions. Lots of love is extended to my lab sisters Caroline, Dahlia, Lisa, Alexa, Erin, Michele, and Danielle who made it fun to come into the lab. To the many other friends I have made throughout this process, I'll never forget your kindness and endless support. Special thanks to Marie-Pierre who kept me on track and Lina who helped me keep my head on straight. Endless love and thanks go out to Barbie, Natasha, Courtenay, and Nina for their listening ears and sturdy shoulders which I unabashedly leaned on throughout this crazy process.

This task would never have been possible without the support of my family. To Marcos, thank you for providing me with endless amounts of comic relief and true innocence when this process would make me cynical and for helping me keep the things that are really important in perspective. I know you're going to accomplish great things in your lifetime, never give up on your dreams or forget your roots. To Dad, thank you for teaching me that the best things in life are worth the work, for your regular phone calls, and for pretending not to notice when I was up to my ears in work and neglected to call you. To Mom, my inspiration. You've taught me the value of hard work and honesty and that if you put your mind (and heart) into something, not only will you succeed, but you might even look like you know what you're doing! To all of you who have become my extended family in Montreal, thank you for keeping me fed, feeling loved, and providing much needed distraction over the years. To Peter, you've truly been my rock. You've taught me to step outside my comfort zone and not only to dare to dream, but to dare to live whatever I decide to dream. And finally I'd like to thank Birdie, my tandem-partner in the free-falling world of academia. Birdie, your love for research is infectious and your drive to live life to the fullest has allowed me to believe that female academics can have it all. I will be forever grateful to you for the windows and doors you've opened...even if sometimes I had to be pushed out of them. The tide is high but I'm holding on!

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Contributions of the Authors

Paula L. Ruttle developed the research questions, designed, performed, and interpreted the statistical analyses, and wrote and edited all chapters included in the current thesis. Drs. Lisa Serbin, Elizabeth Shirtcliff, Dahlia Ben-Dat Fisher, Dale Stack and Alex Schwartzman provided commentary on these manuscripts.

General Introduction

The association between stress and health has been studied since Ancient times; however, it was only in the 20th century that the realm of stress research, as we know it to today, began to emerge (Fink, 2010). With the progression of science, various diathesis-stress theories were proposed to help explain the development of mental and physical health problems (Ingram & Luxton, 2005). These theories suggest that an individual is more likely to develop an illness if he or she is has a particular vulnerability and is faced with a stressor rather than if he or she does not have a vulnerability and just faced the stressor alone. Furthermore, it is posited that multiple factors, including early life stress, genetics, biological make-up, and psychobiological factors, can interact with one another to determine an individual's risk for developing health problems. While there is no doubt that the construct of health is etiologically heterogeneous, promising research in the area of physiology has emerged suggesting that certain patterns of stress hormones may be implicated in the development and course of mental and physical health problems (van Praag, de Kloet & van Os, 2004).

Cortisol, the main hormonal by-product of the stress response, has been identified as a precursor to the development of various health problems. Atypical cortisol levels have been linked to concurrent mental (Board, Persky & Hamburg, 1956; Landau, 1975; Sachar, Hellman, Fukushima & Gallagher, 1973) and physical health problems (Rose, Jenkins, Hurst, Kreger, Barrett, & Hall, 1986) for several years; however, more recent studies have identified atypical cortisol levels as a precursor and possible contributor to the development of certain mental health

problems. More specifically, high levels of cortisol have been linked to internalizing problems both at the clinical (Goodyer, Hebert, Tamplin, & Altham, 2000) and sub-clinical level (Halligan, Herbert, Goodyer & Murray, 2007). While there is some evidence suggesting that cortisol may be related to the onset of externalizing problems (Alink et al., 2008; Shoal, Giancola, Kirillova, 2003), and some physical health problems (Ball, Anderson, Minto, & Halonen, 2006), the majority of this research is correlational in nature. More recent work has suggested that time since onset of the stressor (Gustafsson, Anckarsäter, Lichtenstein, Nelson, & Gustafsson, 2010; Miller, Chen, & Zhou, 2007) or problem (Shirtcliff & Essex, 2008) may play a key role in determining how cortisol is associated with various mental and physical health problems. Although the abovementioned research has set the groundwork for future studies in the area, more work needs to be conducted to determine how cortisol may be differentially related various mental and physical health problems.

The papers included in the present dissertation aim to examine the association between cortisol in early adolescence and mental and physical health outcomes in childhood, early adolescence and mid-adolescence in an at-risk sample of individuals. Discussions will emphasize the importance of examining time since onset and chronicity of the problem when trying to determine the association between cortisol and mental and physical health problems. These papers aim to help make sense of the seemingly divergent findings in previous research and guide future studies to make the results of subsequent research more evident.

Mental and Physical Health Problems

Mental and physical health problems are global phenomena that affect billions of people world-wide and place huge tolls on an individual's productivity and livelihood. In addition to taxing the resources of the individual, health problems drastically affect the economy, costing the government hundreds and billions of dollars annually in treatment and service fees, and even more after considering costs of lost productivity and wages (see Kessler, 2002, Stephens & Joubert, 2001; Canadian Institute for Health Information, 2009). Although the mind and body reciprocally influence each other and are inextricably linked, the concept of health problems are generally divided into two categories: 1) mental health problems (which can be further divided into internalizing and externalizing problems) and 2) physical health problems.

Internalizing problems. Generally speaking, the term internalizing problems refers to a wide range of problems characterized by disordered mood or emotion (Kovacs & Devlin, 1998). More specifically, this term typically refers to symptoms of anxiety, depression and social withdrawal. Prevalence rates of clinical levels of these types of problems vary but results from a large birth cohort have suggested that between the ages of 11-15, rates of depression range from 1.2-18% and rates of anxiety range from 7.5-19.7% (Anderson, William, McGee, & Sliva, 1987; McGee et al., 1990; Newman et al., 1996). Internalizing disorders typically follow a developmental trajectory where rates start to increase at approximately 13 years of age and continuing to rise until late adolescence (Hankin, Abramson, Moffitt, Silva, McGee & Angell, 1998). Along with developmental differences, there are also gender differences in the rates of internalizing disorders that appear at

approximately age 13, such that by age 15 females are twice as likely as males to display internalizing problems (see Rutter, Caspi, & Moffitt, 2003; Zahn-Waxler, Crick, Shirtcliff, & Woods, 2006, for reviews).

Psychological, biological, social and environmental factors have been associated with the development of internalizing problems including temperament (Williams et al., 2009), presence and type of friends (for a review see Vitaro, Boivin, & Bukowski, 2009), genetics (Hicks, DiRago, Iacono, McGue, 2009), and being raised in a non-optimal environment (Hicks et al., 2009; Mrug & Windle, 2010; Williams et al., 2009). While it is likely that all of these variables make unique contributions to the development of internalizing problems, one variable underlying all of these factors is that they have the potential to increase the level of stress experienced by the individual, either by predisposing them to be more sensitive to the effect of stress or by increasing the amount of stress in their lives. Unsurprisingly, high levels of stress and its physiological correlates have been shown to lead to the development and maintenance of internalizing problems (Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001; Natsuaki, Klimes-Dougan, Ge, Shirtcliff, Hastings, & Zahn-Waxler, 2009; Vigil, Geary, Granger, & Flinn, 2010)

Externalizing problems. Externalizing problems are characterized by severe dysregulation in behaviour (Kovacs & Devlin, 1998) including oppositional, defiant and aggressive acts. Common types of externalizing problems seen in individuals in early to mid-adolescence include oppositional defiant disorder and conduct disorder. Prevalence rates of clinical levels of externalizing problems vary with age but generally tend to follow a curvilinear pattern such that low rates are

seen in childhood, rates increase to approximately 12% in adolescence, and then generally decline in late adolescence or young adulthood to rates observed in childhood (Anderson, William, McGee, & Sliva, 1987; McGee et al., 1990; Newman et al., 1996). Youth with these types of problems often display high levels of comorbidity with other behaviour problems including internalizing behaviour (Angold, Costello, & Erkanli, 1999).

Similarly to internalizing problems, various psychological, biological, social and environmental factors have been associated with the development of externalizing problems. Poor parenting, exposure to violence, associating with deviant peers, and poor self-concept have been associated with high levels of externalizing problems (Essex et al., 2006; Klimes-Dougan et al., 2001; Mrug & Windle, 2010; Silver, Measelle, Armstrong, & Essex, 2005). Much like internalizing problems, high levels of externalizing problems have also been linked to high levels of stress and altered stress physiology in youth (Klimes-Dougan et al., 2001; Marsee, 2008; Snoek, Van Goozen, Matthys, Buitelaar, & Van Engeland, 2004).

Physical health problems. The term "physical health problems" refers to a wide range of impairments in physical functioning or well-being. Physical health problems in youth are often quite different than those seen in adults and usually consist of acute health problems, including injuries and infections. While chronic health problems do exist in children and adolescents, these tend to be rarer than chronic health problems in adults and include problems such as asthma and allergies (Cookson, & Moffatt, 1997; Seaton, Godden, & Brown, 1994). Physical health problems in children also follow developmental trajectories which vary by

health problem. The highest rates of infections are typically seen in infancy and early childhood (Monto & Ullman, 1974) and these generally decrease with age whereas chronic health problems such as asthma and allergies are typically not formally diagnosed until early childhood or later when the problems become more apparent, presumably due to repeated symptom presentation (Midodzi, Rowe, Majaesic, Saunders & Senthilselvan, 2010).

Stress, the HPA Axis, & Cortisol

Although stress has been defined in many ways over the years, one widely accepted conceptualization of stress, created by Lazarus and Folkman (1984), states that stress is an ongoing process that varies from moment to moment because of constant cognitive appraisals related to both potential harms and benefits. While a certain level of stress is healthy and necessary for an organism's survival, stress becomes unhealthy when it occurs for a prolonged period of time, too frequently, at a high intensity or involves the accumulation of many minor stressors (Sapolsky, 1992). Sustained exposure to unhealthy levels of stress has been related to the development of various health problems (van Praag et al., 2004).

The notion that stress is both adaptive and maladaptive was first highlighted by Hans Selye (1936). He observed that the systems designed to help the body deal with stress in the short-term actually caused damage and accelerated the disease process if chronically activated. He identified three stages of stress response: alarm (in which the body is made aware of the stressor), resistance (in which the body defends itself against the stressor), and exhaustion (in which the body is no longer able to fight off the stressor), and coined this process "general adaptation

syndrome". More recently McEwen (1998) proposed that the final stage of Selye's model was caused by the body constantly responding to repeated stressors by releasing stress hormones in order to restore balance within the body. The toll the stressors take on the body due to chronic stress or the inability to modulate the stress response is called "allostatic load".

Along this same line of thought, one proposed pathway linking stress to health is via the physiological systems activated during a stress response (McEwen, 2000). One of the two primary systems to be activated during a stress response is the HPA axis, a self-regulating system that works via a negative feedback loop. Once a stressor is experienced, neurons in the hypothalamus secrete corticotropinreleasing hormone (CRH), which travels to the anterior pituitary and stimulates the release of adrenocorticotropin hormone (ACTH). ACTH is then transported through the blood stream to the adrenal gland where the glucocorticoid hormone, cortisol is released. Various feedback circuits are present within the HPA axis to ensure that a sufficient amount of cortisol is released so the body is prepared to deal with the stressor. After producing a sufficient amount of cortisol to deal with the stressor, elevated levels of cortisol signal to suppress the release of CRH and ACTH by feeding back information to the glucocorticoid receptors of the hypothalamus, pituitary, and hippocampus (Sapolsky, 1992, 2003).

In the absence of a stressful situation, cortisol is also present in the body at resting basal levels. Basal levels of cortisol are largely influenced by the sleep/wake cycle and, as such, follow a diurnal rhythm. The diurnal pattern of cortisol is characterized by high levels of cortisol soon after awakening (cortisol awakening

response; CAR) followed by a decline throughout the course of the day (Ice, Katz-Stein, Himes, & Kane, 2004; Kirschbaum & Hellhammer, 1994). A surge in morning cortisol levels followed by a steady decline helps initiate waking activities and primes the body for the demands of pending day (Klimes-Dougan et al., 2001; Smyth, Ockenfels, Gorin, Catley, Porter, Kirschbaum et al., 1997). While the depicted diurnal rhythm and slight increases in response to a stressor are healthy patterns of cortisol output, extreme levels of cortisol can negatively impact the brain and body.

Associations between Mental and Physical Health and Cortisol

Cortisol and internalizing behaviour. A large debate in the cross-sectional cortisol literature is whether the levels of cortisol in individuals with internalizing problems are elevated or blunted. While some studies report high levels of internalizing behaviour with high levels of cortisol (Cicchetti & Rogosh, 2001; Kaufman, 1991; Perez-Edgar, Schmidt, Henderson, Schulkin, & Fox, 2008), others suggest that high levels of internalizing behaviours are associated with lower levels of cortisol (De Bellis, Dahl, Perel & Birmaher, 1996; Granger, Serbin, Schwartzman, Lehoux, Cooperman, & Ikeda, 1998). A meta-analysis examining the association between cortisol and depression in children and adolescents revealed that high levels of internalizing behaviour were related to high levels of cortisol (Lopez-Duran, Kovacs, & George, 2009); however this study only examined basal level of cortisol, not diurnal rhythm.

Cortisol and externalizing behaviour. Although not to the same extent, the results of the externalizing literature are also mixed. While the majority of studies suggest there is a negative association between cortisol and externalizing

behaviours both concurrently (Fairchild et al., 2008; Kariyawasam, Zaw, & Handley, 2002; Moss, Vanyukov, & Martin, 1995; Pajer, Gardner, Rubin, Perel & Neal, 2001; Popma et al., 2007; Shirtcliff, Granger, Booth & Johnson, 2005) and longitudinally (McBurnett, Lahey, Rathouz, & Loeber, 2000) some studies have found no association (Azar et al., 2004; Klimes-Dougan et al., 2001; van Bokhoven et al., 2005) or even a positive association (Gerra et al., 1997; McBurnett et al., 2005; van Bokhoven et al., 2005). Furthermore, a meta-analysis on the topic suggests that both elevated and low levels of cortisol are related to symptoms of externalizing behaviour but that this association may differ as a function of age (Alink et al., 2009).

Cortisol and physical health problems. Persistently high levels of cortisol due to chronic stress are thought to contribute to the development of various physical and mental health problems (Jameson, 2003; McEwen, 1998). Similar to the divergent findings in the mental health literature, research examining the association between cortisol and certain common health problems in youth, such as asthma and allergies, is seemingly mixed. While some studies suggesting high levels of asthma and/or allergies are related to low levels of cortisol (Landstra et al., 2002; Kauffman et al., 1999; Wamboldt et al., 2003), others suggesting that these health problems are related to higher levels of cortisol (Ball et al., 2006; Fujitaka et al., 2000) or even reveal mixed findings (Buske-Kirschbaum, et al., 2003). Additionally, very little research has empirically examined the link between cortisol and infections so no conclusive statement can be made on the direction of this association.

Beyond Concurrent Associations between Health and Cortisol

Although informative, cross-sectional research is only able to reveal associations between phenomena rather than provide directionality to the association. The majority of cross-sectional literature examining the association between cortisol and mental and physical health problems reveal mixed findings. Examining how long the individual has had the problem or the chronicity of the problem may be able to help shed some light on these seemingly divergent findings. Longitudinal research can help clarify these types of associations. While there has been relatively little research examining the longitudinal associations between cortisol and externalizing behaviour or cortisol and physical health problems commonly seen in youth, a substantial amount of literature has examined the association between cortisol and internalizing problems such as depression. Studies have reliably shown that cortisol levels in the morning are able to predict the onset of depression and related symptoms. Goodyer and colleagues examined the cortisol levels of secondary school children at various levels of risk for depression (Goodyer et al., 2000). Results indicated that individuals with high levels of morning cortisol were more likely to develop depression. Similar findings were also revealed in a study of adult women identified as being at-risk for the development of depression (Harris et al., 2000). The same phenomenon seems to hold true when examining symptoms of internalizing behaviour, rather than clinical diagnoses of depression. High morning levels of cortisol were found to precede the onset of later depressive symptoms in adolescents who were at an increased risk of developing depression even after controlling for previous depressive symptoms and other potential

confounding factors including sex and pubertal status (Halligan et al., 2007). To date, studies examining the predictive association between cortisol and internalizing or depressive symptoms suggest that elevated morning cortisol levels precede the onset of clinical depression or increased symptoms of depression but how do we account for the low levels of cortisol sometimes found in individuals with similar mental health problems?

A promising potential clarification of the divergent cross-sectional findings comes in the form of a theory proposed by Fries and colleagues in 2005. It has been suggested that both increased and decreased levels of cortisol can be present in the same individual but that the direction of the association depends on how long they have experienced the stressor. More specifically, while more acute exposure may result in increased levels of cortisol, long-term exposure to excessive levels of stress may result in decreased levels of cortisol as a result of a "counter-regulatory" or "down regulation" of the HPA axis (Fries, Hesse, Hellhammer, & Hellhammer, 2005). Miller, Chen and Zhou (2007) conducted a meta-analysis which applied this theory to various studies examining stress and cortisol. In line with the theory of down-regulation, they found a negative association to exist between the amount of time since the onset of the stress and cortisol secretion (Miller et al., 2007). More specifically, individuals who had recently experienced their stressful situation had higher morning and overall average cortisol levels whereas individuals who had experienced their stressor for a prolonged period of time had lower morning and overall average cortisol levels. The authors suggested that recent exposure to a chronic stressor may result in elevated levels of cortisol but that after extended

exposure to chronic stress, the HPA axis develops a "counter-regulatory response such that cortisol levels rebound below normal" (Miller et al., 2007, pp.26). Furthermore, they suggest that the cross-sectional literature may only seem contradictory because the studies were assessing participants at different times of their personal stressor onset. While these results were not specific to individuals with mental or physical health problems, it makes intuitive sense to explore this avenue in an attempt to determine if various factors can also explain the divergent findings in the literature on cortisol and health problems.

To date two studies have empirically explored the phenomenon of blunting of the HPA axis due to extensive exposure to negative behaviours or situations. Shirtcliff and Essex (2008) looked at the association between cortisol and symptoms of mental health in grades 5 and then re-assessed mental health symptoms at grade 7. They found evidence of hypercortisolism such that high levels of cortisol were predictive of the development of high levels of mental health symptoms at grade 7. However, they also found evidence of hypocortisolism as individuals who had severe levels of symptoms at grade 5 and grade 7 had flattened diurnal cortisol slopes. Additionally, although not longitudinal in nature, Gustafsson and colleagues (2010) found that children who were exposed to a moderate level of adversity displayed a higher cortisol awakening response compared to children who had not been exposed to adversity; however children who experienced a high amount of adversity displayed cortisol levels similar to the children who had not been exposed to adversity (Gustafsson et al., 2010). The authors suggested that the children

exposed to a high level of adversity were displaying blunted HPA axis activity due to exposure to chronic stress.

While these studies are innovative and informative, they each only provide a portion of the total picture. While Shirtcliff and Essex (2008) take a longitudinal approach, they did not measure the cortisol awakening response (CAR), a measure shown to be relevant to the development of depression and other health issues (Adam et al., 2010; for a review see Clow, Hucklebridge, Stalder, Evans & Thorn, 2010). This study also examined mental health symptoms as a whole and did not explore how internalizing and externalizing symptoms differentially related to cortisol. And, although Gustafsson and colleagues (2010) measured the cortisol awakening response, they only compared children at one point in time, rather than exploring the association longitudinally and did not include any health variables in their investigation. Furthermore, both studies only examined cortisol at three different time points during the day which may not allow for the exploration of various intricacies (e.g. quadratic and cubic functions) of the diurnal slope. Additionally, the association between cortisol and health problems in youth (i.e. infections, asthma, and allergies) has not been examined as a function of chronicity of the problem.

The Current Studies

The data for the three studies was drawn exclusively from the Concordia Longitudinal Risk Project, a project initiated between 1976-1978 that involved over 4000 children in schools sampled from neighbourhoods in working class areas of Montreal, Québec. Children in grades 1, 4 and 7 were peer-nominated on levels of

aggression, social withdrawal and likeability. A final sample included an oversampling of children based on high ratings in aggression, withdrawal, or both as well as a comparison sample of typically developing children. These individuals are now adults and have had offspring of their own who are the primary focus of the following three studies.

Families were contacted and asked to participate in subsequent waves of the study. Demographic information regarding age, sex and socio-economic status were collected at all waves of the study as were mother and teacher reports of internalizing and externalizing behaviours. Additionally, youth's diurnal cortisol and archived medical records from birth were collected in 2003 when youth were in within the early adolescent age range. The wealth of data mentioned above provided the unique opportunity to examine several previously unexplored domains.

The current studies explore the concurrent and longitudinal associations between mental and physical health behaviours and a variety of measures of diurnal cortisol (i.e. morning level, CAR, linear slope, quadratic slope, and cubic slope). The first study expands on previous studies by dissecting the combined phenomenon of mental health symptoms to explore how internalizing and externalizing symptoms are differentially related to the diurnal rhythm of cortisol depending on whether the association is studied concurrently or longitudinally. The second study is a replication and extension of the first study as it follows the same children into mid-adolescence to explore whether hypocortisolism is related to later internalizing and externalizing behaviours. The third study expands to include physical health outcomes in association with cortisol. This study explores how rates of various

physical health problems typically seen in youth, more specifically infections, asthma and allergies, relate to various aspects of their diurnal cortisol profiles and whether chronicity of the problem plays a role in this association.

