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SEX AND ETHNIC DIFFERENCES IN THE ASSOCIATION OF RELATIONAL AGGRESSION AND EXTERNALIZING BEHAVIOR AND THE DEVELOPMENT OF DIURNAL CORTISOL VARIATION, AN EXAGGERATED STRESS RESPONSE AND OVERWEIGHT/OBESITY IN DIVERSE YOUTH: A CONSIDERATION OF THE DYNAMIC INTEGRATION PERSPECTIVE

A Dissertation in

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Abstract

An interactionist framework, as proposed by Magnusson (1999), was used to examine the cross-sectional and longitudinal association of relational aggression and externalizing behavior in the development of atypical hypothalamic-pituitary-adrenal (HPA) axis activity and overweight/obesity at puberty. Sex and ethnic differences were considered in relation to overweight and obesity. The sample for the first two studies was drawn from an Eastern US city and consisted of children and adolescents (N=135), aged 8-13 years, who participated in a longitudinal study on puberty and behavior. Girls were aged 8, 10 and 12 years and boys were aged 9, 11 and 13 years at study entry. The Eastern sample, along with a multiethnic West Coast sample (N=454), which included participants aged 9-12 years, drawn from a longitudinal study on neglect and behavior, was used in the third study. The first study examined the cross-sectional relationship of diurnal variation in cortisol, an exaggerated stress response, relational aggression and externalizing behavior, as moderated by timing of puberty and depressive symptoms. Results show that earlier maturing adolescents with a low diurnal cortisol variation, as well as adolescents with a heightened response to a stressor and less diurnal cortisol variation, were most relationally aggressive. Sex differences were found when externalizing behavior was examined, with depressed girls with a low diurnal cortisol variation and either heightened or attenuated cortisol reactivity exhibiting the most externalizing behavior problems. Later maturing boys with higher numbers of depressive symptoms and heightened cortisol reactivity also exhibited higher levels of externalizing behavior problems. The second study investigated the prediction of diurnal

cortisol variation by relational aggression and externalizing behavior over 12 months. Relational aggression predicted less diurnal cortisol variation over 12 months in earlier maturing adolescents with higher numbers of depressive symptoms. Externalizing behavior predicted a steadily decreasing diurnal cortisol variation over 12 months in earlier maturing adolescents with lower numbers of depressive symptoms. Between-group differences indicated that girls who practiced relational aggression and externalizing behavior also had less diurnal cortisol variation over 12 months. The final study examined the interaction of cortisol reactivity and externalizing behavior in the prediction of weight status 12 months later in a multiethnic sample of children and adolescents. Eastern adolescents with lower levels of externalizing behavior and West Coast non-Hispanic Black girls with higher levels of externalizing behavior and a diminished cortisol output at baseline (0 months) had a higher weight status a year later. Only non-Hispanic Black girls with higher levels of externalizing behavior and heightened cortisol reactivity at baseline (0 months) had a higher weight status a year later. These studies highlight a need for additional studies to further examine the unique, integrative role that aggressive behavior can play in the development of atypical HPA axis activity and overweight/obesity in multiple ethnic youth. Such studies may elucidate the pathways leading to the development of health disparities in chronic disease during the adolescent period.

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Chapter 1

Introduction

Aggressive behavior is recognized as a major public health concern, undermining both health and well-being. At the same time, current and available intervention programs focus on risk factors without sufficient research with which to understand the mechanisms that lead to the use of aggressive behavior and the consequences of this behavior for the health and well-being of the individual (Moffitt, 2005). In the current study, the role that two types of aggressive behavior, relational aggression and externalizing behavior – typified by physical aggression, can play in child and adolescent health is investigated.

Physical aggression uses weapons with intent to harm and has physical dominance as its goal (Cairns, Cairns, Neckerman, Ferguson, & Gariepy, 1989). Relational aggression uses the social relationship as the vehicle of harm and uses strategies such as social exclusion and gossip (Crick, 1995), with the goals of peer group maintenance and punishment avoidance (Delveaux & Daniels, 2000). Relational aggression is also closely associated with depression and internalizing behavior problems (Crick, Grotpeter, & Bigbee, 2002; Crick, Ostrov, & Werner, 2006; Werner & Crick, 1999; Zahn-Waxler, Klimes-Dougan, & Slattery, 2000). Studies on aggressive behavior and adolescent health and development have included mainly measures of physical aggression and few have considered relational aggression.

Investigation of all types of aggressive behavior and their consequences for health and development is especially important considering that externalizing and internalizing behavior problems have been associated with an atypical stress response and overweight/obesity in children and adolescents. The physiological stress response is responsible for re-establishing equilibrium or allostasis within the human body.