Disentangling psychobiological mechanisms underlying internalizing and externalizing behaviours in youth: Longitudinal and concurrent associations with cortisol

Paula L. Ruttle, Elizabeth A. Shirtcliff, Lisa A. Serbin, Dahlia Ben-Dat Fisher, Dale M. Stack, & Alex E. Schwartzman

Abstract

Research examining cortisol dysregulation is seemingly contradictory with studies showing that both internalizing and externalizing behaviours are related to high and low cortisol. One extant theory to explain divergent findings in the stress literature is that both hypo- and hyper-arousal of the hypothalamic-pituitary-adrenal (HPA) axis may be present depending on time since onset of the stressor. This theory may extend to the onset of internalizing and externalizing behaviours. Data from 96 youth participating in a longitudinal project were used to examine this possibility. Composite measures of internalizing and externalizing behaviours at both childhood and early adolescence were formed using mother and teacher reports. Multiple salivary cortisol samples were also collected over two consecutive days during early adolescence. Problematic behaviours were associated with cortisol and the direction of the association was dependent on amount of time passed since onset of the behaviours. When examined concurrently in adolescence, youth with more internalizing behaviours had *higher* morning cortisol; however, when examined longitudinally, youth with more internalizing behaviours in childhood had *lower* morning cortisol levels as adolescents. Youth with more externalizing behaviours in childhood had flattened diurnal cortisol rhythms as adolescents, and this finding persisted when examined in adolescence. Cortisol dysregulation was greatest in children with the most severe behaviour problems. Findings support the theoretical model of blunting of the HPA axis over time. While the HPA axis may show hyper-arousal when youth first display behaviours, long-

term exposure may lead to a hypo-arousal of the HPA axis which culminates in a dysregulated diurnal rhythm.

Disentangling psychobiological mechanisms underlying internalizing and externalizing behaviours in youth: Longitudinal and concurrent associations with cortisol

Behaviour problems are often debilitating with profound social, emotional, and psychological ramifications. Children who exhibit problematic behaviours often perform more poorly at school (Ansary & Luther, 2009; Aunola at al., 2000), are more socially rejected by their peers (Hymel et al., 1990; Pederson et al., 2007), have more strained relationships with their parents and siblings (Richmond & Stocker, 2006), and lower self-esteem (Aunola et al., 2000). Children's problem behaviours typically fall into two broad categories: internalizing problems (e.g. depression and anxiety) and externalizing problems (e.g. aggression and oppositional behaviours) and often these disorders are co-morbid (Angold et al., 1999). Given that children who demonstrate higher levels of internalizing and externalizing behaviours are at an increased risk of developing clinical level-disorders (Kroes et al., 2002; Mesman & Koot, 2002; Petty et al., 2008), it is critical to identify mechanisms associated with such behaviours before they develop into more severe disorders.

While there is little doubt that both internalizing and externalizing problems are etiologically heterogeneous, promising research in the area of psychobiology has helped provide a window into the onset and persistence of these types of behaviours. Sustained exposure to unhealthy levels of stress and/or an accumulation of daily hassles has been related to the development of various psychopathologies (Monroe & Hadjiyannakis, 2002; van Praag et al., 2004). Activity

of the hypothalamic-pituitary-adrenal (HPA) axis, frequently measured noninvasively via the stress hormone cortisol, is elicited by a variety of stressors (Dickerson and Kemeny, 2004). While a certain level of stress is healthy and necessary for an organism's survival, stress becomes unhealthy when it occurs for a prolonged period of time, at a high intensity, or involves the accumulation of many minor stressors (Sapolsky, 1992). Thus, dysregulated HPA axis activity may provide a window into the exposure to life stressors or accumulation of daily hassles over time and serve as a psychobiological mechanism whereby stressors manifest as a risk for the onset and persistence of mental health problems (van Praag et al., 2004).

In addition to its role as a hormone that helps individuals cope with a stressful situation, cortisol is also present in the body at resting levels, with basal activity modulated largely by different receptor subtypes than cortisol reactivity. Basal levels of cortisol are greatly influenced by the sleep/wake cycle and, as such, follow a diurnal rhythm. The diurnal pattern is characterized by a peak in cortisol levels approximately 30 minutes post-awakening which is followed by a decline throughout the course of the day (Ice et al., 2004; Kirschbaum & Hellhammer, 1994; Klimes-Dougan et al., 2001). A surge in morning cortisol followed by a steady decline helps initiate waking activities and primes the body for the demands of the pending day (Klimes-Dougan et al., 2001; Smyth et al., 1997). The morning peak appears to be biologically influenced while afternoon or evening levels are largely influenced by environmental and individual factors such as eating, sleeping and mood (Schreiber et al., 2006). The robust diurnal rhythm (and slight increases in response to a stressor) is thought to be a healthy cortisol pattern; in contrast,

extremely low morning or high evening cortisol may signify stress dysregulation (Shirtcliff and Essex, 2008) or a mismatch between an individual's biorhythm and social context (Stetler et al., 2004).

Both internalizing and externalizing behaviours have been linked to dysregulated cortisol levels in youth. The findings in the externalizing literature are somewhat mixed. While the majority of research suggests that externalizing problems in middle childhood and adolescence are associated with low levels of basal or diurnal cortisol, both concurrently (Fairchild et al., 2008; Kariyawasam et al., 2002; Moss et al., 1995; Pajer et al., 2001; Popma et al., 2007; Shirtcliff et al., 2005) and longitudinally (McBurnett et al., 2000) some studies suggest that there no association (Azar et al., 2004; Klimes-Dougan et al., 2001; van Bokhoven et al., 2005) or even a positive association (Fairchild et al., 2008; Gerra et al., 1997; McBurnett et al., 2005; van Bokhoven et al., 2005). However, it must be noted that the studies reporting positive or null associations typically measured externalizing/aggressive behaviour or cortisol in an alternative form than the one used in this study (e.g. induced aggression, reactive cortisol).

While the association between cortisol and externalizing behaviours appears to be somewhat consistent in older children and adolescents, the association between cortisol and internalizing and/or depressive behaviours has not been as consistent. In the concurrent literature some studies report that higher cortisol levels are associated with internalizing behaviours (Cicchetti and Rogosh, 2001; Kaufman, 1991; Perez-Edgar et al., 2008), while others suggest that lower cortisol levels are associated with internalizing behaviours (De Bellis et al., 1996; Granger et

al., 1998). The longitudinal literature is much clearer with nearly all of the longitudinal studies reporting that elevated basal cortisol levels precede the onset of later internalizing symptoms in children (Smider al., 2002) and depression in adolescents (Goodyer et al., 1996, 2000, 2001; Halligan et al., 2007). One exception is a longitudinal study that found high levels of anxiety to be related to high levels of cortisol secretion (Greaves-Lord et al., 2007*)*; however these divergent findings are most likely because they controlled for symptoms of depression. The adult depression literature shows a similar pattern with mostly divergent findings in the concurrent literature (Board et al., 1956; Gold et al., 1988; Peeters et al., 2004; Posener et al., 2004; Sachar et al., 1973) but a positive association longitudinally (Harris et al., 2000). Taken together, these studies suggest that the association between cortisol and internalizing problems may differ depending on the time lag between when cortisol is measured and the onset of depression. At least one prior study (Shirtcliff and Essex, 2008) illustrated that the association between cortisol and general mental health symptoms within the same sample differed depending on whether it was examined concurrently or longitudinally. Nevertheless, the authors point out that the developmental mechanism that links low concurrent cortisol, yet high cortisol over an extended period of time, with mental health is not yet fully understood. The present study will provide a unique contribution to the literature as it involves examining a different sequence: the occurrence of internalizing and externalizing behaviours before and then at the same time as diurnal cortisol.

One theory in the stress literature which may possibly extend to, and account for, the divergent findings in the internalizing and depression literature is that low

or declining basal cortisol levels may be the product of a "counter-regulatory" or "down regulation" response due to long-term exposure to excessive cortisol levels (Fries et al., 2005; Miller et al., 2007). The HPA axis is a regulatory system; therefore, it modulates the amount of cortisol released to achieve a favorable physiological state (Koob & Le Moal, 2001; McEwen, 2000; Sapolsky, 2003). Miller and colleagues (2007) suggest that recent exposure to a severe stressor may initially result in elevated cortisol, but that after extended exposure to severe stress, the HPA axis develops a counter-regulatory response whereby cortisol rebounds below normal (Koob & Le Moal, 2001; Miller et al., 2007). This theory is ground-breaking in the stress literature but has only linked stress, not behaviour problems in particular, to blunting of the HPA axis.

Although basic research such as this is essential for establishing theory and helping develop our general understanding of various phenomena, it is crucial to extend these ideas to applied research in order to explore how basic mechanisms function in real life settings. While it seems plausible that internalizing and externalizing behaviours may be implicated in this theory, it has yet to be sufficiently empirically investigated. Miller, Chen and Zhou (2007) conducted a meta-analysis which explored various factors that could potentially influence the HPA axis to produce either elevated or blunted levels of cortisol after experiencing an extreme stressor. They found a negative association between the amount of time since the onset of the stress and cortisol secretion (Miller et al., 2007). More specifically, individuals who had experienced a recent stressful situation had higher morning and overall average cortisol levels; however, individuals who had

experienced a stressor for a prolonged period of time had lower morning and overall average cortisol levels. Miller and colleagues suggest that the cross-sectional literature on cortisol and depression may only seem contradictory because the studies were assessing participants at different time frames from stressor onset. Since these results were not specific to children with internalizing behaviours, in an attempt to better explain ambiguous findings in the youth internalizing literature, it is worthwhile to explore this avenue that a similar mechanism may link behaviour-onset with HPA axis functioning. We propose that internalizing and externalizing behaviours may also be implicated in this theory as a developmental mechanism, either as an outcome of stress or acting as the stressors themselves.

The present paper was designed around the observation that the cross-sectional literature examining internalizing behaviours and cortisol provides mixed results, but there appears to be a consistent longitudinal pattern of elevated morning cortisol levels in individuals at risk for expressing later internalizing behaviours or depression. Following the theory put forth by Miller and colleagues (2007), we expected that *higher* cortisol would be associated with the development of new internalizing behaviours when examining cross-sectional findings. However, when internalizing behaviours have been present for a prolonged period of time we anticipated that high levels of internalizing behaviours would be related to *low* cortisol levels due to the subsequent down-regulation of the HPA axis and corresponding re-organization of the HPA axis set point in response to persistently elevated cortisol levels. Demonstrating both hypo- and hyper-arousal of the HPA axis within the same population over time provides a strong test of Miller and

colleagues (2007) model. Our focus was largely on internalizing behaviours because it had more bearing on the hypothesis by Miller and colleagues (2007), and because the literature findings are more ambiguous when examining internalizing rather than externalizing behaviours. Nevertheless, we felt it important to extend our analyses to include both externalizing and comorbid behaviours due to the high rates of comorbidity of internalizing and externalizing behaviours within youth (Angold et al., 1999). Studying the possibility of comorbidity in children and adolescents is particularly important because developmental trajectories are still in the process of being established and therefore, behaviours are likely to manifest as comorbid (Boyce and Ellis, 2005; Boyce et al., 2002). Similarly, we also determined if youth with the most behaviour problems had the greatest evidence for distinct diurnal cortisol patterns.

Method

Participants

The participants were part of the Concordia Longitudinal Risk Project, a three generation study. The project began in 1976 and examined a community-based sample of over 4000 French speaking children (and their families) in grade 1, 4, or 7 at public schools, serving economically disadvantaged neighbourhoods in Montreal, Quebec, Canada. For a complete description of the original sample population and procedures, see Ledingham (1981) and Schwartzman and colleagues (1985).

Current sample.

Participants were the offspring of original participants, were primarily Caucasian, and spoke French as their first language. The data were collected at two time points. For the purpose of this paper these will be referred to as "childhood" and "early adolescence". Youth and their legal guardians gave informed, written consent before participation and were reimbursed for their time and involvement in the study. Although 109 participants agreed to engage in the saliva sampling procedure, only those who were able to provide sufficient saliva to assay for cortisol were included in the present analyses (n=96). The 96 youth included 56 females and 40 males. During the childhood testing period, participants ranged in age from 6.3-10.8 yrs (M=7.7, SD=0.91) and during early adolescence they ranged from 9.3-13.5 yrs (M= 10.87; SD=0.90). The majority of participants were able to provide samples over two consecutive days (n=91) with an average of 8.43 samples per day. The data used in these analyses was collected as part of a larger study that was approved by the Institutional Review Board of Concordia University.

Measures

Written measures.

All of the measures used in the current study were written in French. Translated versions of English measures were used if original French versions were not available.

Children's internalizing and externalizing behaviours at childhood and early adolescence.

Both the mother- and teacher- report versions of the Child Behaviour Checklist (CBCL/TRF; Achenbach, 1991a, 1991b, respectively) were used to

measure children's internalizing and externalizing behaviours at childhood and early adolescence which was approximately three years apart. These sub-scales are normed for both age and sex, so children were rated in relation to same sex peers. Total scores were calculated for internalizing and externalizing subscales with scores above 60 indicating a clinical level of symptomology. When scores were only available for one informant (childhood: *n*=12; early adolescence: *n*=19), that score was used in analyses; however, when participants were missing both mother and teacher report forms (childhood: *n*=5; adolescence: *n*=0) missing values were mean replaced according to participant's sex and age. Although mean replacement is not ideal, it is biased towards a more normative score and thus would decrease the probability of obtaining significant findings (Engels & Diehr, 2003). During childhood 28.1% of participants (*n*=27) demonstrated clinical levels of behaviours (internalizing: *n*=9; externalizing: *n*=9; comorbid: *n*=9). During early adolescence 32.3% of youth (*n*=31) demonstrated clinical levels of behaviours (internalizing: *n*=20; externalizing: *n*=6; comorbid: *n*=5). Of the participants who, in childhood, scored above the clinical cut-off in a particular subscale, twelve youth remained above the clinical cut-off for the same subscale as adolescents and nine remained above a clinical cut-off in at least one subscale, but not the same one as in childhood.

In order to create a variable that best captured a child's internalizing behaviours, both the total scores from the mother's and the teacher's reports were examined in a factor analysis. This principle component analysis extracted the shared variability across informants separately from the informant bias following Kraemer and colleagues (2003). See Table 1 for more information. This strategy

was employed to produce a measure that more accurately reflects the child's behaviours and reduces informant bias. This PCA produced two factors: one measuring the trait of internalizing behaviours and one measuring rater differences. Only the factor measuring the trait of internalizing behaviours was used in subsequent analyses. The same process was used to create a variable that best captured the child's externalizing behaviours.

Salivary cortisol samples in early adolescence.

Participants were instructed to provide saliva samples over two consecutive school days at specified time periods: upon awakening, 20 minutes post-awakening and then every two hours until bedtime, while refraining from eating within the 30 minutes prior to sampling. Saliva samples were collected via salivettes. Youth were instructed to remove the cotton swab from a plastic vial, chew on it for 30-45 seconds until it was saturated with saliva, and then place it back in the vial, trying to touch it as little as possible. Saliva samples were kept frozen until they were assayed for cortisol at the Douglas Hospital Research Laboratories (DHRL). The detection limit of the assay was .01 μg/dL and the mean intra-assay and inter-assay variability coefficients were 4.0% and 4.6% respectively. In order to normalize cortisol scores, a log transformation was performed. Remaining outlying scores (n=9) were winsorized to within three standard deviations of the mean. In conjunction with the saliva sampling, participants completed daily diaries for each of the two days of sampling that recorded time of saliva sampling, mood, stress, health, food consumption and time of eating, exercise, time of awakening, and

bedtime. Medication intake (*n*= 14) was also recorded with over half of this subsample (*n*=8) taking methylphenidate (e.g. Ritalin, Concerta).

Analytical Strategy

Exploring comorbidity of internalizing and externalizing behaviours.

Internalizing and externalizing behaviours have a high rate of co-occurrence in children and adolescents (Angold et al., 1999) which can raise challenges when trying to understand the contribution of each symptom type. Although the comorbidity of behaviours is acknowledged, many studies have examined the association between cortisol and internalizing or externalizing disorders in childhood and adolescence individually but not comorbidly (Shirtcliff & Essex, 2008). In the present sample, internalizing and externalizing behaviours were correlated in both childhood (r=.60, p<.001) and in adolescence (r=.35, p<.001). In order to fully examine comorbidity, two factors were created. In order to obtain a measure of the total number of internalizing and externalizing behaviours, *Severity*, was created by obtaining the average of both of the factors of internalizing and externalizing behaviours. In order to determine whether there was a preponderance of externalizing over internalizing behaviours (or vice versa), a second measure, *Directionality,* was created by obtaining the half difference of the internalizing and externalizing scores. For the purpose of this study, higher scores on directionality indicate a preponderance of externalizing behaviours. (For a more complete review of this process see Essex et al., 2003, 2006 and Shirtcliff and Essex, 2008). Severity and directionality variables were calculated from the internalizing and externalizing factors for both time points.

Additionally, standardized residuals were computed to measure change in participants' behaviours over time (Llabre et al., 1991). Residuals were created for internalizing and externalizing behaviours as well as severity and directionality. Furthermore, quadratic functions of these variables were computed and examined to determine if children with more extreme behaviour problems had different diurnal cortisol trajectories.

Hierarchical linear modelling.

A three-level hierarchical linear model separated within-the-day (*n*=1578 samples) day-to-day (*n*=187 days), and between individual (*N*=96 participants) sources of cortisol variability (see Bryk & Raudenbush, 1992 for more information). Level 1 included Time Since Waking as a within the day predictor capturing the diurnal rhythm. The intercept thereafter reflects the level of cortisol upon awakening. Also, since the diurnal rhythm is steeper in the morning than afternoon, this model included quadratic and cubic functions of Time Since Waking to allow for the examination of curvature in the slope. Another variable representing Cortisol Awakening Response (CAR), was also included at level 1 as a dummy code (1=CAR, 0=not CAR), to distinguish the CAR from cortisol level or slope. All time-related variables were found to be significant in the model (See Table 2.). Additional Level 1 predictors were entered into the model to explore if mood, stress, health, food intake and exercise influenced cortisol levels; however, none of these variables had a significant effect on cortisol (See Table 2).

Level 2 captures the day-to-day variability in cortisol levels upon awakening and the diurnal slope. The model showed substantial variability in cortisol intercept

levels between the two days, χ^2 (91)=141.27, p<.001, as well as variability in slope from one day to another, χ^2(91)=135.09, p<.01. Youth reported whether they had taken medication on each day of cortisol assessment. Medication (coded as a yes/no for the day), was significantly related to cortisol slopes such that youth who were on medications had a less steep slope on the day they took medications (B=.016, t=3.071, p<.01).

Level 3 captures between individual variation, or the stability in cortisol levels across time. Cortisol intercept levels varied across individuals, χ^2(95)=227.47, p<.001 and the slopes varied linearly, χ^2(95)=156.98, p<.001, as well as quadratically, χ^2(95)=146.99, p<.001, suggesting that each individual had their own diurnal slope, and the degree to which the morning decline was steeper than the afternoon decline was different from one person to another. Predictors of variability between individuals were entered at Level 3. First, we entered the control variables of age and sex into the model to assess if these variables influenced cortisol. There was no effect of sex on either intercept or any of the slope variables. There was a significant effect of age on cortisol slope (B=.005, t=2.316, p<.05) such that older children had less steep slopes.

According to this base model, day-to-day fluctuations accounted for 10.0% of the total variance in the level of cortisol, within-the-day fluctuations accounted for 60.1% of the total variance, and between individual fluctuations accounted for 29.9% of the total variance in cortisol levels.

As described below, *Severity* and *Directionality* of behaviours were then examined as Level 3 predictors of cortisol levels and the diurnal rhythm. Next we

entered childhood and early adolescence measures of internalizing and externalizing behaviours and their residual scores in an attempt to clarify the severity and directionality findings; however due to brevity only the most pertinent of these results will be presented. After each set of analyses we examined quadratic functions of the aforementioned predictor variables to see if youth with more extreme behaviour problems had distinct cortisol profiles. Unless otherwise mentioned, all variables were entered independently to predict the overall cortisol intercept and various aspects of diurnal slope. Additionally, cortisol intercept will now be referred to as morning cortisol level.

Results

Longitudinal Findings: Behaviours in Childhood Predicting Morning Cortisol Levels in Early Adolescence

Upon examining total internalizing and externalizing behaviours in childhood (i.e. severity) as a predictor of cortisol in early adolescence, results suggest that youth with a greater number of behaviour problems in childhood developed lower levels of morning cortisol in adolescence, particularly when using the severity standardized residual which controlled for concurrent behaviour severity during adolescence (B=-0.044, t=-2.23, p<.03).

To determine if this finding was specific to one behaviour type, we next examined internalizing and externalizing behaviours separately. When using the standardized residual that controlled for internalizing behaviours in adolescence, youth with more internalizing behaviours in childhood developed *lower* morning cortisol levels by the time they reached adolescence (B=-0.042, t=-2.11, p<.04). In

contrast, there was no relation between externalizing behaviours in childhood and morning cortisol levels (B=-.035, t=-1.55, p<.20). These findings suggest that the association between severity of problem behaviour in childhood and the development of blunted morning cortisol levels by adolescence was driven largely by internalizing behaviours.

Directionality scores (in which higher scores indicate a preponderance of externalizing behaviours regardless of severity of behaviours) were not associated with any either morning levels of cortisol or diurnal cortisol slope, either longitudinally or concurrently and thus will not be mentioned in future subsections.

Concurrent Findings: Adolescent Behaviours Predicting Concurrent Morning Cortisol Levels

Severity of adolescent behaviours was not a significant predictor of adolescent morning cortisol. However, when controlling for internalizing behaviours in childhood, we found that youth who had more internalizing behaviours as adolescents had *higher* concurrent morning cortisol levels (B=0.039, t=1.95, p=.05). This relation did not exist with concurrent externalizing behaviours and morning cortisol in adolescence (B=0.013, t=0.46, p<.70). When coupled with the longitudinal association between internalizing and morning cortisol, these findings suggest that internalizing behaviours are influential in helping to shape morning cortisol levels in adolescence.

To help disentangle whether hypo- or hyper-arousal of the HPA axis was more robust, both internalizing behaviours at childhood and adolescence were entered simultaneously. Greater internalizing behaviours in childhood

longitudinally predicted the development of low morning cortisol by adolescence (*B*=-0.045, *t*=-2.103, *p*<.04), but greater concurrent internalizing behaviours in adolescence were associated with higher morning cortisol (at a trend level) (*B*=0.043, *t*=1.911, *p*<.06).

Longitudinal Findings: Behaviours in Childhood Predicting Cortisol Slope in Early Adolescence

When examining the *longitudinal* association between a greater number of total behaviours (i.e. severity) and amount of decline in cortisol secretion across the day, or *slope*, youth with a greater number of total behaviours in childhood developed flatter diurnal cortisol slopes by adolescence (*B*=0.006, *t*=2.437, *p*<.02). To determine if the predictions of the slope were driven by internalizing or externalizing behaviours, the model was re-examined with behaviour type separately tested. Youth with externalizing behaviours in childhood had a flatter diurnal cortisol slope by adolescence (*B*=0.006, *t*=2.62, *p*<.01); however, there was no relation between internalizing behaviours in childhood and diurnal cortisol slopes (*B*=.003, *t*=1.63, *p*<.20).

Concurrent Findings: Concurrent Behaviours Predicting Cortisol Slope in Early Adolescence

Although there was no association between concurrent severity of behaviours and cortisol slope in adolescence, upon examining internalizing and externalizing behaviours separately we found that the only significant associations were found with externalizing behaviours. Adolescents with more externalizing behaviours had flatter concurrent diurnal cortisol slopes (*B*=.005, *t*=2.209, *p*<.03)

which parallels the longitudinal association between externalizing behaviours and diurnal cortisol. When externalizing behaviours at both time points were entered into the model, externalizing behaviours at childhood predicted the amount of decline in cortisol across the day years later when the child was an adolescent (B=.005, t=2.02, p<.05), but externalizing behaviours at adolescence did not (B=.002, t=0.62, p<.60). This suggests that the relation between the slope of diurnal cortisol and externalizing behaviours may be primarily due to behaviours that were established earlier in childhood.

Associations between Cortisol and Behaviours after Including Control Variables

Next, we determined if associations of interest persisted after including control variables. Although there was a significant main effect of age, including age in the models did not significantly change the findings. For sex, there was no main effect on cortisol levels or slope and controlling for sex did not substantially change models.

After controlling for medications at Level 2, the significant association between morning cortisol levels and internalizing behaviours persisted (p<.05), although the association between severity of behaviours (which was primarily driven by internalizing behaviours) decreased to a trend level (p<.07). In contrast, the associations between slope of cortisol and externalizing behaviours diminished (ps>.16 to ps>.25), although there was still a trend for high externalizing behaviours in childhood to predict flatter slopes in adolescence, p<.08. While medications may have influenced the concurrent association between externalizing behaviour and

decline of cortisol across the day, the persistence of the longitudinal association between externalizing behaviour and cortisol slope (at a trend level) after controlling for medications suggests that this association was not entirely eliminated by the inclusion of medication in the model.

Longitudinal Associations between Cortisol and Behaviours when Examining Youth with the Most Extreme Behaviour Problems

Next, we wanted to determine if participants who had the most behaviour problems had distinct diurnal cortisol trajectories. Linear and quadratic functions of the predictor variables were entered into the model simultaneously which allowed for examination of HPA trajectories belonging to the participants with the most extreme behaviour problem scores. Given the large number of predictors, non-significant effects were removed through backwards elimination procedures to arrive at parsimonious models.

To determine if youth with the most internalizing problems in childhood had a distinct diurnal cortisol pattern in adolescence, the quadratic function of internalizing behaviour was examined. Adolescents with the most extreme internalizing behaviours had a trend for the lowest morning cortisol levels (B= -.085, t= -2.40, p<.02 for linear internalizing, B= 0.018, t= 1.93, p<.06 for quadratic internalizing). Compared to youth with average internalizing symptoms in childhood, youth with the most extreme internalizing behaviours had less steep diurnal slopes (B= .042, t= 2.45, p<.02 for linear internalizing, B= -0.013, t= -2.98, p<.004 for quadratic internalizing), as well as a greater curvature to their diurnal slope (B= -.008, t= -2.95, p<.005 for linear internalizing, B= .003, t= 3.64, p<.001 for

quadratic internalizing), and less of a cubic curve to their diurnal slope (*B*= .0003, *t*=3.25, *p*<.002 for linear internalizing, *B*= -.0001, *t*= -3.86, *p*<.001 for quadratic internalizing) which culminated in the flattest overall slopes for youth with the most internalizing behaviours in childhood (See Figure 1). Controlling for medication usage had no significant effect on the abovementioned associations between internalizing problems and diurnal cortisol profile.

To determine if youth with the most externalizing problems in childhood had a distinct diurnal cortisol pattern in adolescence, linear and quadratic functions of externalizing behaviour were examined and non-significant predictors were eliminated. The parsimonious model continued to find that adolescents with more externalizing behaviours in childhood had lower morning cortisol levels (*B*= -.055, *t*= -2.31, *p*<.02), but this was not more pronounced in the adolescents with the most extreme externalizing behaviours. Nonetheless, adolescents with the most extreme externalizing behaviours in childhood had less steep diurnal slopes at some points in the day (*B*= .051, *t*=3.09, *p*<.003 for linear externalizing, *B*= -0.016, *t*= -3.09, *p*<.003 for quadratic externalizing), greater quadratic curvature to their diurnal slope (*B*= -.007, *t*= -2.68, *p*<.009 for linear externalizing, *B*= .003, *t*= 2.90, *p*<.005 for quadratic externalizing), and less of a cubic curvature to their diurnal slope (*B*= .0001, *t*=2.61, *p*<.01 for linear externalizing, *B*= -.0003, *t*= -2.89, *p*<.004 for quadratic externalizing) which culminated in the flattest overall slopes for youth with the most extreme externalizing behaviours in childhood (See Figure 2). Contrary to the findings of the previous linear model where adding medication decreased the association between externalizing behaviour and diurnal cortisol slope, including

medication in the quadratic model had no significant effect on the abovementioned associations between externalizing problems and any aspect of the diurnal cortisol profile.

Similarly to the linear findings, there was no effect of the quadratic function of *Directionality* either concurrently or longitudinally.

Concurrent Associations between Cortisol and Behaviours when Examining Youth with the Most Extreme Behaviour Problems

To determine if youth with the most internalizing problems in adolescence had distinct concurrent diurnal cortisol patterns, the linear and quadratic functions of internalizing were examined. Quadratic effects of internalizing problems were not significant and did not emerge after eliminating all non-significant effects, culminating in the linear model as described above (see Figure 3). Adding medication to the model did not significantly decrease the significance of the association between internalizing and cortisol.

To determine if youth with the most externalizing problems in adolescence had a distinct concurrent diurnal cortisol pattern, linear and quadratic functions of externalizing behaviour were entered. A parsimonious model revealed a significant quadratic effect of externalizing on the linear cortisol slope (B= 0.022, t= 2.83, p<.006), quadratic cortisol slope (B= -0.003, t= -2.79, p<.007), and cubic cortisol slope (B= 0.000, t= 2.92, p<.004), such that youth with the most extreme behaviour problems had a distinct, flat diurnal rhythm (See Figure 4). Controlling for medication usage eliminated the significant quadratic effect of externalizing

behaviour on the linear slope (p<.12) and decreased the effects on the quadratic and cubic cortisol slopes to trend levels (p<.08 and p<.06, respectively).

Conclusions & Discussion

For many years, research has suggested the existence of altered HPA activity in the presence of internalizing and externalizing behaviours, although the directions of the effects have been inconsistent and the developmental mechanisms that alter HPA axis activity remained elusive. The present investigation revealed systematic longitudinal and concurrent associations between HPA activity and problematic behaviours which may be associated with the development of later mental health problems. Even within the same sample, evidence for both hypo- and hyper-arousal of HPA axis functioning was found. Comorbidity of internalizing and externalizing behaviours is often present in youth as was the case in this sample; therefore, we examined whether the total number of behaviours in general, or specifically a preponderance of one type of behaviour, influenced cortisol levels and the diurnal rhythm. The total number of behaviours was important, but closer inspection revealed that internalizing and externalizing behaviours predicted separate, but not necessarily opposing, components of the HPA axis. Internalizing behaviours were particularly influential for morning levels of cortisol whereas diurnal cortisol slope reflected the influence of externalizing behaviours. Interestingly, the stability and direction of these associations over time was different for internalizing behaviours depending on whether they had been present for an extended period of time or only appeared more recently. Furthermore, examination of the youth with the most behaviour problems (internalizing, externalizing, or combined) revealed that these

youth had distinct cortisol patterns, particularly the youth who had extreme levels of symptoms for a prolonged period of time.

For decades, research has linked internalizing behaviours and altered HPA functioning with varying success. The present study found that higher internalizing behaviours in children longitudinally predicted lower morning cortisol later when youth were adolescents. Yet, when internalizing and cortisol were measured concurrently in adolescence, an increased number of internalizing behaviours were expressed in adolescents with higher levels of morning cortisol. These seemingly opposing findings may be best understood through the theory proposed by Miller and colleagues (2007). Stress exposure may initially cause hypercortisolism, but after prolonged exposure to elevated levels of stress (and thus persistently elevated cortisol levels), the HPA axis down-regulates, resulting in a blunting effect or hypocortisolism. In their meta-analysis, Miller and colleagues illustrated that the findings from cross-sectional studies initially appeared divergent. Yet, separating the studies by time since onset of stress revealed that the majority of the studies focusing on recent and ongoing stress typically displayed higher levels of cortisol in stressed individuals, whereas individuals whose stress exposure began several years beforehand typically displayed lower cortisol levels. Our findings are in line with this notion such that more recent internalizing behaviours were related to a higher level of morning cortisol. This pattern was particularly evident after controlling for previous behaviours, a statistical practice which would essentially exclude individuals who had developed mental health problems earlier in childhood. Conversely, higher levels of internalizing behaviours in childhood (controlling for

the effects of current levels of internalizing behaviours in adolescence) were related to the development of lower cortisol levels approximately three years later when the children were adolescents.

These findings may fit into Miller and colleagues' theory of "down-regulation" through two possible psychobiological mechanisms. First, internalizing behaviours may, in and of themselves, be operating as a stressor. This may be evident especially for individuals who recently developed behaviours which may precede the onset of a subsequent disorder, events which are commonly included on subjective stressful life events checklists (Rudolph & Clark, 2001; Rudolph et al., 2006). The present study examined internalizing behaviours and not necessarily clinical diagnoses. Approximately 30% of our sample demonstrated clinical levels of behaviours, these youth and those within a more moderate range may interpret internalizing emotions as a stressor which may lead to the development of a variety of internalizing behaviours and subsequent dysregulated morning cortisol levels.

The second explanation is not mutually exclusive with the first. Individuals may experience an objective stressor which manages to dysregulate their HPA axis; then, this altered physiological state (combined with the stressful situation) induces feelings of depression, particularly if the stressor triggered mixed internalizing emotions (Rudolph et al., 2006). The result may include heightened internalizing behaviours and heightened HPA axis activity around the time of the stressor (Miller et al., 2007) or the first experience with heightened depression symptoms. Regardless of the original pathway, both environmental forces would be expected to lead to blunting of the HPA axis over time (Koob & Le Moal, 2001). In the present

study, internalizing, and not externalizing, behaviours mapped onto the blunting theory. These findings may help refine the model so that it applies primarily to internalizing symptomology. Alternatively, it is possible that this same mechanism occurs (or had already occurred) in youth with externalizing problems, but our study did not capture it. One possible explanation for this, discussed below, may be due to the typical developmental period when externalizing behaviours first present.

The presence of high cortisol followed by blunting over time in individuals with internalizing behaviours may be specific to morning cortisol levels for several reasons. Individuals with environmental or personality characteristics that predispose them to developing depressive symptoms have been shown to possess high levels of morning cortisol (Ockenfels et al., 1995; Polk et al., 2005; Portella et al., 2005; Pruessner et al., 2003; Schulz et al., 1998; Steptoe et al., 2005). Cortisol levels are at their peak in the morning part of the diurnal rhythm, and thus, occupy the most cortisol receptors at this time point, especially mineralocorticoid (MR) receptors. If individuals with predispositions for developing internalizing problems (e.g. high levels of stress) produced an excessive amount of morning cortisol, it could chronically exceed the capacity of the MR receptors in particular, potentially straining glucocorticoid receptor binding as well. Over time, elevated morning cortisol in youth experiencing stress may no longer be able to sustain hyper-arousal of the HPA axis. Our study provides some evidence that expression of internalizing behaviours leads to the development of more severe or persistent internalizing behaviours and the down-regulation of the HPA axis.

When examining the relation between cortisol *slope* and behaviour, youth with more externalizing behaviours had flattened diurnal cortisol rhythms as adolescents, regardless of whether they had expressed these behaviours as children or as adolescents. While the finding appeared stable over time, the longitudinal extension from childhood appeared slightly stronger than the concurrent findings. Although certain studies have not found this association (Azar et al., 2004; Klimes-Dougan et al., 2001; McBurnett et al., 2005; van Bokhoven et al., 2005), our findings are consistent with the majority of the literature which suggests that older children and adolescents with externalizing behaviours display lower basal or diurnal HPA activity both concurrently (Pajer et al., 2001; Shirtcliff et al., 2005; Snoek et al., 2004) and longitudinally (McBurnett et al., 2000). The pattern of low morning cortisol levels in youth with high externalizing behaviours was also present in our data, though the association was not significant. Our findings are also consistent with two previous studies (Fairchild et al., 2008; Popma et al., 2007) which found evidence of a flattened diurnal cortisol slope in some subtypes of aggressive or delinquent individuals; however, these studies only examined this association concurrently.

Initially, the model proposed by Miller and colleagues (2007) does not seem to apply to externalizing behaviours. However, it may be possible that blunting of cortisol over time is also applicable to youth with externalizing disorders (Susman, 2006). A meta-analysis by Alink and colleagues showed that externalizing problems are related to higher cortisol levels in very young children but not older children (Alink et al., 2008; Bakermans-Kranenburg et al., 2008; see also Kestler & Lewis,

2009). As alluded to above, externalizing behaviours often emerge very early in development (particularly compared to internalizing behaviours). Therefore, elevated levels of cortisol may only exist in children with externalizing problems when they are very young (Alink et al., 2008). This notion is supported by studies which have found an inverse or null association between cortisol and externalizing behaviours, depending on the stage of this psychobiological developmental process (Pérez-Edgar et al., 2008; Spinrad et al., 2009). In sum, it may be that children with externalizing behaviours experience blunting of the HPA axis earlier in development than those with internalizing behaviours due to the timing of behaviour onset. Given that our study examined processes in mid- to late- childhood and early adolescence, we would not have been able to assess initial HPA hyper-arousal because we did not study children early enough to capture the developmental stage in which hyperarousal first emerged.

Again, multiple psychobiological mechanisms may be operating at this earlier developmental point. A temperamental predisposition to aggression may act as a stressor which produces initially elevated levels of cortisol very early in development and consequently results in later externalizing behaviour problems which exposes a person to more and more stressors (Rudolph & Hammen, 1999; Rudolph et al., 2000). Alternatively, high levels of oppositional or defiant behaviours and activities may cause emotional distress due to the negative consequences of those behaviours. This emotional distress may initially produce elevated cortisol levels; however, further exposure to high levels of cortisol may then result in a blunted HPA system linked with the manifestation of externalizing

behaviour problems which is consistently seen in late childhood and throughout adolescence (Kariyawasam et al., 2002; McBurnett et al., 2000; Moss et al., 1995; Pajer et al., 2001; Shirtcliff et al., 2005). Excessive blunting of cortisol levels in externalizing youth, when developmentally evident for a longer period of time, may continue to exert its toll on the HPA axis. Further dysregulation may be demonstrated as lack of a typical diurnal decline over the course of the day. It is anticipated that this would especially be true for individuals who had evidence of HPA axis dysregulation for a longer period of time across early development (Susman, 2006). Although it still needs to be empirically validated, this perspective would serve as a good model to explain the preponderance of low cortisol levels typically associated with externalizing behaviours expressed persistently across child development and into adolescence (Caspi et al., 1995; Moffitt, 1990).

Further examination of the youth with the most extreme levels of behaviour problems revealed the existence of additional dysregulation of the HPA axis, such that the greatest dysregulation was present in youth who demonstrated the most extreme symptoms, particularly when they were younger. It must be noted that this is not simply an additive effect whereby youth who experience severe symptoms for an extended period of time demonstrate the same altered profile as youth who experience symptoms for a prolonged period of time, just to a greater degree. Rather, these youth demonstrate a distinct diurnal cortisol profile not demonstrated in the other participants with lower levels of symptoms. Youth with the most symptoms in childhood demonstrate discernible profiles in adolescence, identifiable by low morning levels, atypical diurnal curves throughout the day, and elevated

evening levels. Such a profile seems to be a product of an HPA axis that is not able to self-regulate in order to produce healthy levels of cortisol at nearly any point in the day. While the additional dysregulation may be a function of excessive exposure to cortisol which can occur at any point in the lifespan, it is possible that HPA dysregulation is a function of exposure to greater levels of behaviour problems in a developmentally time-sensitive period. Although a normal pattern of diurnal cortisol production is typically established within the first year of life (de Weerth et al., 2003), the HPA axis continues to develop into early childhood and may continue to be sensitive to external influences during the formative early years. Behaviours or stress during this period may have a substantially greater influence on the development of diurnal cortisol patterns thus producing dysregulation of the HPA axis in childhood not seen in later developmental time periods.

Secondary analyses revealed a significant effect of medication on the association between externalizing behaviour and cortisol levels. Although the initial association between externalizing behaviours and a less steep slope remained at trend level for the longitudinal association after controlling for medication, this effect disappeared when examining the relation concurrently with medication in the model. This may be because stimulant medications used to control externalizing behaviours can sometimes increase cortisol levels (Hibel et al., 2007; Kariyawasam et al., 2002). Since children typically take this medication in the morning, by including the effect of medication in the model it may have resulted in more elevated morning levels of cortisol which would then produce a steeper slope. However, although taking medication may have been associated with a less steep

cortisol slope when examining the larger sample, it had a limited association with the diurnal cortisol profiles of youth with more severe levels of behaviour problems. Use of medication only reduced the association between concurrent quadratic externalizing behaviour and linear diurnal cortisol slope in adolescence; however, it only reduced the quadratic and cubic slopes to a trend level. Furthermore, it had no significant effect on any longitudinal associations involving quadratic functions of behaviour problems. This suggests that internalizing behaviours in both childhood and adolescence and extreme levels of both types of behaviour problems in childhood have stronger influences on the diurnal cortisol profile in adolescence than the medications youth were taking in adolescence.

While this study provides an important starting point for future research to continue studying the possibility of blunting of the HPA axis in response to behaviour problems, it is not without its limitations. The design employed in the current study could have included additional waves of assessment to be ideal. Furthermore, researchers who wish to examine this association should employ a design that that involves at least two time points, one shortly after symptom onset and one after symptoms have been experienced for a prolonged period of time, at which both cortisol and behaviour problems are measured. Although examining this association in a community sample is important, studying this phenomenon in a larger, more at-risk population may help clarify ambiguities.

Further research needs to be conducted in younger children to establish whether or not blunting occurs in response to externalizing behaviours or if it is specific to internalizing behaviours. Furthermore, research is needed to determine

if blunting and additional dysregulation is due to cumulative exposure to symptoms regardless of developmental period or if it only occurs early on in development. Blunting of the HPA axis may have initially served as a protective mechanism for youth who would otherwise be subjected to chronically high cortisol levels; however, research suggests that atypical cortisol levels (e.g. too high or too low) are detrimental to the brain (Lupien et al., 2005). More research is required to determine the potential costs and/or benefits of hypo-regulation of the HPA axis (blunting) or hyper-regulation of the HPA axis as a function of behaviour problems, and their potential effects on future physical and psychological health. In summary, this study helped disentangle the complex association between cortisol and internalizing and externalizing behaviours. More specifically, it complimented basic research findings by adding empirical support to the theoretical notion of blunting of the HPA axis over time in relation to internalizing behaviours and added new findings to support the notion of lowered HPA activity in youth with externalizing behaviour.

Hypocortisolism in youth with extended exposure to elevated levels of internalizing and externalizing behaviour

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Abstract

Internalizing and externalizing behaviours have been related to both high and low levels of cortisol. Recent findings suggest that this may be due to how long the individual has experienced these behaviours, such that high levels of diurnal cortisol were evident if a shorter period of time had passed between behaviour onset and cortisol measurement whereas blunted levels of cortisol were evident if an extended period of time had passed. Results were specific to behaviours measured in childhood and adolescence therefore this study aims to extend these findings to examine behaviours in mid-adolescence. Data from 96 youth participating in a longitudinal project were used to examine this possibility. Composite measures of internalizing and externalizing behaviours at childhood, early adolescence, and mid-adolescence were formed using mother and teacher reports. Behaviours in mid-adolescence, controlling for previous behaviours, were examined in analyses. Multiple salivary cortisol samples were also collected over two consecutive days during early adolescence. As hypothesized, a blunted diurnal cortisol slope in early adolescence was associated with high levels of both internalizing and externalizing behaviours in mid-adolescence. When examining youth with the most severe behaviour problems, rather than displaying a more severely blunted diurnal cortisol slope, these youth displayed unique patterns of blunted cortisol. Findings support the theoretical model of HPA axis blunting whereby long-term exposure to mental health symptoms may lead to a hypo-arousal of the HPA axis.

Hypocortisolism in youth with extended exposure to elevated levels of internalizing and externalizing behaviour

Although altered levels of cortisol have been related to behaviour problems for several years (McEwen, 2000), there is controversy as to whether the association is positive or negative. Within the stress literature, the same debate was occurring and a possible explanation was proposed by Fries and colleagues (2005) who suggested that both hypo- and hyper-cortisolism may be present in an individual, but that the association depended on length of exposure to the stressor. A recent meta-analysis (Miller et al., 2007) divided stress studies according to time since onset of the stressor and found that studies containing individuals who had experienced a stressor for a short period of time were more likely to display elevated levels of cortisol whereas studies involving individuals who had experienced stressors for a prolonged period of time typically demonstrated decreased levels of cortisol. Additional support for this theory comes from an empirical study that found that children who were exposed to a moderate level of adversity displayed a higher cortisol awakening response whereas the children who experienced a high amount of adversity displayed cortisol levels similar to the children who had not been exposed to adversity (Gustafsson et al., 2010).