Allostasis is the maintenance of stability through physiological change (McEwen, 1998). The physiological stress response is made up of the sympathetic-adrenal-medullary (SAM) axis and the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol is a steroid hormone that is the final product of a cascade of physiological reactions within the HPA axis and is released in response to environmental disruptions (Tsigos & Chrousos, 2002). Chronic stress, either from traumatic experiences as is the case of war veterans and Holocaust survivors, or prolonged, non-traumatic stress as is the case of teachers and caregivers, causes frequent hypothalamic arousal, leading to elevated cortisol secretions (Miller, Chen, & Zhou, 2007; Preussner, Hellhammer, & Kirschbaum, 1999; Yehuda, Halligan, Grossman, Golier, & Wong, 2002). However, prolonged hypothalamic arousal can cause allostatic load, the wear and tear in bodily systems due to frequent cycles of allostasis (McEwen, 1998). It also leads to the inhibition of endocrine systems necessary for successful pubertal development such as those responsible for growth, reproduction and metabolism (Chrousos & Gold, 1992). Over time, the normal diurnal secretion of cortisol is affected, with the highest levels in the morning on awakening and decreasing to the lowest levels at night. Chronic elevation of cortisol levels can result in a flat diurnal rhythm with steady cortisol secretion and little diurnal variation (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Heim, Ehlert, & Hellhammer, 2000; Miller, Chen, & Zhou, 2007). Chronic elevations of cortisol, as well as a low diurnal cortisol variation, carries implications for health and are associated with overweight/obesity and related diseases (Bjorntorp, 2001; Bjorntorp, Holm, & Rosmond, 1999; Bjorntorp & Rosmond, 2000; Chrousos, 2000) and aggressive behavior (McBurnett et al., 2005; Popma et al., 2007; Susman et al., 2007). Therefore, it is vital to examine the role of

these HPA axis perturbations in aggressive behavior and overweight/obesity within the pubertal context if the development of weight-related chronic disease in adolescence and adulthood is to be understood.

Few studies have considered simultaneously the role that biological, psychological and social factors can play in the development of an atypical stress response and overweight/obesity, especially in the developmentally important period of puberty. Furthermore, these relations rarely have been examined in diverse ethnic groups in order to increase understanding of ethnic disparities in health. Within the U.S., Whites, African American/Blacks and Hispanic/Latino share three of the top ten leading causes of death. These are heart disease, stroke and diabetes (Mortality Rates by Race/Ethnicity, Health, U.S., 2006, Table 29). Although heart disease and diabetes mellitus are among the leading causes of death in the United States, disparities in mortality exist between ethnic groups. In 2004 (latest figures), using the age-adjusted death rate per 100 000 of the population, Blacks died in greater numbers from heart disease (280.6) and diabetes mellitus (48.0) than Hispanics/Latino (heart disease: 158.4, diabetes mellitus: 32.1) and Whites (heart disease: 213.3, diabetes mellitus: 22.3). Homicide was also among the top 10 leading causes of death in Blacks (20.1) and Hispanics (7.2) but not for Whites. Therefore it is pertinent to investigate the development of chronic disease and aggressive behavior during adolescence, a developmentally important period of the lifespan, in diverse ethnic groups.

Overweight/obesity in children and adolescents is more prevalent today than it was three decades ago. Data from two National Health and Nutrition Examination Surveys (NHANES), conducted in 1976-1980 and 2003-2004, shows the prevalence of

overweight increasing from 6.5% to 18.8% in children aged 6-11 years and 5.0% to 17.4% in adolescents aged 12-19 years (Ogden, Flegal, Carroll, & Johnson, 2002). Further investigation revealed that adolescent non-Hispanic black girls have the highest prevalence of overweight (25.4%) compared to Mexican American (14.1%) and non-Hispanic white (15.4%) adolescent girls (Ogden et al., 2006). Overweight/obesity in children and adolescents is related to cardiovascular risk factors (Freedman, Dietz, Srinivasan, & Berenson, 1999; Must & Anderson, 2003) and early-onset Type 2 diabetes mellitus (Marcovecchio, Mohn, & Chiarelli, 2005). Early-onset Type 2 diabetes mellitus is more aggressive than later onset Type 2 diabetes mellitus and is reflected by a more adverse cardiovascular risk profile and a higher relative risk of myocardial infarction and cardiovascular death (Song & Hardisty, 2008). It is especially disquieting to recognize that although overweight/obesity prevalence is on the increase, mortality rates from heart disease and Type 2 diabetes mellitus, both obesity-related diseases in childhood and adolescence, are also increasing. In addition, heart disease and Type 2 diabetes are the leading causes of death in all ethnic groups, but mortality rates from these diseases are highest in Blacks.