While these studies definitely contribute to a revolutionary way of thinking about stress, they do not empirically explore the association longitudinally nor do they consider the association between cortisol and mental health. Two recent studies have applied this theory to the longitudinal examination of behaviour problems and found that while initial experiences of behaviour problems may be

associated with hypercortisolism, prolonged exposure to problematic behaviours may be associated with hypocortisolism (Ruttle et al., 2011; Shirtcliff & Essex, 2008). Given that there has been only minimal research conducted on this topic, replication is needed. Furthermore, given that these two studies both examined youth in early adolescence, research needs to be conducted with individuals of different ages to determine if the same phenomenon occurs at other developmental periods. The current study uses the same sample as Ruttle and colleagues (2011) but follows the participants into mid-adolescence to examine the prospective development of subsequent behaviour problems. Following the theory put forth by Miller and colleagues (2007) and previous results (Ruttle et al., 2011; Shirtcliff & Essex, 2008), it was expected that a blunted cortisol pattern in early adolescence, as evidenced by low morning levels and/or a less steep decline in the diurnal cortisol pattern, would be related to high levels of internalizing and externalizing behaviours in mid-adolescence after controlling for previous levels of the behaviour.

2. Method

2.1. Participants

One hundred nine adolescents participating in the Concordia Longitudinal Risk Project were contacted and agreed to participate in the study. See Schwartzman and colleagues (1985) for a full description of the study. Exclusion criteria for this study included failure to fulfil the requirements of the saliva sampling procedure in early adolescence which resulted in a final sample of ninety-six adolescents (56 females; 40 males). Three waves of testing at approximately 3-year intervals, were used in this study: childhood, early adolescence, and mid-

adolescence. During the childhood testing period participants ranged from 6.3-10.8 yrs (*M*=7.70, *SD*=0.91), during the early adolescence testing period participants ranged from 9.3-13.5 yrs (*M*= 10.87; *SD*=0.90) and during the mid-adolescence testing period they ranged from 12.0-15.8 yrs (*M*= 13.46; *SD*=0.91). Behaviours across the three study periods were highly correlated (See Table 3). Internalizing and externalizing behaviours examined in this study are from mid-adolescence, controlling for behaviours at early adolescence and childhood. Study procedures were approved by the Concordia University Institutional Review Board and all participants and their parents provided informed consent.

2.2. Measures

2.2.1. Saliva sampling

During the early adolescence period (age 9-13 years), participants were asked to provide saliva samples upon waking, 20 minutes post-waking and then every two hours until bedtime. Participants were asked to refrain from eating 30 minutes prior to sampling as well as complete a daily diary recording time of waking and bedtime, time of eating and food consumption, exercise, mood, health, and medication intake. The majority of participants provided samples over two days (*n*=91) with an average of 8.43 samples per day. For a more detailed description of the saliva sampling procedure please see Study 1 of this dissertation.

2.3. Analytical Strategy

2.3.1. Cortisol

A log transformation was used to normalize positively skewed cortisol scores. Nine scores remained as outliers and were therefore brought down to within three standard deviations of the mean.

2.3.2. Exploring comorbidity of internalizing and externalizing behaviours.

Due to the high rate of behaviour problem comorbidity in general, as well as within our sample (See Table 3), it was important to examine this phenomenon in our sample. In order to explore comorbidity, two factors were created. The first factor, *Severity* provided an average of both internalizing and externalizing behaviours, and the second factor, *Directionality*, provided a measure of whether or not one behaviour, internalizing or externalizing, was more prevalent than the other. For the purpose of this study, a higher score of *Directionality* indicates a preponderance of externalizing behaviour. For a more detailed description of the calculations see Essex et al., 2003 and Shirtcliff & Essex, 2008).

Additionally, standardized residuals were computed to measure change in participants' behaviours over time (Llabre et al., 1991). Residuals were created for internalizing and externalizing behaviours as well as *Severity* and *Directionality* so that the resulting score was a measure of the respective behaviour in mid-adolescence, respective of the level of behaviour at childhood and early adolescence. Furthermore, quadratic functions of these variables were computed and examined to determine if children with more extreme behaviour problems had different diurnal cortisol trajectories. The resulting standardized residuals were used in all subsequent analyses.

Hierarchical linear modelling.

A three-level hierarchical linear model separated the variability in the cortisol data according to within-the-day, day-to-day, and between-individual sources of variability (see Bryk & Raudenbush, 1992 for more information). Given that cortisol is influenced by a number of variables such as time, food intake, etc., these measures were entered as Level 1 predictors. All time-related variables were found to be significant in the model and all other possible predictors had non-significant effects on cortisol (See Table 2).

Level 2 captures the day-to-day variability in cortisol levels upon awakening and the diurnal slope. Substantial variability between the two days was detected in cortisol intercept, χ^2 (91)=141.27, p<.001, and slope, χ^2(91)=135.09, p<.01. Medication intake was recorded on both days (coded as a yes/no for the day), and youth who were on medications had a less steep slope the day they took medications (B=.016, t=3.071, p<.01).

Level 3 captures how much cortisol levels vary between individuals across time. Cortisol levels varied by individual for intercept, χ^2(95)=227.47, p<.001, linear slope, χ^2(95)=156.98, p<.001, and quadratic slope, χ^2(95)=146.99, p<.001, suggesting that the starting point and pattern of decline was different for each individual. Predictors of variability between individuals were entered at Level 3. First control variables were entered. There was no main effect of sex or age in mid-adolescence on any level of cortisol. Following the control variables, all behaviour-related variables were entered separately to predict the cortisol intercept (which reflects the morning level of cortisol and will now be referred to as such) and the pattern of diurnal cortisol decline.

Results

Longitudinal Findings: Association between Cortisol in Early Adolescence and Behaviours in Mid-Adolescence

Upon examining the association between cortisol in early adolescence and total number of internalizing and externalizing behaviours in mid-adolescence (i.e. *Severity*), results suggest that youth with a less steep slope had a greater number of behaviour problems in early adolescence (B= 0.006, t= 2.37, p<.03) compared to adolescents with less problem behaviours. There was no significant effect of *Directionality* suggesting that when examining the association between type of behaviour problems and cortisol longitudinally, there was no impact of type of behaviour (i.e. internalizing or externalizing) on cortisol.

To further examine the findings related to *Severity* of problem behaviours, internalizing and externalizing behaviours were analyzed separately. Results revealed that individuals with a less steep diurnal cortisol slope in early adolescence were at an increased risk of having internalizing problems in mid-adolescence, regardless of what their behaviour profile was in childhood or early adolescence (B= 0.005, t= 2.11, p<.04). Furthermore, a less steep diurnal cortisol slope is also more likely in individuals who displayed high levels of externalizing behaviour in mid-adolescence regardless of their behaviour profile in early adolescence (B= 0.005, t= 2.15, p<.04). See Figures 5 and 6. These results suggest that individuals with blunted HPA axis activity in early adolescence may have higher levels of internalizing and/or externalizing behaviours later in development compared to those individuals with typical cortisol profiles.

Associations between Cortisol and Behaviours after Including Control Variables

Demographic variables were next included as in the model. Adding sex to the model did not influence cortisol levels or slope, nor did it significantly change the models. There was no significant main effect of age in mid-adolescence on any level of cortisol and adding age to the model did not significantly change any associations; however, there was a significant interaction effect of age X internalizing behaviours on the linear slope of cortisol (B=0.005, t=2.15, p=.05), which suggests that within the group of adolescents with low levels of internalizing symptoms, there was a trend for the younger participants to have a slightly steeper slope than the older participants, whereas in the group of adolescents with high levels of internalizing symptoms, the older participants tended to have a slightly steeper slope than the younger participants.

After controlling for medications at Level 2, the associations between severity of behaviour and slope of cortisol remained significant (p<.05); however, the associations between cortisol slope and youth's internalizing and externalizing behaviours diminished to non-significant associations (ps>.21 to ps>.24). Additionally, the interaction between age X internalizing behaviours diminished to a trend level (p<.10).

Longitudinal Associations between Cortisol and Behaviours when Examining Youth with the Most Extreme Behaviour Problems

Next, we aimed to determine if participants who had the most severe behaviour problems had distinct diurnal cortisol trajectories. Linear and quadratic

functions of the predictor variables were entered into the model simultaneously which allowed for examination of HPA trajectories belonging to the participants with the most extreme behaviour problem scores. Non-significant effects were removed through backwards elimination to produce more parsimonious models.

Upon examining youth with the most extreme internalizing behaviours in mid-adolescence, we found that individuals with the most internalizing symptoms had less of a cubic curve to their diurnal cortisol slope (B= -0.000, t= -2.38, p<.02). Youth with high levels of externalizing problems had a significant effect of externalizing on the linear cortisol slope (B= .010, t= 3.47, p<.001), and the quadratic slope of cortisol (B= -.0006, t= -2.50, p<.02) which culminated in flattened diurnal cortisol slopes for youth with the most externalizing behaviours in mid-adolescence. All findings remained significant after including medication in the abovementioned models examining the children with the most extreme behaviours (ps<.04).

Discussion and Conclusions

This study replicates and extends the previous work on the blunting of the HPA axis as it relates to behaviour problems (Ruttle et al., 2011; Shirtcliff & Essex, 2008). We demonstrated that there is a negative association between HPA activity and behaviour problems when this association is examined longitudinally. More specifically this study revealed that less of a decline in the typical diurnal pattern of cortisol in early adolescence is associated with higher levels of internalizing behaviour in mid-adolescence and this pattern is the same for externalizing behaviours. Furthermore, upon specific examination of the children with the most

severe behaviour problems, we found that they displayed unique diurnal cortisol profiles. Medication was a significant predictor of the slope of cortisol and, as such, when added to the model, it reduced the significant effect of internalizing and externalizing behaviours on the cortisol slope. However, including medication in the model which examined youth with combined behaviour problems as well as in the model examining youth with the most severe behaviour problems did not change significant findings, which speaks to the robust association between extreme or multiple behaviour problems and HPA axis dysregulation.

In combination with our previous findings, these results add support to the hypothesis that prolonged exposure to experiences of behaviour problems may result in hypocortisolism (Fries et al., 2005; Miller et al., 2007; Pervanidou, 2008). This phenomenon has only recently been empirically studied however findings are consistent with others suggesting that youth who experience symptoms of mental health problems (Shirtcliff & Essex, 2008) or are exposed to adverse circumstances (Gustafsson et al., 2010) may initially experience elevated levels of cortisol but after extended exposure they demonstrate hypocortisolism. Although this theory seems to explain the phenomenon of HPA axis blunting, the sequence of events is not entirely clear and it is likely that both behaviour and cortisol work in a bi-directional manner to exacerbate the other rather than a unidirectional association with symptoms causing behaviour.

For many years, research has suggested the existence of altered HPA activity in the presence of internalizing and externalizing behaviours, however the exact processes behind this association remained elusive. Future research is needed to

determine if blunting is more severe if exposure to stressors occurs at an earlier age versus a later age. Furthermore, longitudinal studies should explore the possibility of altering hypocortisolism via intervention techniques tailored to address the problem and explore the role of hypocortisolism in treatment effectiveness and long-term prognosis. In conclusion, research examining hypocortisolism should consider how long the problem has existed in order to truly comprehend this phenomenon.

Associations between physical health outcomes and diurnal cortisol in early adolescence

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Abstract

Youth experience different physical health problems than adults, both in terms of acute and chronic health problems. Two chronic conditions, asthma and allergies, have been linked to atypical diurnal cortisol patterns but the research is inconsistent. Very little research has examined the association between cortisol and more acute health problems in youth such as infections. This project aims to link the aforementioned health problems to hyper- or hypocortisolism depending on the chronicity of the problem. Data from 96 early adolescents participating in a longitudinal project were used to examine this possibility. Participants provided multiple salivary cortisol samples from awakening until bedtime over the course of two consecutive days during early adolescence. Medical records were drawn from government databases (from a universal single-payer healthcare system) indicating how frequently these individuals presented at healthcare facilities for infections and allergies from infancy to early adolescence, as well as the presence or absence of asthma. Results showed that individuals with high rates of infections experienced higher levels of awakening cortisol. In contrast, individuals who had increased healthcare visits for allergies from infancy through early adolescence had blunted diurnal cortisol slopes across the day as did individuals with asthma. These associations were moderated by age, sex and socio-economic status. Findings support the theoretical model of HPA axis down-regulation with more transient stressors resulting in elevated levels of cortisol but more chronic exposure to health problems resulting in blunted adrenocortical activity across the day.

Associations between physical health and diurnal cortisol in early adolescence

Health problems observed in youth generally tend to be different than those observed in adults. Children generally receive more medical services for asthma, allergies and infections compared to older individuals (Monto & Ullman, 1974; Waters, Davis, Nicolas, Wake & Lo, 2008). Asthma is a disease in which an increased immune sensitivity to typically non-harmful irritants causes the tissues of the airway to become inflamed and this subsequently impairs breathing. Allergies are also characterized by increased sensitivity of the immune system to irritants which results in the stimulation of the body's defence mechanisms. These two health conditions are often studied together and, although the exact aetiology of asthma and allergies is unknown, it is expected that they are multifactorial, involving both environmental and genetic factors as well as interactions between the two (Daruna, 2004). Furthermore, both asthma and allergies have common trigger irritants such as pollen, dust, and mold. Rates of both diseases have seen drastic increases in Western societies in the past few decades (Ring, Kramer, Schafer, & Behrendt, 2001), with prevalence rates in youth ranging from 17%-23% for asthma and 9-14% for allergies.

A variety of infections including the common cold, influenza, pneumonia, and ear infections are also fairly common in youth. Children under five years of age typically average three to six episodes of acute respiratory illness each year, regardless of their social and economic situation (Monto & Ullman, 1974). In contrast to asthma and allergies, rates of infection have seen a dramatic decline due to increased sanitary conditions and medical advancements (Braun-Fahrlander,

Riedler, Herz, Eder, Waser, Grize, et al., 2002; Check, 2004; Ponsonby, Couper, Dwyer, Carmichael, & Kemp, 1999). Although several factors have been identified in the development of the aforementioned physical health problems in youth, including genetic predisposition and alterations in the immune system, stress has also been associated with all three types of health problems.

Stress is suspected to play a role in the development and persistence of several immune-based diseases (McEwen & Stellar, 1993), including infections (Shephard & Shek, 1994; Sheridan, Dobbs, Brown & Zwilling, 1994), asthma, and allergies (Koh & Hong, 1993; Michel, 1994; Parker, 1991). While there is a clear association between stress and the prevalence or exacerbation of these health problems, what remains unclear are the physiological correlates associated with these problems. Physiological response systems associated with stress may help to explain the processes whereby stress impacts physical health. While some research to date has supported the role of stress and its physiological correlates in the development of asthma, allergies, and infections in specialized clinical subsamples (Ball, Anderson, Minto, & Halonen, 2006; Buske-Kirschbaum, Jobst, & Hellhammer, 1998; Fujitaka, Nomura, Sakura, Ueda, Matuura, & Yumiba, 2000; Kallenbach, Panz, Girson, Joffe, & Seftel, 1998; Landstra, Postma, Boezen, & van Aalderen, 2002; Tromp, 1968), very little research has attempted to examine this association in a representative population sample. Studying these associations could provide much needed insight into patterns of childhood morbidity in the general population.

The hypothalamic pituitary adrenal (HPA) axis is one of the primary systems activated during the stress response. When a situation is perceived to be stressful,

the hypothalamus releases corticotrophin-releasing hormone (CRH), which then triggers the pituitary to secrete adrenocorticotropin hormone (ACTH). ACTH travels through the bloodstream to reach the adrenal gland and stimulates the production of the glucocorticoid hormone, cortisol (Sapolsky, 1994). Cortisol is a steroid released by the adrenal cortex and, during times of stress, its role is to help the body cope with the stressor by mobilizing resources such as focusing attention, regulating metabolism and blood pressure, and inhibiting the inflammatory response of the immune system (Buckingham, 2010), thereby reducing the body's ability to fight infections during times of stress when levels of cortisol are high in the body. In addition to being released in response to a stressor, cortisol is also present in the body at rest and it follows a diurnal pattern. Levels peak approximately 20-40 minutes post-awakening (more commonly known as the cortisol awakening response; CAR) followed by a decline over the course of the day, steeper at first and then more gradual until bedtime (Ice, Katz-Stein, Himes, & Kane, 2004; Kirschbaum & Hellhammer, 1994; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001). Experiencing moderate or extreme levels of stress can influence the diurnal cortisol profile and altered diurnal cortisol patterns have been linked to both mental (Adam, Doane, Zinbarg, Mineka, Craske & Griffith, 2010; Goodyer, Hebert, Tamplin, & Altham, 2000; Halligan, Herbert, Goodyer, & Murray, 2007; Harris, Borsanyi, Messari, Stanford, Cleary, Shiers, et al., 2000) and physical health problems including diabetes and arthritis (Bruehl, Wolf & Convit, 2009; Chrousos & Gold, 1992).

Cortisol and infections. The association between cortisol and prevalence rates of infections in the general population is an understudied phenomenon; however results from one study suggest there is a positive association between basal and reactive cortisol levels and the incidence of infections in infants: that is, elevated cortisol appears to suppress the immune system's overall ability to cope with infections (Wilson et al., 2003).

Although there is little empirical research on this topic in the medical literature, there is strong basic research to support the existence of this association. A bi-directional association exists between cortisol and the immune system such that high levels of activity in one system exert an effect on the other system. Although high levels of immune system activation generally stimulate increased HPA axis activity, the HPA axis acts differentially on various part of the immune system, increasing activity in some portions and suppressing activity in others (Elenkov & Chrousos, 1999). While the HPA axis influences the immune system in a very complex manner, it seems likely that high levels of stress reduce immunity to various infectious agents responsible for respiratory infections (Cohen, Tyrrell & Smith, 1991; Elenkov & Chrousos, 1999). Although empirical evidence is lacking, a more holistic picture implies that a positive association between children's infections and cortisol may be expected.

Cortisol and allergies. The literature examining the association between cortisol and allergies is also quite sparse. Generally speaking, research examining the association between cortisol and allergies also includes individuals with asthma. One study found that infants at risk for developing allergies and asthma because

their mother had allergies had lower morning levels of cortisol in combination with a greater cortisol response to a stressor (Ball, Anderson, Minto, & Halonen, 2006). In other words, infants at risk for atopic disorders displayed both high levels of stress reactivity and also a lowered (blunted) diurnal rhythm of cortisol secretion. One of the few studies directly examining atopic disorders and diurnal cortisol sampled cortisol four times across a single waking day and found no difference in diurnal cortisol patterns between adolescents with atopic disorders and healthy controls (Wamboldt, Laudenslauger, Wamboldt, Kelsay & Hewitt, 2003). Additional research has been conducted on eczema, a chronic inflammatory skin disease. Eczema is often considered under the classification of allergies as frequently individuals will experience an allergic reaction in the form of eczema. While some studies find that individuals with eczema also display low levels of diurnal cortisol (Heubeck, Schonberger, Hornstein, 1988; Lonne-Rahm, Rickberg, El-Nour, Marin, Azmitia, Nordlind, 2008), others find no such association (Rupprecht, Hornstein, Schluter, Schafers, Koch, Beck, & Rupprecht, 1995). Although the limited empirical research to date does not identify clear patterns of association between these chronic illnesses and cortisol levels, given that both allergies and asthma involve a hypersensitive immune system, one would expect that both health conditions would have similar, although possibly not exact, underlying physiology.

Cortisol and asthma. There is a much more extensive amount of research examining the association between cortisol and asthma. Given that cortisol provides anti-inflammatory signals to immune cells (Sapolsky, Romero, & Munch, 2000), low levels of cortisol would be related to the increased inflammation and

immune response typically seen in individuals with asthma; however, this finding is not consistent within the asthma literature. Some studies have found the expected negative association between cortisol levels and asthma. For example, Kauffmann and colleagues (1999) found that individuals with asthma had lower levels of basal cortisol than individuals without asthma. Similarly, Landstra and colleagues (2002) collected cortisol from children at six different time points throughout the day and found that children with asthma had lower mean levels of cortisol when compared to non-asthmatic age-matched controls. Additionally, a comprehensive study by Tromp (1968) followed patients for a 24-hour period once each week for one month and found that patients with asthma had lower cortisol levels than controls. While the majority of studies linked asthma to low levels of cortisol, results are not as clear in other studies. One study comparing 118 individuals with asthma to healthy controls found no difference in morning versus afternoon levels of cortisol in individuals with moderate asthma but found higher afternoon compared to morning levels of cortisol in individuals with severe asthma (Fujitaka et al., 2000). Results from Buske-Kirschbaum and colleagues (2003) also show great variation by day when examining the diurnal pattern of cortisol over 3 days in adolescents with asthma. Compared to controls, youth with asthma displayed higher levels of cortisol on some days and other days there was no difference. Although the majority of literature examining reactive cortisol in individuals with asthma and/or allergies finds a negative association between reactive cortisol and the presence of asthma/allergies (Buske-Kirschbaum, von Auer, Krieger, Weis, Rauh & Hellhammer, 2003; Kallenbach et al., 1998) or atopic disorders in general (Wamboldt et al.,

2003), one study found that infants at risk of developing atopic disorders had higher levels of reactive cortisol (Ball et al., 2006). Although these differences may seem contradictory, one theory used to account for differences observed in the association between cortisol and stress may be useful in helping interpret these findings.

Stress researchers have proposed that elevated and blunted levels of cortisol may be associated with length of exposure to the stressor (Heim et al., 2000; Fries et al., 2005). More specifically, while transient stressors seem to be associated with increased cortisol, prolonged exposure to stressful situations may actually result in lower levels of cortisol. That is, extreme levels of prolonged stress may result in blunted HPA axis activity, expressed by low levels of cortisol in the morning due to an inability to express the morning peek and/or high levels in the evening due to an inability to decline (Heim, Ehlert, & Hellhammer, 2000; Fries et al., 2005). This down-regulation process has been demonstrated in research examining the association between cortisol and prolonged symptoms of mental health problems (Shirtcliff & Essex, 2008), and more specifically, prolonged internalizing problems (Ruttle, Shirtcliff, Serbin, Ben-Dat Fisher, Stack, & Schwartzman, 2011). This finding can be applied to the current study in two ways. First, infections are a transient stressor. When the individual experiences an infection, it acts as a stressor on the body for a limited period of time until the infection is cleared. However, when an individual has either allergies or asthma, these diseases are in the individual's system and are constantly reacting, albeit most frequently not in a severe manner, to mild irritants. This constant influence, combined with more severe attacks, takes

a toll on the body in a manner similar to allostatic load and would this be expected to be associated with blunted levels of cortisol. Furthermore, this theory may be used to clarify the divergent findings in the asthma literature such that while individuals who are at risk for developing or have only recently developed asthma and allergies may initially display increased levels of cortisol, these levels may become blunted once the condition persists. This seems plausible given the results from a study conducted by Nomura and colleagues (1998) which support this notion. They found that during an asthma attack, children's cortisol levels increased and typically decreased to the levels of healthy controls after a 24-hour period, however, children with prolonged attacks lasting more than 5 days had significantly lower cortisol levels (Nomura, Fujitaka, Sakura, & Ueda, 1998).

In summary, although transient stressors or the initial experience of extreme levels of stress may result in increased levels of cortisol, prolonged or chronic exposure to high levels of stress may result in the down-regulation of the HPA axis resulting in an atypical diurnal cortisol pattern as demonstrated by lower morning levels, higher evening levels, and/or lack of a typical diurnal curve. Although this theory is empirically supported, given that not all individuals who experience high levels of stress or chronic health problems will develop hypocortisolism, one must explore individual differences that may predispose certain individuals to develop a blunted cortisol response (Boyce & Ellis, 2005; Boyce, Essex, Woodward, Measelle, Ablow, Kupfer, 2002; Shirtcliff & Essex, 2008).

Factors Influencing the Association between Cortisol and Physical Health Outcomes

There is wide consensus in the adult literature that socioeconomic status (SES) has a profound impact on health (see Adler, Boyce, Chesney, Folkman, & Syme, 1993; Adler, Boyce, Chesney, Cohen, Folkman, Kahn et al., 1994; Anderson & Armstead, 1995, for reviews). Reviews examining this association in children and adolescents have also found that living in an environment characterized by low SES is also related to poor health (for reviews see Aber, Bennett, Conley, & Li, 1997; Jolly, Nolan, Moller & Vimpani, 1991; Shah, Kahan & Krauser, 1987); however the association between SES and general health in children is not nearly as robust as that found in adults (see Chen, Matthews, & Boyce, 2002 for a review). Additionally, research typically finds a strong association between SES and dysregulated levels of cortisol; however low SES has been linked to high levels of cortisol, low levels of cortisol, and occasionally, no association was detected (for a review see Dowd, Simanek, & Aiello, 2009).

In addition to SES, several other variables may impact physical health, cortisol, or the association between these two variables. Individual characteristics, such as sex and age, have been associated with various aspects of health and cortisol. For example, in youth, asthma is typically more prevalent in males and tends to decrease in severity with age, particularly in males (Bjornson & Mitchell, 2000; Mccallister & Mastronarde, 2008; Waters et al., 2008). Also, there are various inconsistencies in the literature as to the association between sex and HPA function (Gunnar & Vasquez, 2006; Spinrad, Eisenberg, Granger, Eggum, Sallquist, Haugen et al., 2009). Both age and sex will be examined as possible moderators. Additionally, we felt it was important to control for mother's own healthcare seeking behaviours

as most children and adolescents receive healthcare services because services are sought by their mother (Broadhurst, 2003; Logan & King, 2001).

The current investigation aims to explore the understudied association between cortisol and common health problems in a group of early adolescents from a representative lower income community sample. More specifically, research examining the association between cortisol and infections in typically developing youth is significantly lacking. We hypothesize that overall, participants who had more infections across their lifespan would have higher cortisol levels due to the acute nature of infections, with the duration of typical infections lasting a few days before treatment is sought and the problem remediated. An additional purpose of the investigation was to help clarify the literature examining the association between cortisol and asthma and allergies within a non-clinical sample. We hypothesize that overall participants with asthma and allergies would display a blunted diurnal cortisol pattern due to the more chronic nature of these problems. We examined the association between cortisol and these two atopic disorders separately as we aimed to determine if asthma and allergies have different, albeit presumably slightly different, associations with cortisol. Additionally, we aimed to explore the role of variables that may potentially mediate or moderate the associations between the aforementioned physical health outcomes and cortisol, including SES, age and sex.

Method

Participants

The participants were part of a longitudinal intergenerational study. Beginning in 1976, the Concordia Longitudinal Risk Project examined a community-based sample of over 4000 French-speaking school children (and their families) in grade 1, 4, or 7. The children were recruited from schools serving economically disadvantaged neighbourhoods in Montreal, Quebec, Canada. A subset of these individuals were selected for a more in depth follow-up, including some individuals who displayed high levels of behavioural problems and a control group of typically developing individuals. For a complete description of the original sample population and procedures, see Ledingham (1981) and Schwartzman and colleagues (1985).

Current sample. The participants examined in subsequent analyses were the offspring of original participants, primarily Caucasian, and spoke French as their first language. One hundred-twenty children were eligible for participation based on the appropriate age range (9-13 years of age) but only 109 adolescents agreed to participate in the saliva sampling procedure. Of which only 96 participants were able to successfully meet sampling requirements (e.g. sufficient saliva, daily diary, etc.) and included in the present analyses. Based on original selection criteria (i.e. low SES neighbourhoods and increased sampling for behaviour problems), the current sample can be considered an at-risk, community sample.

Cortisol and demographic data were collected between 2003 and 2007, when children ranged in age from 9.3-13.5 years (M= 10.87; SD=0.90). Testing sessions were arranged according to age, such that older children were tested first. The health variables were drawn on from the provincial healthcare database (see below

for more information) on December 31, 2003 when children ranged in age from 7.35 to 13.5 years (*M*= 10.32; *SD*= 1.65). Youth and their legal guardians gave informed, written consent before participation and were reimbursed for their time and involvement in the study. Although 109 participants agreed to engage in the saliva sampling procedure, only those who were able to provide sufficient saliva to assay for cortisol were included in the present analyses. The remaining 96 individuals included 56 females and 40 males. The majority of participants were able to provide samples over two days (*n*=91) with an average of 8.43 samples per day. The data used in these analyses was collected as part of a larger study that was approved by the Institutional Review Board of Concordia University.

Measures

Cortisol. During early adolescence, participants were instructed to provide saliva samples over two consecutive days upon awakening, 20 minutes post-awakening and then every two hours until bedtime. Salivettes were used to collect saliva samples. Participants were instructed to chew on a cotton swab for 30-45 seconds and then place the swab in a plastic container, trying to refrain from touching it. Participants were also asked to refrain from eating for 30 minutes before sampling as well as to keep a record of the time of saliva sampling, mood, stress, health, food and medication intake, any exercise performed, as well as time of eating, time of awakening, and bedtime for both days. Participants were instructed to freeze saliva samples until they were picked up by researchers and samples were kept frozen until brought for assay at the Douglas Hospital Research Laboratories (DHRL). The detection limit of the assay was .01 μg/dL and the mean intra-assay

and inter-assay variability coefficients were 4.0% and 4.6% respectively. Cortisol data was skewed so log transformations were performed to normalize the distribution. Nine scores remained as outliers after this process and were brought down to within three standard deviations of the mean.

Physical health. Self-reports of health are typically the most common way of measuring physical health; however, these assessments are subject to negative affectivity, social desirability, and recall biases (e.g. Brett, Brief, Burke, George, & Webster, 1990; Edwards, 1953; Parkes, 1990; Weinhardt, Forsyth, Carey, Jaworski, & Durant, 1998). Less commonly used are medical records of visits to health professionals because of inaccessibility due to a variety of reasons including privacy or convenience. Using such records would eliminate the potential biases associated with self reports and may provide a more accurate picture of an individual's physical health and health seeking behaviours.

Due to the sensitive nature of healthcare data, permission was required from the "Commission d'accès à l'information du Québec" in order to obtain access to participants' healthcare service usage records. Following a careful review of identity and privacy protecting procedures to ensure confidentiality, permission was granted to retrieve service usage information. Provincial medical records regarding service usage from infancy until 2003 were obtained from the Régie de l'Assurance-Maladie du Québec (RAMQ) and the "Ministère de la Santé et des Services Sociaux" (MSSS), which act as a "single-payer" system and cover the costs incurred by medical services for all permanent residents of the province of Québec. These records contained numeric codes pertaining to various medical acts,

assessments and diagnostic information. Although there was a wealth of information available, three predictor variables were chosen due to increased prevalence rates in childhood: (1) Total number of medical acts performed due to infections, (2) Total number of medical acts due to asthma and (3) Total number of medical acts performed due to allergies. Please see Appendix A for a list of health care codes considered in each category. All variables were skewed and required transformations in order to normalize the distributions. Neither square root nor log transformations were successful in normalizing the data therefore participants were grouped into categories depending on the number of times he or she had experienced a given health problem. Given that on average children have 3-6 infections per year before age 5 (Mono & Ullman, 1974), children who had received a score under 10 visits to a healthcare facility over the course of their lifetime were deemed to have too infrequent of visits, presumably not due to lack of infections but rather due to lack of access to healthcare providers. Coding for this variable is as follows: too few visits for infections (0-9), average visits for infections (10-28), above average visits for infections (29-46), and many visits for infections (47+). "Total number of medical acts due to asthma" was divided into a "yes/no" dichotomous variable based on the presence or absence of asthma, and "Total number of medical acts due to allergies" was divided into the following categories based on how frequently the individual received medical services for their allergies: none (0), some (1-2), and many (3+).

Mother's health and healthcare seeking behaviour. Healthcare usage reflects both actual health needs (e.g. frequency and severity of illnesses) and

patterns of care usage (i.e. likelihood of consulting a physician when ill or for preventive care). Parents' and their children's health are likely to be related statistically, in the sense that aspects of health are inherited genetically and also because parents can impact children's health via shared environment, nutritional patterns, health-related behaviours (such as patterns of hand washing), convenience and availability of care, and many other factors. In addition, parent's patterns of seeking healthcare are likely to carry over to their children, given that youth generally receive medical attention because their primary caregiver brings them (Broadhurst, 2003; Logan & King, 2001). For these reasons we felt it was important to control for mothers' pattern of healthcare usage over time. To do this, the average number of times that the mother had seen the doctor in the three years before her child was conceived, minus all obstetric and gynecological visits, was computed and controlled in all analyses examining the relation between children's health variables and cortisol patterns.

Socioeconomic status (SES). Various measures of socio-economic status have been used when examining the association between SES and health including individual or family income (McDonough, Duncan, William, & Hauser, 1997), education (Ross & Wu, 1995), average income level of the neighbourhood (Hou & Myles, 2005), and occupation or ratings of occupational prestige (Marmot, Shipley, & Rose, 1984). Although mother's years of education, family income, and level of neighbourhood poverty have all been shown to independently predict youth's health outcomes (for a review see Chen et al., 2002), a factor was created with these variables via principal components analysis to provide a more comprehensive

measure of the phenomenon. Factor loadings for these variables were in the moderate to high range (0.52-0.73) and had 44% variability.

Procedure

Participants were contacted by telephone between 2003 and 2007 to request their participation in the current wave of the study. Those interested in participating affirmed consent at this time and a home visit was scheduled. As part of a larger study, research assistants visited homes during which time parents were asked to fill out a demographics questionnaire and participants were given instructions on the saliva collection procedure. Both mother and child were financially compensated with a small honorarium once the questionnaires and vials were returned. Information pertaining to the participant's postal code, educational attainment and income was requested in the questionnaire package. Using the first three letters and digits of the postal code, census track data on the participants' neighbourhoods were retrieved and a variable measuring quality of neighbourhood was created and used in subsequent analyses. Finally, medical data was collected from the RAMQ database.

Design and analysis: Hierarchical linear modelling. A three-level hierarchical linear model was created using HLM 6.0 (Bryk & Raudenbush, 1992) to examine various sources of variability within diurnal cortisol: within-the-day (*n*=1578 samples), day-to-day (*n*=187 days), and between individual (*N*=96 participants). The default of HLM provides users with an intercept and slope coefficient which, in the current study, represented the level of cortisol at awakening and the amount of linear decline over the course of the day; however, several additional predictors

were added to the model in order to best capture within-the-day cortisol variability. As previously mentioned, a healthy diurnal cortisol pattern is depicted by high levels of cortisol in the morning, a peak in cortisol approximately 20 minutes post-awakening, and the a decline over the course of the day. The decline tends to be steeper in the morning hours and then more gradual in the afternoon. In order to best model the peak after waking, samples occurring approximately 20 minutes post-awakening were identified as the CAR using a dummy variable (coded 0= not CAR and 1=CAR). Furthermore, in order to capture the intricacies associated with the curvatures in the decline in cortisol across the waking day, quadratic and cubic functions of time since waking were included as Level 1 predictors. This was done to model slopes best depicted by one bend (quadratic) and two bends ('s' shaped; cubic). All time-related variables were found to be significant predictors of the actual cortisol levels (See Table 2). Variables measuring mood, stress, health, food intake and exercise were also examined as Level 1 predictors; however, none of these variables had a significant effect on any level of diurnal cortisol (See Table 2).

Changes in day-to-day cortisol were captured by Level 2. The model showed substantial variability in cortisol intercept levels, χ^2 (91)=141.27, p<.001 and slope, χ^2(91)=135.09, p<.01 between these two days. Medication intake (coded as a yes/no for the day), was examined as a Level 2 predictor of cortisol variability. Medication intake was significantly related to cortisol slopes such that participants who were on medications had a less steep slope on the day they took medications (B=.016, t=3.071, p<.01).

Level 3 captures between individual changes in cortisol, and examination of this variability revealed that cortisol intercept ($\chi^2(95)=227.47$, $p<.001$), linear slopes ($\chi^2(95)=156.98$, $p<.001$), and quadratic slope ($\chi^2(95)=146.99$, $p<.001$) varied across individuals. This suggests that each individual had differences in the morning level of cortisol as well as their own diurnal slope. Also, the degree to which the morning decline in slope was steeper than the afternoon decline was different from one person to another.

Next, predictors of between-individual cortisol variability were entered at Level 3. First, we entered the control variables of sex, age, SES and mother's visit to healthcare facilities together into the model to assess if these variables influenced cortisol. As described below, total number of visits to health care professionals for infections, asthma, and allergies were then examined independently (along with demographic variables) as Level 3 predictors of cortisol levels and the diurnal rhythm. Associations between demographic variables and health outcomes are included in Table 4. Next we explored possible moderation effects of the demographic variables by exploring interactions between all health and demographic variables. Statistically significant interactions are presented below. Finally, we explored whether medication intake changed the association between health variables and cortisol. Given the large number of predictors, non-significant effects were removed through backwards elimination procedures to arrive at parsimonious models. This is a standard procedure when analyzing data with hierarchical linear modelling (Kreft & DeLeuw, 1998).

Results

Association between Demographic Variables and Diurnal Cortisol

Demographic variables, including sex, age, and SES and mothers' frequency of healthcare visits were examined to explore possible associations with cortisol regardless of the influence of children's health variables. After including all demographic variables into the equation, only age was significantly related to cortisol such that older participants had a slightly more quadratic curve and less of a cubic curve to their diurnal cortisol pattern (B=0.0003, t=2.16, p<.05; B=-0.006, t=-1.99, p=.05, respectively). None of these other predictors were associated with any other aspect of the diurnal curve.

Association between Acute Versus Chronic Health Problems and Diurnal Cortisol

Upon examining the association between acute health problems, more specifically infections, and diurnal cortisol in early adolescence, results suggest that individuals with more infections had higher levels of cortisol. Participants who presented more frequently at healthcare facilities for infections had higher cortisol awakening response (CAR) samples in early adolescence than individuals who had fewer infections (B=0.052, t=1.99, p<.05), such that for every unit increase in frequency of infections, youths' CAR increased by .05 (see Figure 7).

Next we examined possible moderating effects of demographic variables on the association between infections and cortisol. In addition to the main effect of infection on the CAR, we found a significant interaction between SES and infections on morning level of cortisol (B=0.049, t=2.52, p<.02). Upon closer examination, participants from low SES backgrounds who presented more frequently with

infections had higher levels of morning cortisol before the CAR. Combined with the previous finding, these results suggest that youth who received more services for infections had higher CARs and that youth from low SES backgrounds who presented more frequently at healthcare facilities for treatment of infections also had high levels of cortisol before the CAR.

Conversely, individuals who had chronic health problems had blunted cortisol patterns. Specifically, participants who had asthma had less of a cubic curve to their diurnal cortisol slope as demonstrated by elevated afternoon and evening levels of cortisol (B=0.0001, t=2.08, p<.04). See Figure 8.

Next we examined possible moderating effects of demographic variables on the association between asthma and cortisol. In addition to the main effect of asthma on the cubic slope of cortisol, we found interactions between sex and asthma on the linear and quadratic functions of slope (B=-0.014, t=-2.03, p<.05; B=0.0009, t=2.05, p<.05, respectively). Upon closer examination, girls with asthma were more likely to have more of a quadratic slope and less of a cubic slope to their diurnal cortisol profiles. These findings suggest that participants who presented at healthcare facilities for asthma had less steep cortisol slopes and this effect was primarily driven by girls.

The next health problem examined was allergies. Youth who presented most frequently at healthcare facilities with allergy-related problems also had a blunted cubic slope. Participants with more visits for allergies between infancy and early adolescence had less of a cubic bend in their diurnal slope than individuals who presented less frequently (B=0.0001, t=2.03, p<.05), such that for every degree

increase in frequency of allergy-related problems (i.e. when individuals moved from a lower category to a higher category), the diurnal cortisol pattern flattened out by .0001. This effect ultimately resulted in a flattened diurnal slope as evidenced by lower levels in the afternoon and higher levels in the evening (see Figure 9). Possible moderating effects of demographic variables on the association between allergies and cortisol were explored; however none of these associations were significant.

Finally, we examined the possible effect of medication on the different associations between cortisol and physical health problems. Medication was recorded on as a between-day variable (i.e. was medication taken on each day of sampling). Three different medication-related variables were entered separately into the model: total medications, steroid medication, and stimulant medication. None of the abovementioned associations changed after including these variables into the model.

Conclusions & Discussion

Although stress has been implicated in a variety of physical health issues in children and adolescents, little research has thoroughly examined how physiological correlates of stress relate to such issues. Further, the limited research examining this association has primarily been conducted in specific clinical populations. Based on a theory suggesting that the duration of the stressor may be an important factor when considering the association between cortisol and stress-related issues (Heim et al., 2000; Fries et al., 2005; Miller et al., 2007), the present investigation revealed systematic associations between HPA activity and common health

problems in youth depending on the type of the problem (acute or chronic). Physical health problems in youth that were of a more acute or transient nature, in effect infections, were related to elevated levels of cortisol. More specifically, youth who had more visits to healthcare providers from infancy to early adolescence for infections had higher cortisol awakening responses in early adolescence. Conversely, common physical health problems in youth that were of a more chronic or persistent nature, in effect allergies and asthma, were related to blunted levels of cortisol. Results reveal that both asthma and allergies were associated with a blunted cubic bend in their diurnal rhythm.

Research has consistently linked stress to the development and maintenance of a variety of physical health problems. The present study sought to empirically test a theory developed in the stress literature (Miller et al., 2007) by applying it to physical health problems. This theory states that although initial exposure to stress may be associated with elevated levels of cortisol, prolonged exposure to high levels of stress may result in the down-regulation of the HPA axis, ultimately resulting in a blunted pattern of cortisol. Applying this theory to physical health, we hypothesized that youth exposed to more transient or acute health problems would have increased levels of cortisol whereas youth with more chronic conditions would have blunted diurnal cortisol patterns. Our findings seem to support this hypothesis such that youth who presented to the doctor with a higher number of infections had higher levels of cortisol at the awakening response than did youth who had fewer infection-related visits. However, youth who presented at the doctor for more chronic health conditions, such as asthma and allergies, had blunted patterns of

diurnal cortisol as evidenced by less steep slopes. This pattern was evident even after controlling for including variables such as mother's health seeking behaviours, SES, age and sex in the model.

The HPA axis and the immune system are closely linked. Although it was initially thought that elevated activity of the HPA axis suppressed the immune system (Munk, Guyre, & Holbrook, 1984), more recent research suggests that the HPA axis is immunomodulating rather than immunosuppressing (Elenkov & Chrousos, 1999), as HPA activation can suppress some aspects of the immune system but enhance other aspects. Although the immune system differentially acts on components of the immune system, research suggests that it may act in an inhibitory manner on aspects of the immune system that defend against infections. More specifically, hyperactivation of the HPA system has been shown to create profound inhibitory effects on proteins and cytokines implicated in protection against infections, such as Interleukin-12 and Interferon-γ, thus resulting in decreased immunity against viruses linked to acute respiratory infections (Elenkov & Chrousos, 1999). Consistent with this idea, researchers have found that psychological and social stress is associated with an increased risk of developing an acute respiratory infection after experimental exposure to different types of common cold viruses (Cohen, Line, Manuck, Rabin, Heise, & Kaplan, 1997; Cohen et al., 1991). This suggests that stress and its physiological correlates may be able to inhibit certain aspects of cellular immunity thus increasing susceptibility to acute infections. It should be noted that this pattern may or may not be present in children who experience *chronic* infections. Although a high number of infections

may be related to high levels of cortisol as high immune system activation may stimulate increased HPA activation and cortisol secretion (Daruna, 2004), repeated stressors from chronic infections may also result in the down-regulation of the HPA axis (Heim et al., 2000; Fries et al., 2005).