Aggressive behavior is associated with overweight/obesity (Judge & Jahns, 2007; Pine et al., 1996; ter Bogt et al., 2006) and aggressive adolescents tend to be more overweight in adulthood (Hasler et al., 2004), when compared with non-overweight adolescents. An investigation of aggressive behavior and overweight/obesity within the pubertal context identifies pubertal timing as a correlate of both (Ge, Brody, Conger, Simons, & Murry, 2002; Ge et al., 2006; Wang, 2002). Puberty is a stressful transition and a time when depression increases with increasing pubertal stage in

normal adolescents (Susman & Rogol, 2004). Furthermore, it is well known that when adolescents mature at a time not comparable to their peers, they are more at risk for the development of depression and behavior problems (Brooks-Gunn, Petersen, & Eichorn, 1985). Pubertal timing is the comparison of pubertal stage within same age and sex peers. Early timing of puberty is defined as pubertal maturation earlier than one's peers and later timing of puberty is defined as pubertal maturation later than one's peers. Both earlier and later timing carry implications for biology and behavior (Brooks-Gunn, Petersen, & Eichorn, 1985; Dorn, Susman, & Ponirakis, 2003). For example, early timing of puberty is associated with overweight and high blood pressure in adulthood (Hulanicka, Lipowicz, Koziel, & Kowalisko, 2007) whereas off-time puberty, both early and late, is associated with more internalizing and externalizing behavior problems than their on-time counterparts (Ge, Brody, Conger, Simons, & Murry, 2002; Ge et al., 2003; Graber, Lewinsohn, Sealy, & Brooks-Gunn, 1997; Michaud, Surio, & Deppen, 2006). Therefore, it is pertinent to include measures of pubertal timing when examining pathways to the development of chronic disease in adolescents, especially those pathways that involve aggressive behavior and physiological health.

The current study uses an overall theoretical framework that employs dynamic integration (Magnusson, 1999) across biological, behavioral development and cultural domains. Dynamic integration recognizes the individual as an active participant in an integrated, complex and dynamic person-environment system (Magnusson & Cairns, 1996). By employing dynamic integration, it is possible to show that certain biological, psychological and social vulnerabilities may arise to produce individual differences in health and behavior. Using this interactionist framework, it is possible to

consider the integration of psychological, biological and social processes in the investigation of the pathways that lead to the development of overweight/obesity and an atypical stress response during the pubertal transition and across adolescence. It should be noted that an atypical stress response and overweight/obesity may singly or together lead to adverse health outcomes in the future.

Dynamic integration is first utilized in Paper 1, wherein a cross-sectional model is examined in a sample of 135 rural Pennsylvania adolescents, aged 8-13 years, recruited for a study on the physiology of puberty and behavior. The paper examines the sex differences in the relationship between the diurnal cortisol variation, cortisol response to a laboratory stressor and relational aggression. This relationship is proposed to be moderated by pubertal timing and depression. There is a paucity of studies on relational aggression and HPA axis activity even though it has been identified as a more potent stressor than academic stress (Crick & Nelson, 2002). Given the wealth of information on externalizing behavior and cortisol, the cross-sectional model was also applied to examine the relationship between diurnal cortisol variation, cortisol response to a laboratory stressor and externalizing behavior. Pubertal timing and depression were examined as moderators of this relationship. This approach allowed a comparison of HPA axis perturbations between the two different types of aggressive behavior, within the pubertal context. In Paper 2, longitudinal effects of, and sex differences in, relational aggression and externalizing behavior on the diurnal cortisol variation were investigated, using all three waves of measurement 6 months apart over the course of one year in the same sample as the sample in Paper 1. Finally, in Paper 3, a longitudinal biobehavioral model was employed that considered the effects of

diurnal variations in cortisol and cortisol response at study entry on weight status a year later. Externalizing behavior was considered a moderator of HPA axis activity and weight status at the 12 month follow-up. Both the sample from the longitudinal study on the physiology of puberty and behavior and a unique sample of West Coast adolescents participating in a longitudinal study on neglect and behavior were examined. Together, the two samples consisted of 589 adolescents, aged 8-13 years and enabled the investigation of ethnic and sex differences in the development of overweight/obesity in a multiethnic sample. Data from the first (0 months) and third (12 months) waves of measurement were used in the Eastern sample. Data from the first (0 months) and second (12 months) waves of measurement were used in the West Coast sample. This approach was adopted to enable an adequate comparison over the course of 12 months in each sample. The integrated conceptual model for the three studies is provided in Figure 1.1.