We found that having a high number of infections was specifically associated with a high cortisol awakening response (CAR). The CAR is a peak in cortisol shortly after awakening; it seems to be particularly susceptible to certain outside influences, in particular, stress (Wright & Steptoe, 2005). Individuals reporting high levels of stress typically demonstrate increased CARs compared to individuals who are less stressed (Pruessner, Hellhammer, Pruessner & Lupien, 2003; Schulz, Kirschbaum, Pruessner & Hellhammer, 1998; Wust, Wolf, Hellhammer, Federenko, Schommer, & Kirschbaum, 2000). In healthy individuals the HPA axis and immune system interact reciprocally to maintain homeostasis; however, in a state of disequilibrium, high levels of cortisol can suppress certain immune functions and increased immune system activation due to persistent infections can elicit HPA axis activity (Tsigos, Kyrou, & Chrousos, 2005). As such, we could expect to find that high levels of cortisol upon awakening to be associated with an increased number of infections. Additionally, children with more infections also had higher levels of morning cortisol before the CAR; however this was specific to individuals from low SES backgrounds. Studies have shown that individuals from low SES background also display a higher CAR (Kunz-Ebrecht, Kirschbaum, Marmot & Steptoe, 2004; Wright & Steptoe, 2005) as well as higher morning levels of cortisol (Brandtstädter, Baltes-Götz, Kirschbaum, & Hellhammer, 1991), although these findings are less

consistent (Lupien, King, Meaney, & McEwen, 2000). Moreover, studies typically find low SES to be associated with more stress (Goodman, McEwen, Dolan, Schafer-Kalkhoff & Adler, 2005) as well as poorer health (Adler, Epel, Castellazzo, & Ickovics, 2000; Belsky, Bell, Bradley, Stallard, Stewart-Brown, 2007; Singh-Manoux, Adler, & Marmot, 2003) including increased risk of developing certain types of infections (Cohen, Alper, Doyle, Adler, Treanor, & Turner, 2008). Given such associations, it is not surprising that individuals with high rates of infections have higher CAR and that this effect is more pronounced and extends to elevated levels of cortisol before the CAR in individuals from low SES backgrounds.

Furthermore, we found that youth with more chronic problems such as asthma and allergies had blunted levels of cortisol. These findings may fit into Miller and colleagues' theory of "down-regulation" through two possible mechanisms. First, asthma and allergies are common chronic condition experienced by youth. It is possible that individuals may have developed blunted levels of cortisol as a function of persistent and repeated exposure to allergic reactions or asthma attacks. Blunted levels of cortisol are observed in individuals with a variety of chronic conditions including chronic fatigue syndrome, fibromyalgia, and arthritis (Tsigos et al., 2005). Individuals with such persistent health problems may have initially had increased levels of cortisol which then became blunted over time. Although speculative in nature, research in very young children at risk for developing asthma and allergies found a positive association between cortisol and asthma (Ball et al., 2006) whereas literature examining this association in older children and adolescents seems to support a negative association (Kauffman et al.,

1999; Landstra et al., 2002; Tromp, 1968) or evidence of a blunted diurnal pattern (Buske-Kirschbaum et al., 1998; Fujitaka et al., 2000). Furthermore, a similar process of down-regulation has been observed in the mental health literature, with initial symptoms of internalizing problems or overall poor mental health being related to elevated levels of cortisol and prolonged symptoms being related to decreased levels of cortisol (Ruttle et al., 2010; Shirtcliff & Essex, 2008).

A second explanation that is equally plausible suggests that this pattern of blunted cortisol is particular to the nature of the problems being observed. Asthma and allergies are both disorders of inflammation and, given that cortisol is an anti-inflammatory, it makes intuitive sense that individuals with these problems would display lower levels of cortisol. Accordingly, a classic study by Funkenstein (1953) examined drug-induced asthmatic reactions in individuals with asthma and found that the reaction was much less severe in individuals who were under a high level of psychosocial stress, possibly because increased levels of cortisol acted as an anti-inflammatory. Given that the present study used a community sample and that the rates of most chronic illnesses in children are relatively low compared to those found in adults, we were only able to examine two chronic disorders and thus it is difficult to determine which mechanism is at work. However, it is not only possible, but also plausible, that both mechanisms are active such that the chronic nature of asthma and allergies place an individual at risk for developing a blunted diurnal cortisol pattern but the nature of these disorders also exerts influence, increasing the possibility that these individuals will indeed develop blunted HPA activity.

Upon examining possible moderating effects of demographic variables on the association between asthma and cortisol we found that girls who had asthma displayed more dysregulated diurnal cortisol slopes than boys. This may be because during childhood and early adolescence, asthma is more often found in boys (Bjornson & Mitchell, 2000; Mccallister & Mastronarde, 2008), thus making it less developmentally appropriate for girls to have asthma and this translates into a more atypical slope. Some research suggests that during adolescence severity of asthma decreases in boys, but not in girls. Further, girls with asthma report lower quality of life than boys (Burkhart, Kolbrun Svavarsdottir, Rayens, Oakley, & Orlygsdottir, 2008) which could indicate increased perceived stress due to asthma which could also impact the diurnal slope. In combination, the less normative symptom presentation in girls combined with the persistence of symptoms into adolescence and lower quality of life due to asthma symptoms may negatively impact their physiological functioning and contribute to the more blunted diurnal cortisol slope found in girls in this study.

While this study provides an important starting point for future research on the possibility of HPA hyper- versus hypo-activity according to severity of disease, it is not without its limitations. An ideal design would have permitted us to assess associations between concurrent cortisol and health outcomes at a very early age and then follow these individuals throughout childhood to determine if HPA activity does indeed become blunted due to prolonged exposure to health problems. This would help determine if blunting only takes place when exposure to symptoms

occurs during a specific developmental period as well as determine directionality of the effects.

Further research needs to examine the long-term outcomes associated with elevated versus blunted diurnal cortisol patterns. Although previous research suggests that extremely high and extremely low levels of cortisol can impact non-health related outcomes such as memory (Lupien, Fiocco, Wan, Maheu, Lord, Schramek, & Tu, 2005), more research is required to determine the potential costs and/or benefits of hypo-regulation of the HPA axis (blunting) or hyper-regulation of the HPA axis. In summary, this study applied the theory of hypocortisolism to acute and chronic health-outcomes to provide a more theoretically substantiated idea behind the existence of atypical cortisol levels in certain health conditions prevalent in youth. Studying these differences could provide insight into patterns of childhood morbidity in the general population and possibly identify children who are at an increased risk of developing various health conditions.

Although this study is the first of its kind to explore various patterns of diurnal cortisol as a function of acute and chronic health problems in youth, it is not without its limitations. One main limitation is that the health variables used in the current study are largely non-specific. Due to limited prevalence rates, only the total number of visits for infections, asthma, and allergies was able to be explored, rather than more specific health information (e.g. diagnosis of asthma by a respirologist rather than visits for asthma-like problems, etc.). Future studies should explore the association between cortisol and more specific aspects of the

health problems mentioned here to determine if these associations are able to be replicated.

Although speculative in nature, it seems probable that blunted cortisol levels would be associated with the development of several long-term health outcomes, particularly autoimmune disorders (e.g. diabetes, scleroderma, multiple sclerosis, etc.). Autoimmune disorders are caused by the body mounting an inappropriate immune system reaction against its own tissues (Daruna, 2004). The cause of autoimmune disorders has long evaded health professionals but most speculate that it is due to a biological predisposition. Given that the HPA axis and the immune system are closely related and that blunted levels of cortisol have been consistently linked to several autoimmune disorders, it is plausible that blunted levels of cortisol may be implicated in the development of various autoimmune disorders, either as a vulnerability or causal factor. As such, it is of the upmost importance to conduct longitudinal studies that are able to determine if and how atypical, and more specifically blunted, levels of cortisol precipitate further dysfunction of other bodily systems.

General Discussion

Measures of internalizing behaviour, externalizing behaviour, and health outcomes were associated with various measures of diurnal cortisol in early adolescence. Four questions were raised in the general introduction: (1) Do the associations between diurnal cortisol and internalizing behaviours differ depending on how long youth have experienced the behaviours? (2) Do the associations between diurnal cortisol and externalizing behaviours differ depending on how long youth have experienced the behaviours? (3) Do associations persist if behaviours are examined at a later point in time (i.e. mid-adolescence)? (4) Do the associations between diurnal cortisol and physical health problems in early adolescence differ depending on the chronicity of the health problem? Individual responses to these questions as well as general strengths and limitations to the studies will be presented in the following sections.

Recently, a theory has been put forth that suggests that while elevated levels of stress initially raises cortisol levels, prolonged exposure to chronic stress results in the down-regulation of the HPA axis and subsequently lower than normal cortisol levels (Miller et al., 2007). The three studies presented here are organized around this principle with the first two studies suggesting that while more recent internalizing and externalizing behaviours may be related to high levels of cortisol, prolonged exposure to these behaviours will result in the down-regulation of the HPA axis and subsequently blunted levels of cortisol. The third study also followed this general premise and suggested that more transient or acute health conditions (i.e. infections) would be associated with elevated levels of diurnal cortisol whereas

more chronic health problems (i.e. asthma and allergies) would be associated with blunted levels of diurnal cortisol.

Associations between cortisol and internalizing behaviours

Previous studies examining the association between cortisol and internalizing behaviours have produced inconclusive findings which may be due to some participants experiencing the symptoms for a prolonged period of time and others only experiencing symptoms for a short period of time. The first study of this dissertation found that when controlling for behaviours in early adolescence, higher internalizing behaviours in childhood longitudinally predicted lower morning cortisol in early adolescence. Yet, when internalizing behaviours in childhood were statistically controlled and only internalizing behaviours in early adolescence were measured in relation to cortisol (which was also measured in early adolescence), the opposite was found: more internalizing behaviours were related to higher levels of morning cortisol in early adolescence.

These findings seem to fit with the notion of down-regulation of the HPA axis (Fries et al., 2005; Miller et al., 2007), with short-term or non-chronic associations between cortisol and internalizing behaviour demonstrating a positive association and long-term or chronic associations demonstrating a negative association. Although the design used in the first study presented here is not optimal to assess down-regulation of HPA axis (this will be discussed in depth later), the results provide a much needed explanation for divergent findings in the current literature. This study is a crucial starting point from which to begin exploring the possibility of blunting of the HPA axis in a variety of chronic mental and physical health disorders.

Associations between cortisol and externalizing behaviours

Although research examining the association between cortisol and externalizing behaviours is much more congruent, with the majority of research suggesting that high levels of externalizing behaviours are associated with lower levels of cortisol, some studies have found the opposite or no association. The first study of this dissertation found that regardless of whether the association was examined longitudinally or concurrently, higher levels of externalizing behaviours in were associated with a less steep cortisol slope. Although this association seems to differ from the theory put forth by Miller and colleagues (2007), it is possible that down-regulation of the HPA axis has still occurred. Given that externalizing behaviours typically occur at an earlier point in development than internalizing behaviours, it is possible that initial externalizing behaviours were associated with elevated levels of cortisol which then became blunted but that behaviours and cortisol were not measured at an early enough time point to detect both hyper- and hypo-cortisolism.

Association between hypocortisolism in early adolescence and behaviour problems in mid-adolescence

The results of the second study of this dissertation are in line with our previous findings and provide evidence of a blunted association between cortisol and chronic behaviour problems when measured longitudinally. While the general premise holds true (i.e. youth who have experienced behaviour problems for an extended period of time have lower levels of diurnal cortisol), there are some slight differences between the second and first studies. Although in both studies there

was a consistent negative longitudinal association between cortisol and behaviour, the aspect of cortisol in the diurnal profile (e.g. morning level or slope) associated with internalizing behaviours differed by study. Internalizing behaviours were associated with the slope of decline in the current study whereas they were only associated with morning level in the previous study. One possible explanation for this difference is that youth in the second study had experienced the behaviours for a prolonged period of time. Even though behaviours at previous time points had been controlled for statistically, it may be that initial chronic behaviour (e.g. internalizing behaviour in early adolescence) impacts morning levels of cortisol but extended or prolonged exposure to internalizing behaviours (e.g. internalizing behaviour in mid-adolescence) further impacts diurnal cortisol levels thus resulting in blunted levels over the course of the waking day. Given that both the first and second study are the first of their kind insofar as they relate prolonged internalizing and externalizing behaviours to various measures of diurnal cortisol, more research needs to be conducted to determine precisely what aspects of the diurnal profile are influenced by prolonged exposure to behaviour problems.

An additional difference between this study and the previous study is the influence of medication on the associations. As mentioned above, in the first study internalizing behaviours were related to blunted morning levels of cortisol and including medication in the model did not influence this association; however, externalizing behaviours were related to a blunted diurnal cortisol slope and including medication in the equation decreased this association to a non-significant level. In the second study, both internalizing and externalizing behaviours were

related to a blunted cortisol slope, the same aspect of the diurnal cortisol profile influenced by medication, and including medication in the model decreased the association between cortisol and both internalizing and externalizing to non-significant levels. This would suggest that medication, while a powerful predictor of the linear slope of cortisol, does not fully account for the association between cortisol and problem behaviour.

Associations between cortisol and physical health outcomes in early adolescence

There has been little work examining the association between cortisol and physical health outcomes within a general population of youth. To our knowledge, no other study has examined the association between cortisol and whether or not youth presented at healthcare facilities for problems related to asthma, allergies and infections. The third study presented here found that youth who presented at healthcare facilities for more transient or acute health problems, such as infections, demonstrated high levels of diurnal cortisol whereas youth who presented at healthcare facilities with more chronic health problems, such as asthma and allergies, demonstrated blunted cortisol patterns. These findings also seem to fit within the notion of down-regulation of the HPA axis (Fries et al., 2005; Miller et al., 2007), albeit in a slightly different manner than the previously mentioned findings. Although this study does not explore the actual process of HPA axis down-regulation through concurrent versus longitudinal associations between cortisol and health problems, it does suggest the possibility of HPA axis down-regulation as a function of more chronic disorders. While this study does not allow for causal conclusions regarding chronic health conditions resulting in blunted patterns of HPA axis

activity, or vice-versa, this is not our intention. Rather, this is a correlational study exploring how acute versus chronic health conditions are differentially related to stress physiology. This study provides a first step for researchers to begin exploring how chronic health conditions may impact stress physiology as well as how altered stress physiology may exacerbate various health conditions, or yet still, how a third variable such as early environment or genetic predisposition may place an individual at increased risk of developing altered stress physiology and health conditions.

Strengths and Limitations

A major strength of the first two studies is the longitudinal design which provides the opportunity to explore time-sensitive associations between cortisol and mental health problems that would not have been able to be explored using a cross-sectional design. Given the divergent nature of the findings between cortisol and health in the cross-sectional literature, it is critical to examine these associations using longitudinal data. While a few studies have examined the relationship between cortisol and internalizing symptoms longitudinally, without knowing how more recent/concurrent measures of cortisol and internalizing behaviours are associated, these studies only provide a portion of the overall picture.

Another strength of these studies is that it involved a general population rather than clinical samples. Most other studies exploring associations between cortisol and mental or physical health outcomes used clinical samples or involved sampling from populations that were at a significantly greater risk for developing

the specified disorder due to genetic predisposition. By examining such associations in a more general, albeit slightly at-risk, population we are afforded the opportunity to explore how associations between cortisol and mental and physical health outcomes would appear in a sample more representative of the population at large.

Cortisol sampling can be an expensive and time consuming endeavour and, as such, it is more the exception than the rule that several different aspects of the diurnal curve are measured. That is, studies often require participants to provide saliva samples at only a few times during the day (e.g. morning and evening) so as to limit attrition rates and this is particularly true with longitudinal studies. The present study provided salivary cortisol data on 96 individuals over the course of two waking days and afforded numerous measures of the circadian rhythm of cortisol to be examined including morning level, CAR, and various measures of slope. By exploring multiple aspects of the diurnal curve, we were able to detect numerous associations that would have gone unnoticed if more limited measures of the cortisol profile had been explored.

Another advantage and possible explanation for our ability to detect previously unreported associations between the diurnal cortisol curve and mental and physical health outcomes was our use of highly sophisticated data analysis programs and techniques. By using HLM 6.0, software designed to model trajectories of change, we were able to explore how cortisol profiles changed over the course of the day rather than being limited to static measures of cortisol. Furthermore, the software used in these analyses is able to estimate missing data

based on sufficient existing data which provided us with the increased power needed to detect small effects. By using such advanced software we were able to detect and report novel, and potentially revolutionary, findings.

While these studies have explored associations between cortisol and mental and physical health outcomes that had yet to be examined, they are not without their limitations. Several limitations will be mentioned including population characteristics, sampling limitations, and alternative explanations for the aforementioned associations. One of the main problems associated with all three studies is the wide age range for each sampling time. Although age was examined as a possible mediator and/or moderator in all analyses, it is possible that the younger participants were in different stages of pubertal development than older participants. Although some researchers have found that age is a suitable proxy for puberty (Stroud, Foster, Papandonatos, Handwerger, Granger, Kivlighan, & Niaura, 2009), a valid measure of puberty may have contributed greatly to our understanding of how physiology and behaviour vary as a function of biological development.

Second, although participants were asked to record the time of day associated with saliva sampling, and this was modelled in all analyses, no objective measure of time sampling was used. It is possible that individuals with high levels of behaviour problems are less compliant in their sampling than individuals without behaviour problems and this may increase their chances of taking saliva samples later than they recorded. If this were true, it would appear that individuals with high levels of behaviour problems had blunted HPA axis activity when such results

would actually be an artefact of naturally decreasing cortisol levels due to the circadian rhythm. While this would explain the longitudinal association between cortisol and behaviour problems, it does not account for the positive association between cortisol and behaviour problems when these are examined concurrently (as observed with respect to behaviours). As such, although an objective measure of sampling time would be ideal, it does not seem likely that non-compliance would be driving both sets of findings in such a systematic manner.

Although numerous variables that have been found to be associated with cortisol were controlled for in this study (e.g. time of sampling, mood, food intake, etc.), several other variables were not including quality of sleep, daily and lifetime measures of stress, and season of the year. Quality of sleep can be a major factor in determining level of cortisol at various points in time across the day (El-Sheikh, Buckhalt, Keller, & Granger, 2008; Hatzinger, Brand, Perren, Stadelmann, von Wyl, von Klitzing, & Holsboer-Trachsler, 2008; Scher, Hall, Zaidman-Zait, & Weinberg, 2010). While the present study took time of awakening and bedtime into consideration, participants were not asked about the quality of their sleep which could have played a significant role in the resulting altered levels of cortisol.

Furthermore, although this study examined cortisol, a physiological outcome of stress, as well as health variables, which have been extensively linked to stress, the study lacks an actual measure of stress. A measure of both daily hassles and significant lifetime stressors would have been important and unique predictors to include in the current study. Daily hassles and lifetime measures of stress have been shown to be related to level of cortisol (Newman, O'Connor, & Conner, 2007; Van

Eck, 1996), as well as in the association between level of cortisol and depression (Sayal, Checkley, Rees, Jacobs, Harris, Papadopoulos, et al., 2002). Furthermore, lifetime measures of stress have also been related to cortisol (Cutuli, Wiik, Herbers, Gunnar, & Masten, 2010; Elzinga, Roelofs, Tollenaar, Bakvis, van Pelt, & Spinhoven, 2008) as well as cortisol and symptoms of depression (Ganzel, Eckenrode, Kim, Wethington, Horowitz, & Teple, 2007). Given the strong associations with the other variables examined in the study, measures of various forms of stress/stressors would have been a valuable addition to this study.

Some research suggests that season of the year may influence peak level of cortisol. This research is less consistent though with some studies showing peak levels of cortisol in the spring (Reinberg, Lagoguey, Cesselin, Touitou, Legrand, Delassalle et al., 1978; Matchock, Dorn, & Susman, 2007), others finding peak levels in the winter (Agrimonti et al., 1982; Del Ponte et al., 1984; Hansen et al., 2001), and still some finding no differences (Bellastella, Criscuolo, Mango, Perrone, Sinisi, Faggiano, 1983). Nonetheless, examining season of the year may have helped clarify some findings.

Additionally, causal statements cannot be made concerning any of the reported associations, including the first two studies which examined time-sensitive, longitudinal differences in the association between cortisol and symptoms of mental health problems. While cortisol levels may very well influence the development of mental and physical health problems, the reverse may also be true. Moreover, it may be possible that both cortisol levels and the development of mental health problems can be attributed to a third variable. Research suggests that

both genetics (for a review see Wallace, Schneider & McGuffin, 2002) and early environment (Essex, Klein, Cho, & Kalin, 2002) have been found to be associated with behaviour problems and altered HPA axis activity. Similarly, research has also suggested that other variables may influence both physical health and HPA axis functioning (for a review see Flinn, 1999). Although exploring such associations is out of the scope of these papers, it is important to examine all possible pathways to determine the fundamental cause of mental and physical health problems so as to not make causal claims in the lack of strong empirical evidence.

Directions for Future Research

Although several studies have been conducted on the association between cortisol and mental health problems as well as the association between cortisol and certain physical health problems, future study is required to better understand this connection both cross-sectionally and longitudinally. More specifically, a consensus needs to be reached among researchers in the field to determine which variables and designs would be most fruitful to explore. Controlling for factors such as time elapsed since onset on the problem and chronicity of the problem may help disentangle the seemingly contradictory findings. Furthermore, it is possible that not all individuals with elevated levels of cortisol will eventually develop blunted levels of cortisol or develop serious mental or physical health conditions. Accordingly, future research needs to explore the role of individual differences in the down-regulation of the HPA axis.

Longitudinal studies should employ cross-lagged designs to examine possible down-regulation of the HPA axis. This would serve as a more ideal method of

detecting HPA axis down-regulation. Furthermore, it is likely that the most fruitful studies will examine multiple aspects of the diurnal cortisol slope when trying to determine relations with mental and physical health problems as this research is still in the exploratory stages. Future research may also wish to examine how various patterns of cortisol reactivity in individuals with blunted diurnal cortisol profiles in order to more fully understand HPA axis functioning.

Additionally, although the present studies always included sex as a predictor of cortisol as well as a possible moderator in the associations between cortisol and health variables, future studies should explore the associations examined in this paper separately for each sex. A substantial body of literature suggests that cortisol patterns may be different for boys than for girls (Gunnar & Vazquez, 2006; Hastings, Ruttle, Serin, Mills, Stack & Schwartzman, in press; Spinrad et al., 2009). Additionally, rates and frequency of health problems differ in boys and girls. For example, in childhood and adolescence, boys are more likely to be diagnosed with asthma (Bjornson & Mitchell, 2000; Mccallister & Mastronarde, 2008) and adolescent girls are more likely to display symptoms of internalizing problems than boys (Hankin et al., 1998). Furthermore, some research suggests that the differences seen in boys' and girls' behaviour patterns may be reflected in their cortisol patterns (Zahn-Waxler, Shirtcliff, & Marceau, 2008). Unfortunately the current study did not have enough power to be able to divide the sample by sex and still detect significant results; however future studies should examine a sufficient number of participants in order to be able to determine if associations differ for males and females.

Although they may seem quite distinct, mental and physical health problems tend to also be co-morbid. Individuals with internalizing and externalizing behaviour problems are at a greater risk of developing health problems in adulthood including cardiovascular problems (Carney & Freedland, 2003) and immune system disorders (Kiecolt-Glaser & Glaser, 2002; Miller, Freeland, Carney, Stetler, & Banks, 2003). Although limited, research also suggests that there is also a high level of comorbidity between mental and physical health problems in youth (Essex et al., 2009). Given that the studies of this dissertation and other research has found that both types of health problems are linked to stress and altered HPA axis functioning, future research should explore if the comorbidity between mental and physical health problems can be explained by altered stress physiology.

Theoretical and Practical Implications

The findings reported in this paper suggest that the association between cortisol and mental and physical health problems may be more complex than previously suggested. Current theories of stress and mental health problems depicting a uniform association, either hyper-or hypo-responsive, may need to be modified to acknowledge the various cortisol profiles displayed by individuals with mental health problems and the factors (e.g. time since onset of the stressor and the nature of the stressor) found to influence the activity of the HPA axis (Miller et al., 2007). If we are ever to identify the pathways through which stress influences health, it is important to include all relevant factors, both person and stressor, into models linking HPA activity to mental and physical health.

In addition to the theoretical implications, practical implications for the prevention of depression arise from the previously described research. First, if atypical levels of cortisol are a risk factor for the development of mental and physical health issues, it is important to focus stress reduction efforts on individuals who are at an increased risk of developing such problems. If stress and atypical cortisol levels lead to devastating mental and physical health issues, it might be possible to reduce the prevalence of these issues by instructing and encouraging at-risk individuals to employ stress-management techniques in their daily lives as a preventative measures.

Additionally, if level of cortisol is indeed a precursor to the development of mental and physical health problems, it may be useful to include saliva sampling as part of an early identification program, particularly for children as they may not be capable of reporting symptoms. Identifying an individual as being at risk for mental and physical health problems before he or she demonstrates severe symptoms, may help health practitioners determine follow-up procedures and course of treatment and may encourage the individual to engage in various preventative lifestyle changes. While many other variables contribute to the development of health problems, if it is determined that cortisol is a causal factor, a simple saliva sample may be an easily obtainable biological predictor of the likelihood of developing future conditions. Although significantly more research would be required before exploring this possibility, incorporating cortisol into treatment regimes may be useful in helping regulate the persistence of various mental and physical health problems. With the proper research, cortisol levels may soon be considered a risk

factor for the development of health problems and used to guide prevention and treatment initiatives with the ultimate goal of improving overall mental and physical health.

Tables

Table 1

Percentage of Variability Accounted for by Factors

Factor	Shared informant variability	Informant bias variability
Childhood Internalizing	53.8	39.0
Childhood Externalizing	59.5	36.3
Adolescence Internalizing	53.9	38.2
Adolescence Externalizing	62.1	33.5

Table 2

Association between Level 1 Predictors and Diurnal Cortisol

Predictor	*B*	*t*	*p*
TSW	-0.086	-22.63	<0.001
TSW^2	-0.065	2.37	0.02
TSW^3	-.00	-1.93	0.05
CAR	0.111	4.59	<0.001
Mood	0.002	0.12	.902
Stress	-0.002	-0.22	.828
Health	-0.004	-0.38	.703
Food	0.008	0.23	.854
Exercise	0.005	0.19	.892

Table 3

Correlations among Internalizing (INT) and Externalizing (EXT) Behaviours across All Three Time Points

Variable	Time 1 INT	Time 2 INT	Time 3 INT	Time 1 EXT	Time 2 EXT	Time 3 EXT
Time 1 INT	---	.43**	.31**	.60**	.19^{t}	.14
Time 2 INT		---	.53**	.26**	.35**	.18^{t}
Time 3 INT			---	.21*	.30**	.51**
Time 1 EXT				---	.56**	.49**
Time 2 EXT					---	.63**
Time 3 EXT						---

^{t}= p<.10; * = p<.05; **= p<.01

Table 4

Correlations among predictor variables

Variable	Sex	Age	Parent visits	SES	Infection	Asthma	Allergies
Sex	---	-.09	.08	-.07	- .29**	-.06	.24*
Age		---	.03	-.07	- .01	-.00	-.12
Parent visits			---	-.15	.28**	.15	.08
SES				---	.20[t]	.04	-.04
Infection					---	.25*	.20*
Asthma						---	.14
Allergies							---

[t]= p<.10; * = *p*<.05; **= *p*<.01

Figures

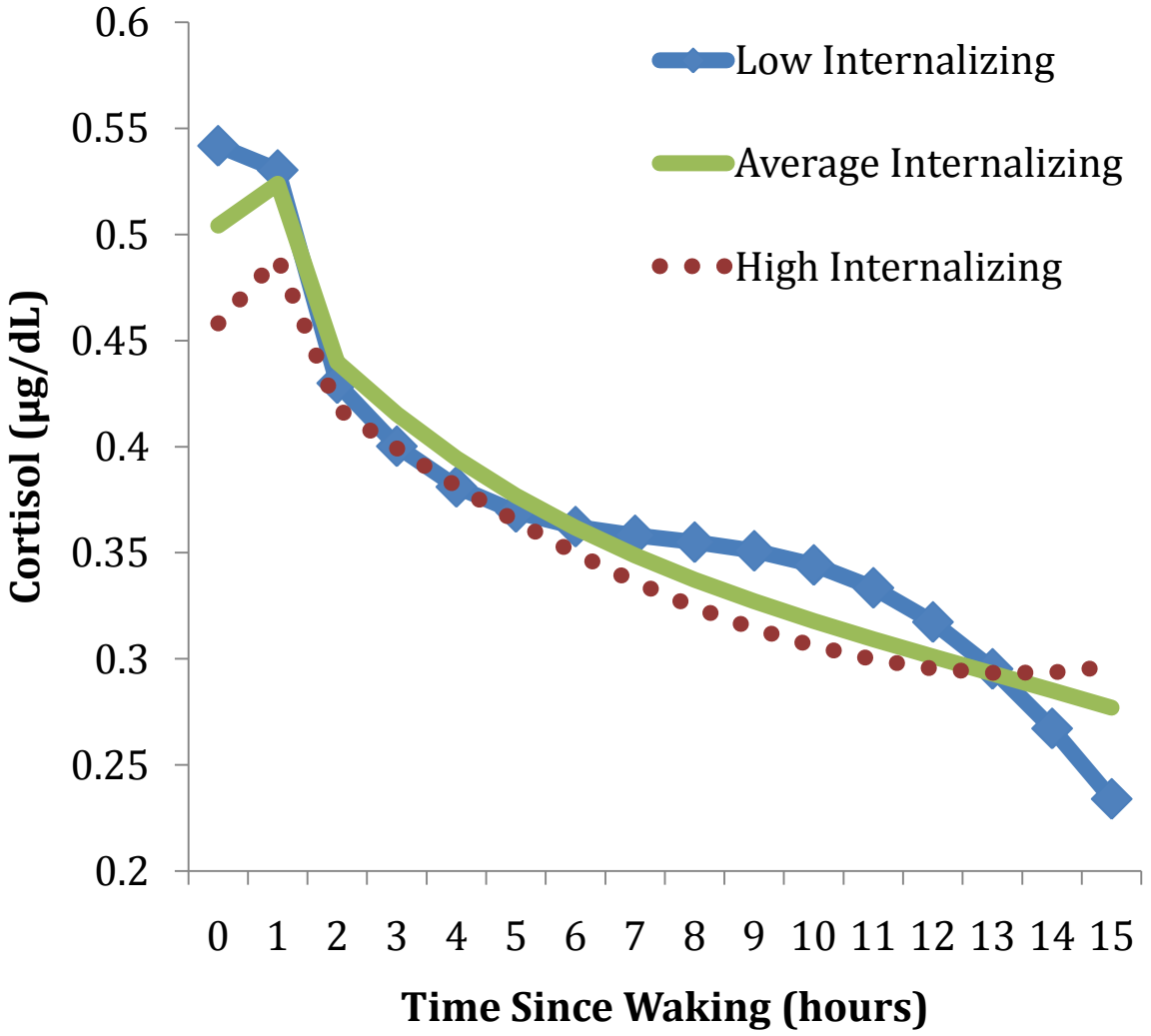

Figure 1. Longitudinal association between level of internalizing behaviours in childhood and diurnal cortisol in early adolescence.

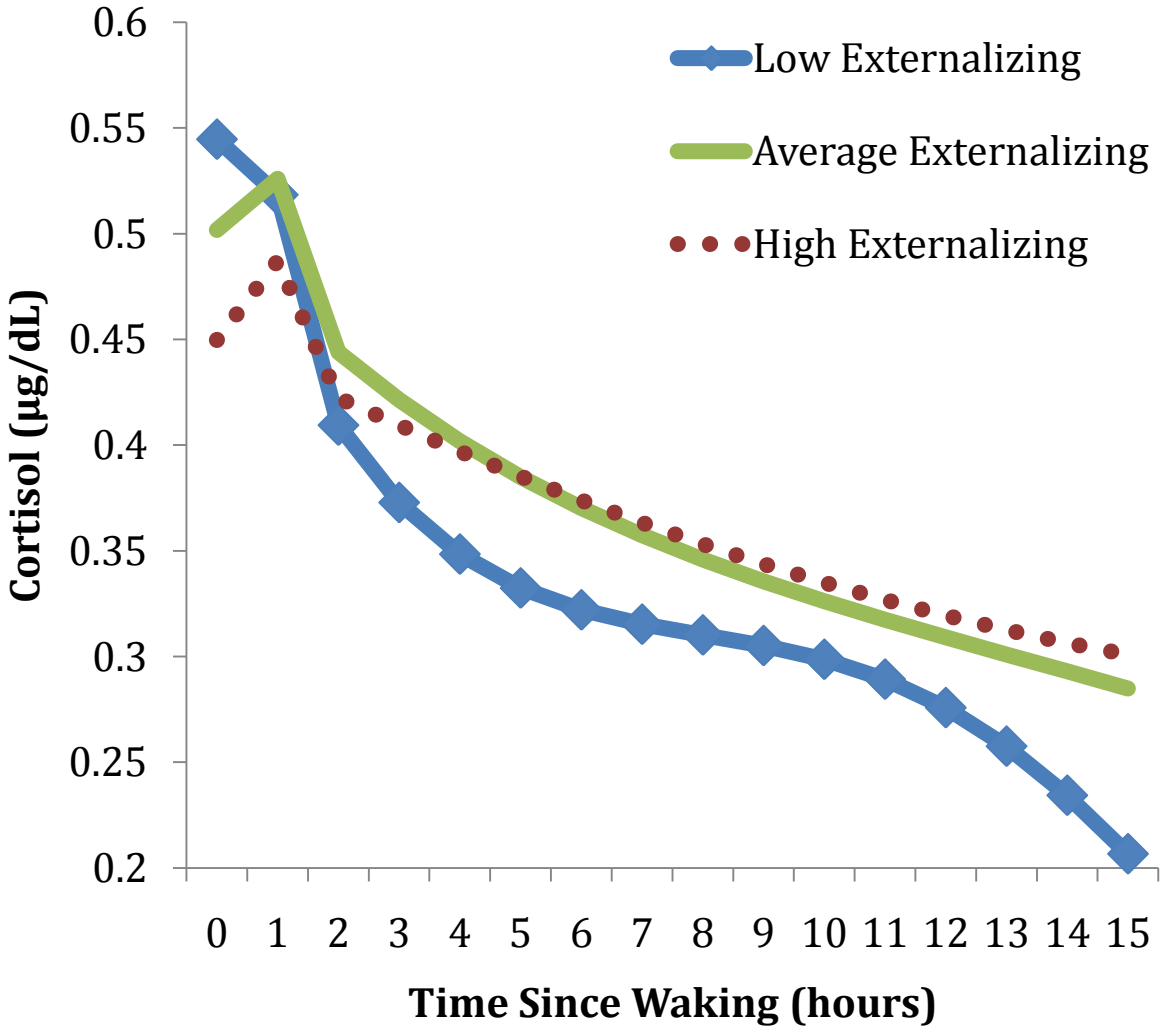

Figure 2. Longitudinal association between level of externalizing behaviours in childhood and diurnal cortisol in early adolescence.

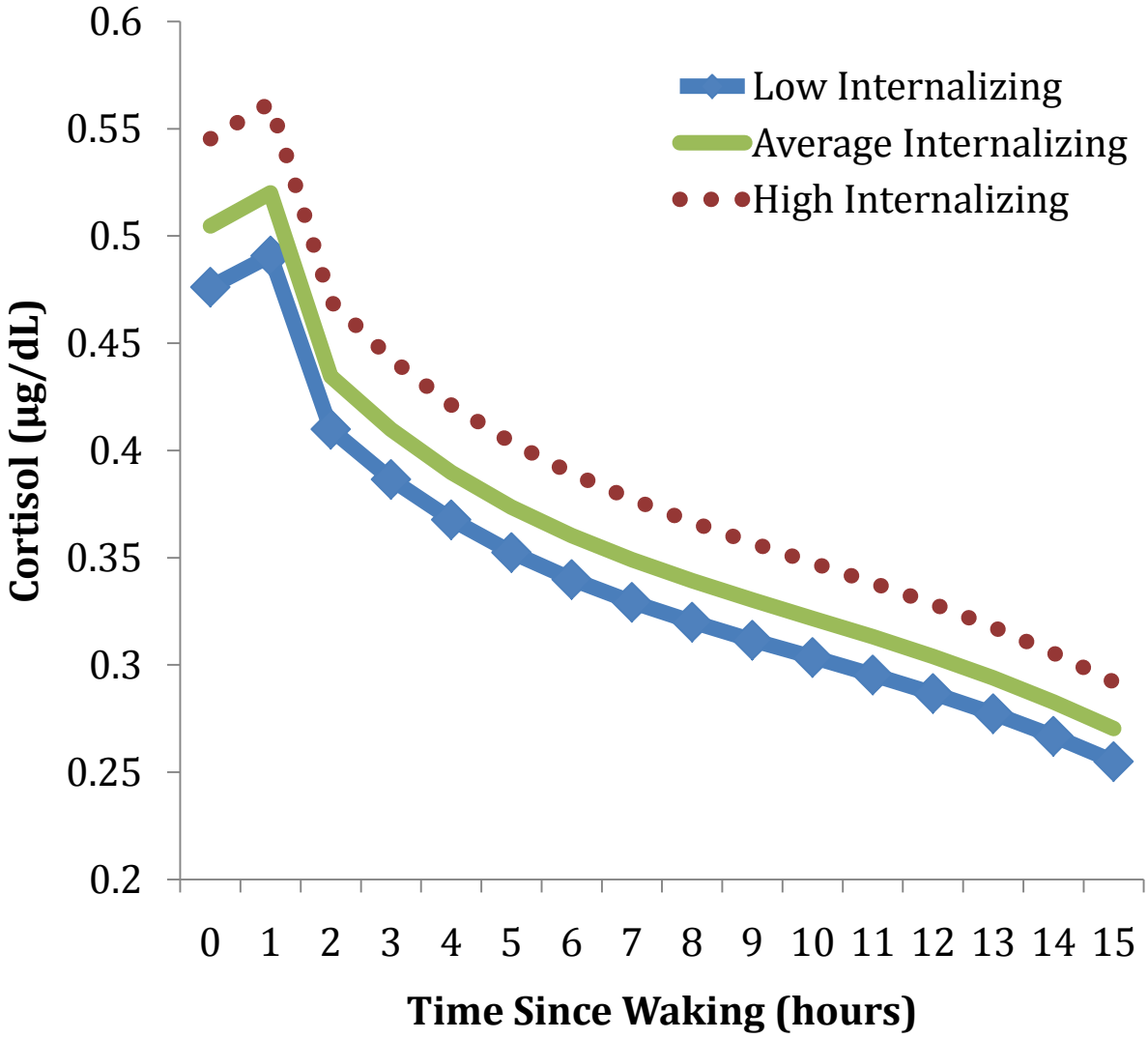

Figure 3. Concurrent association between level of internalizing behaviours and diurnal cortisol in early adolescence.

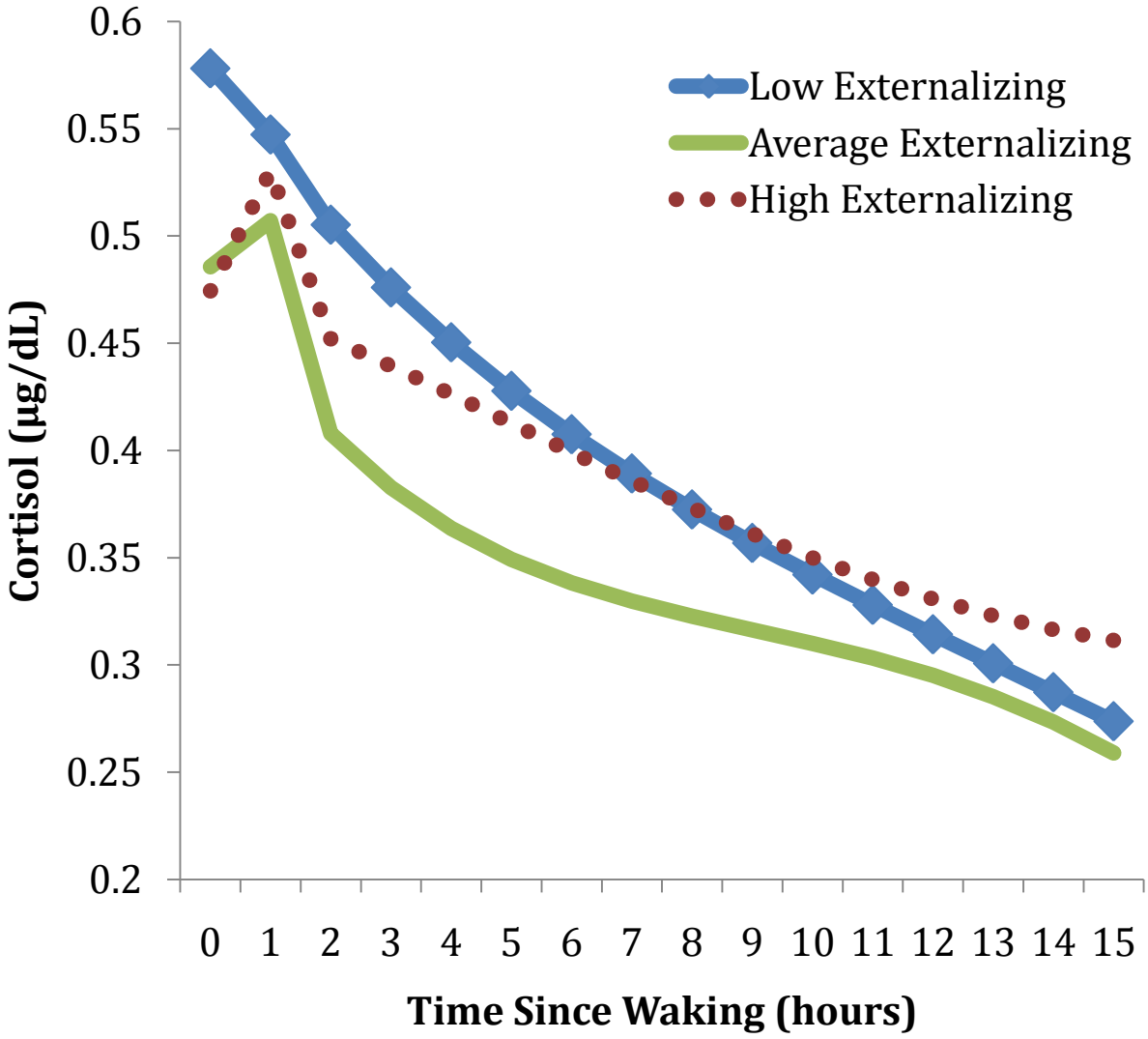

Figure 4. Concurrent association between level of externalizing behaviours and diurnal cortisol in early adolescence.

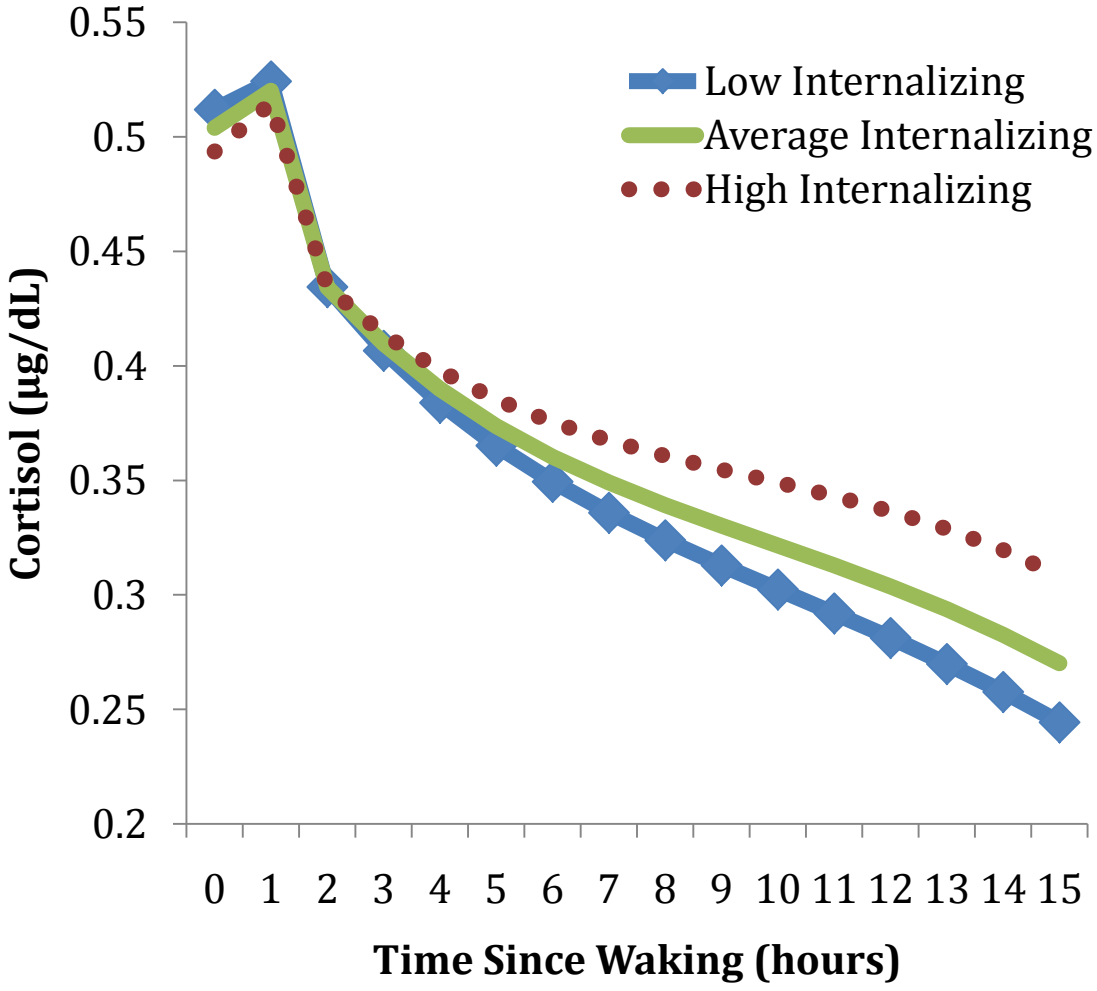

Figure 5. Longitudinal association between diurnal cortisol in early adolescence and internalizing behaviours in mid-adolescence.

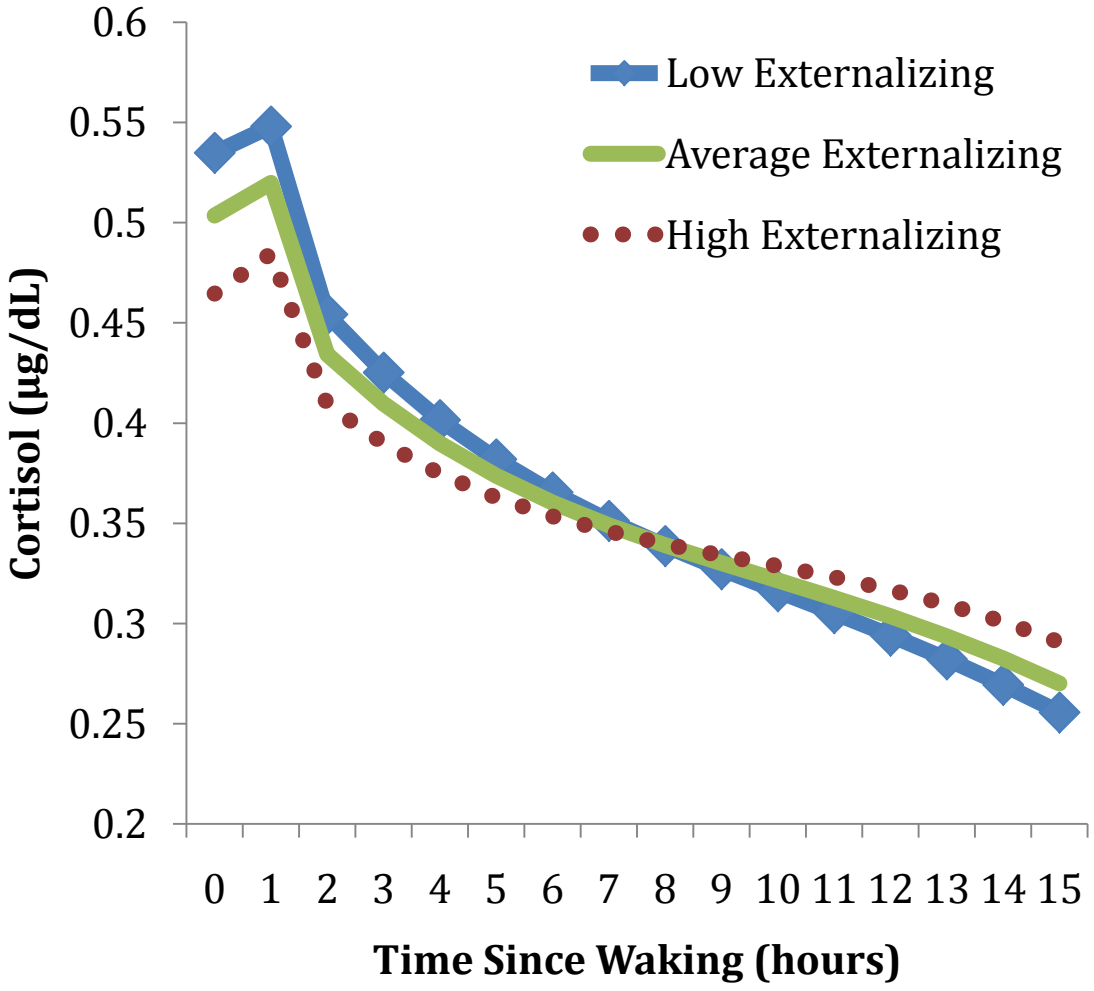

Figure 6. Longitudinal association between diurnal cortisol in early adolescence and externalizing behaviours in mid-adolescence.

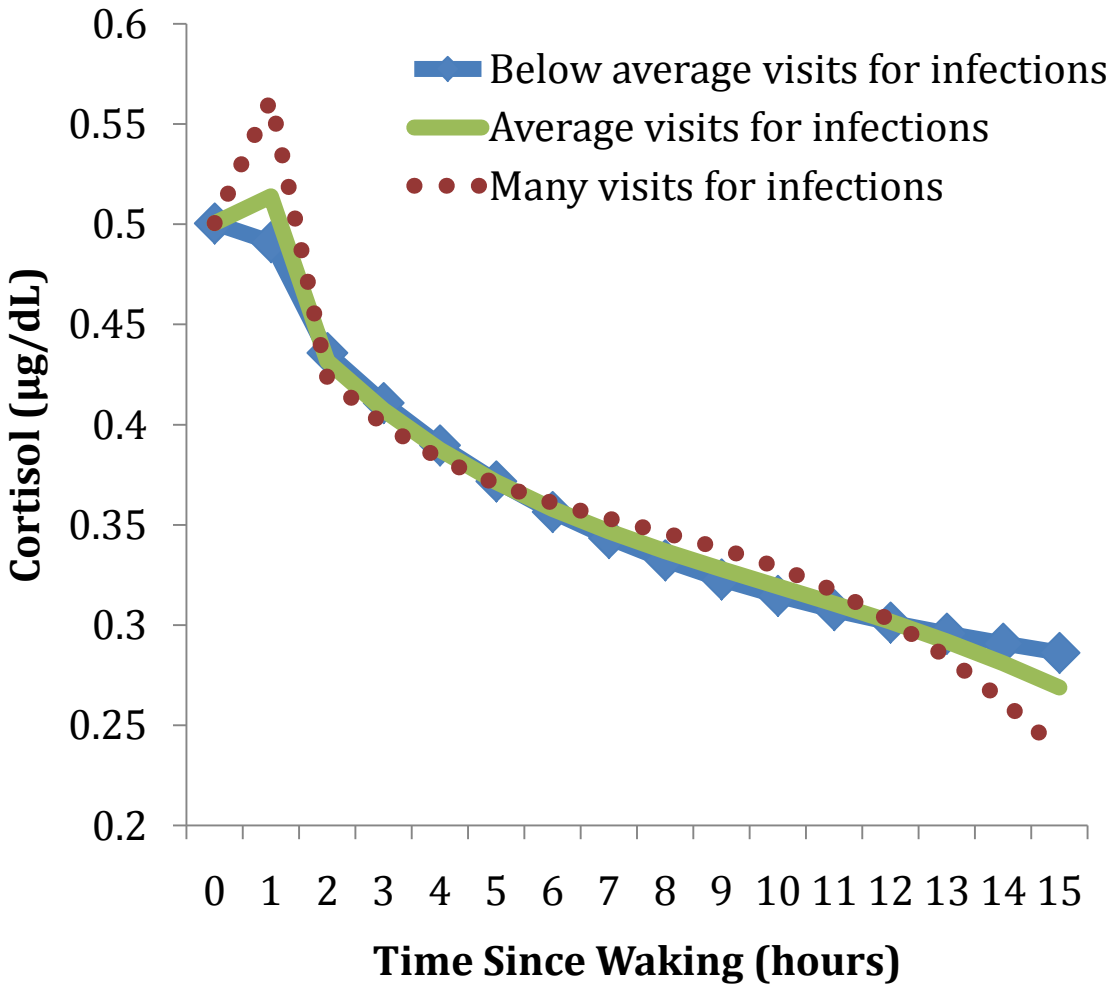

Figure 7. Association between number of healthcare visits from infancy to early adolescence for infections and diurnal cortisol in early adolescence.

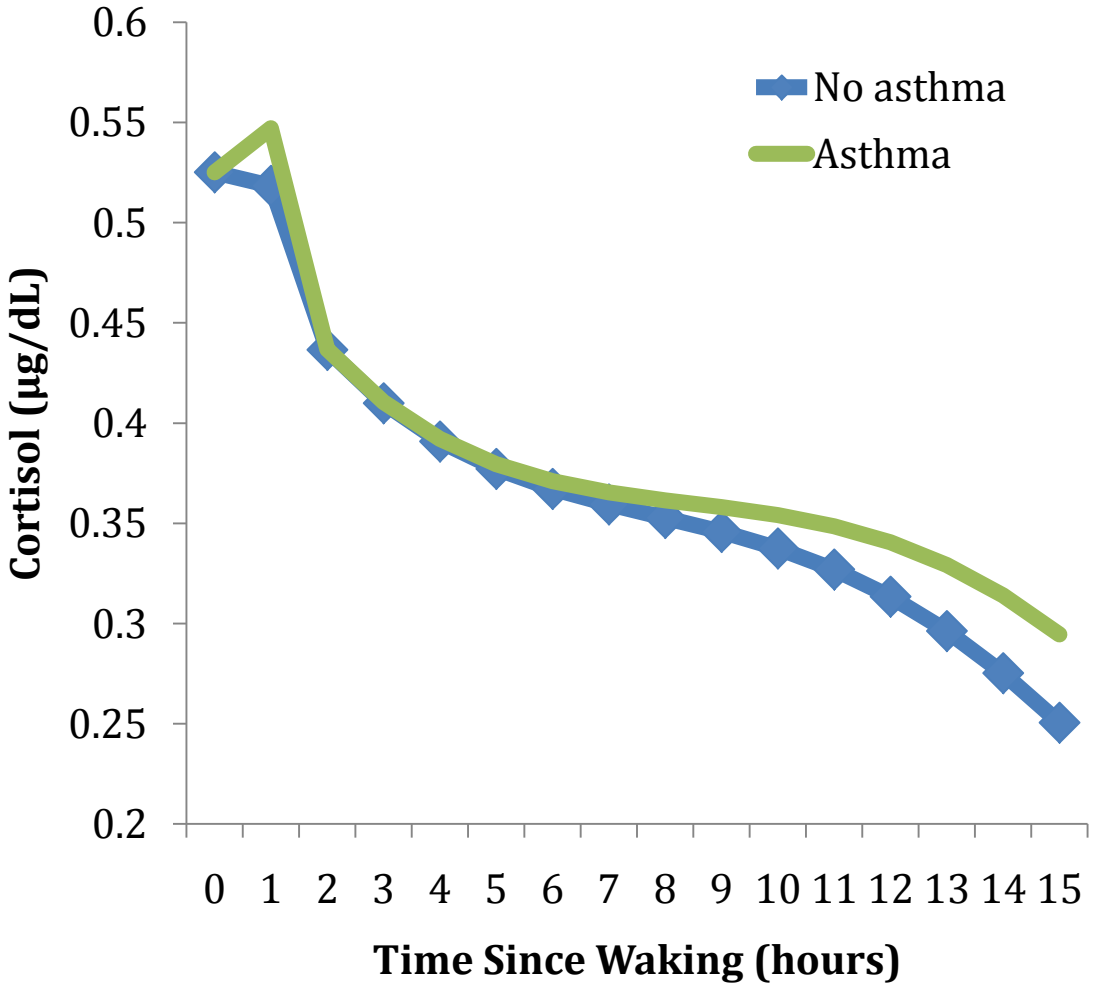

Figure 8. Association between the presence or absence of asthma and diurnal cortisol in early adolescence.

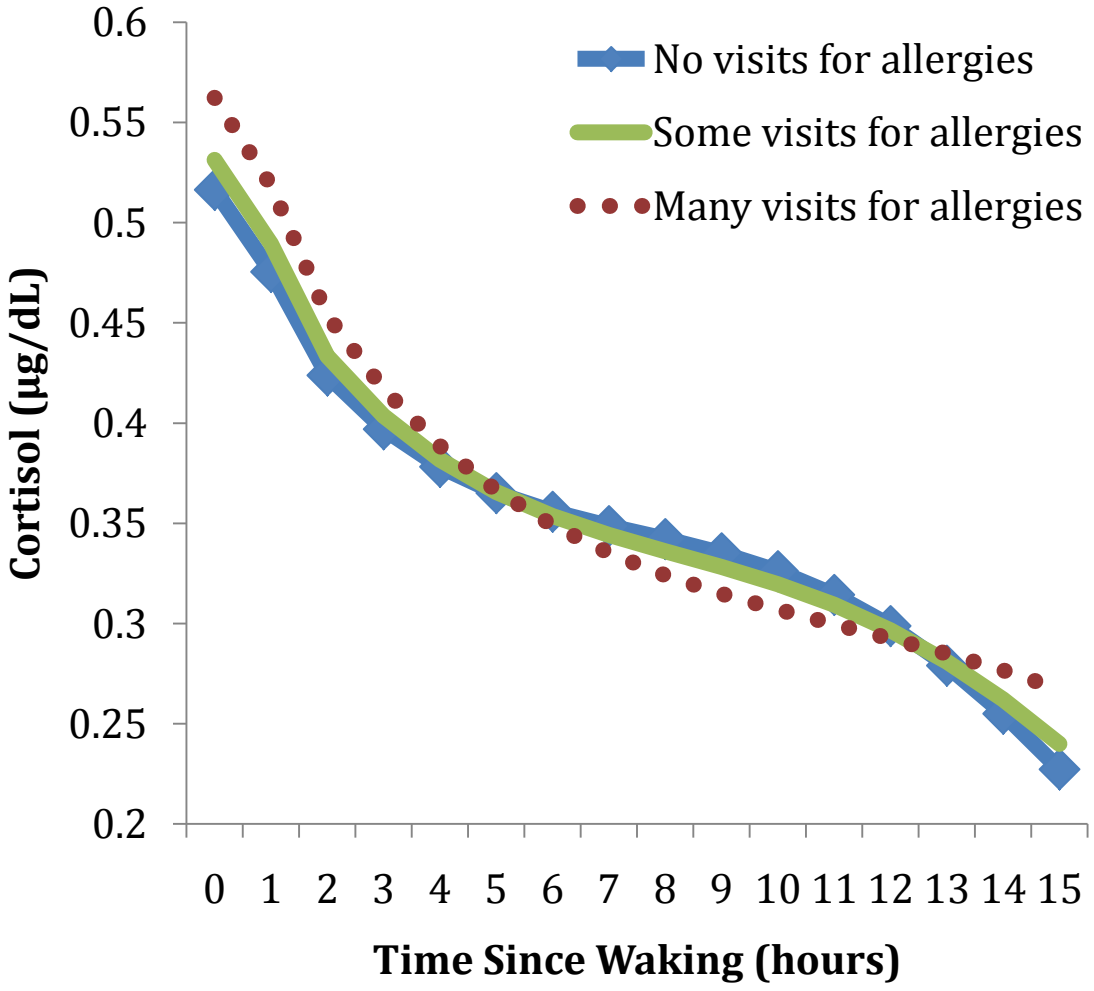

Figure 9. Association between number of healthcare visits from birth to early adolescence for allergies and diurnal cortisol in early adolescence.

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Appendix A.

Codes Included in RAMQ Health Variables

Appendix A- Codes Included in RAMQ Health Variables

Asthma

- 493.0- Extrinsic asthma
- 493.1- Intrinsic asthma
- 493.2- Chronic obstructive asthma
- 493.8- other forms of asthma
- 493.9- Asthma unspecified

Allergies

- 477.0- Due to pollen
- 477.1- Due to food
- 558.3- Allergic gastroenteritis and colitis
- 691.0- Diaper or napkin rash
- 691.8- Other atopic dermatitis and related conditions
- 692.0- Due to detergents
- 692.1- Due to oils and greases
- 692.2- Due to solvents
- 692.3- Due to drugs and medicines in contact with skin
- 692.4- Due to other chemical products
- 692.5- Due to food in contact with skin
- 692.6- Due to plants
- 692.7- Due to solar radiation
- 692.8- Due to other specified agents
- 692.9- Unspecified cause
- 693.0- Due to drugs and medicines
- 693.1- Due to food
- 693.8- Due to other specified substances taken internally
- 693.9- Due to unspecified substance taken internally
- 708.0- Allergic urticaria
- 708.1- Idiopathic urticaria
- 708.2- Urticaria due to cold and heat
- 708.3- Dermatographic urticaria
- 708.4- Vibratory urticaria
- 708.5- Cholinergic urticaria
- 708.8- Other specified urticaria
- 708.9- Urticaria, unspecified
- 995.0- Other anaphylactic shock
- 995.3- Allergy, unspecified
- 995.6- Anaphylactic shock due to adverse food reaction
- 995.7- Other adverse good reactions, not elsewhere classified
- V07.1- Desensitization to allergens

- V15.0- Allergy, other than to medicinal agents
- V72.7- Diagnostic skin and sensitization tests

Infections

- 100- Leptospirosis
- 101- Vincent's angina
- 102-Yaws
- 103-Pinta
- 104- Other spirochetal infections
- 110- Dermatophytosis
- 111- Dermatomycosis, other and unspecified
- 112- Candidiasis
- 114- Coccidioidomycosis
- 115- Histoplasmosis
- 116- Blastomycotic infection
- 117- Other mycoses
- 118- Opportunistic mycoses
- 120- Schistosomiasis
- 121- Other trematode infections
- 122- Echinococcosis
- 123 Other cestode infection
- 124- Trichinosis
- 125- Filarial infection and dracontiasis
- 126- Ancylcostomiasis and necatoriasis
- 127- Other intestinal helminthiases
- 128- Other unspecified helminthiases
- 129- Intestinal parasitism
- 130- Toxoplasmosis
- 131- Trichomoniasis
- 132- Pediculosis and phthirus infestation
- 133- Acariasis
- 134-Other infestation
- 135- Sarcoidosis
- 136- Other and unspecified infectious and parasitic diseases
- 137- Late effects of tuberculosis
- 138- Late effects of acute poliomyelitis
- 139- Late effects of other infectious and parasitic diseases
- 380.0- Perichondritis and condritis of pinna
- 380.0-382.9- Disorders of external ear
- 460- Acute nasopharyngitis (common cold)
- 461- Acute sinusitis

- 462- Acute pharyngitis
- 463- Acute tonsillitis
- 464- Acute laryngitis and tracheitis
- 465- Acute upper respiratory infections of multiple or unspecified sites
- 466- Acute bronchitis and bronchiolitis
- 470- Deviated nasal septum
- 471- Nasal polyps
- 472- Chronic pharyngitis and nasopharyngitis
- 473- Chronic sinusitis
- 474- Chronic disease of tonsil and adenoids
- 475- Peritonsillar abscess
- 476- Chronic laryngitis and laryngotracheitis
- 477- Allergic rhinitis
- 478- Other diseases of upper respiratory tract
- 480- Viral pneumonia
- 481- Pneumococcal pneumonia
- 482- Other bacterial pneumonia
- 483- Pneumonia due to other specified organism
- 484- Pneumonia in infectious diseases classified elsewhere
- 485- Bronchopneumonia, organism unspecified
- 486- Pneumonia, organism unspecified
- 487- Influenza
- 488- Influenza due to identified avian influenza virus
- 490- Bronchitis, not specified as acute or chronic
- 491- Chronic bronchitis
- 492- Emphysema
- 494- Bronchiectasis
- 495- Extrinsic allergic alveolitis
- 496- Chronic airway obstruction, not elsewhere classified
- 500- Coal workers' pneumoconiosis
- 501- Asbestosis
- 502- Pneumoconiosis due to other silica or silicates
- 503- Pneumoconiosis due to other inorganic dust
- 504- Pneumonopathy due to inhalation of other dust
- 505- Pneumoconiosis, unspecified
- 506- Respiratory conditions due to chemical fumes and vapors
- 507- Pneumonitis due to solids and liquids
- 508- Respiratpry conditions due to other and unspecified external agents
- 510- Empyema
- 511- Pleurisy
- 512- Pneumothorax
- 513- Abscess of lung and mediastinum

- 514- Pulmonary congestion and hypostasis
- 515- Postinflammatory pulmonary fibrosis
- 516- Other alveolar and parietoalveolar pneumonopathy
- 517- Lung involvement in conditions classified elsewhere
- 518- Other diseases of lung
- 519- Other diseases of respiratory system