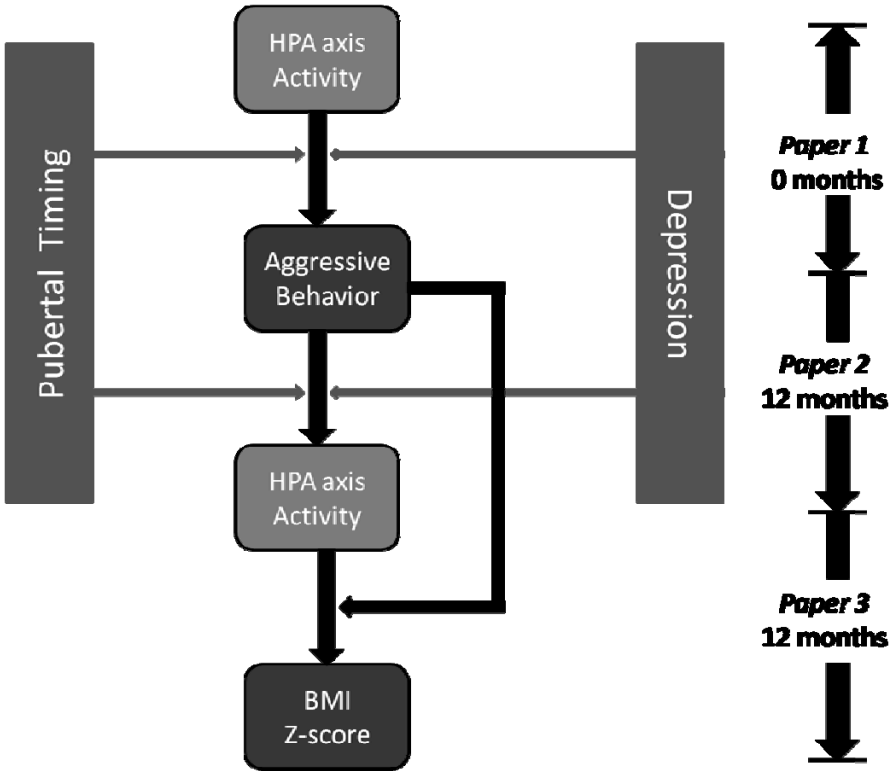

Figure 1.1 An integrated conceptual model of biological and behavioral factors that lead to the development of an atypical stress response and the development of overweight/obesity, when aggressive behavior is considered. Pubertal timing and depression act as moderators of the aggressive behavior and HPA axis activity relations in Papers 1 and 2; aggressive behavior acts as a moderator of HPA axis activity and BMI z-score relationship at 12 months follow-up in Paper 3.

Chapter 2

Paper 1

Cortisol, timing of puberty and depression symptoms are associated with relational aggression and externalizing behavior in young adolescents

Abstract

The present report is based on the theoretical framework that cortisol, an index of the hypothalamic-pituitary-adrenal (HPA) component of the stress system, is a vulnerability for relational aggression and externalizing behavior. Specifically, the following hypotheses were investigated: (1) cortisol will interact with the timing of puberty and depressive symptoms and (2) atypical HPA axis functioning, as demonstrated by a low variation in cortisol levels over the day, and heightened or attenuated cortisol reactivity, will be associated with more use of relational aggression and externalizing behavior. The sample consisted of 8- to-13-year-old boys and girls (N= 135). Timing of puberty moderated the relationship between the variation of cortisol levels across the day and relational aggression, such that early timing adolescents with low diurnal cortisol variation were the most relationally aggressive. At the same time, boys and girls with high cortisol reactivity and low diurnal cortisol variation also reported the most relational aggression. High cortisol reactivity and low reported depression were found in both boys and girls that reported using more relational aggression. In comparison, externalizing behavior was associated with a low diurnal cortisol variation in depressed girls with either heightened or attenuated cortisol reactivity. Consequently, timing of puberty and depressive symptoms were found to moderate the relationship between cortisol reactivity and externalizing behavior only in later timing boys who reported high levels of depressive symptoms. The interaction between components of HPA axis activity, timing

of puberty and depressive symptoms suggests the importance of recognizing an interactionist framework of biopsychosocial processes when investigating relational aggression and externalizing behavior.

Introduction

The interactionist framework, proposed by Magnusson (Magnusson, 1999), provides the conceptual basis for this study and considers the interaction of biological maturation, psychological processes and social contexts in individual development. Within this overall theoretical framework, the specific hypotheses were tested that vulnerabilities of the hypothalamic-pituitary-adrenal (HPA) axis of the stress system will interact with the psychosocial vulnerabilities of pubertal timing and depressive symptoms in young adolescents. The association of these interacting variables with relational aggression and externalizing behavior will be examined.

Cortisol is recognized as the final hormone product of a cascade of events within the HPA axis in a neuroendocrine response to diurnal variations and environmental challenges (Tsigos & Chrousos, 2002). Cortisol levels rise to a peak shortly after awakening and decrease steadily throughout the morning, approaching the lowest levels in the evening hours. Although this is the expected profile of cortisol secretion across the day, there are individuals showing little variation in the levels of cortisol from morning to evening. These individuals have been found to make up from 10% (Stone et al., 2001) to 17% (Smythe et al., 1997) of individuals. Exposure to chronic stress may also give rise to a lowered diurnal cortisol variation, especially when linked with relational loss (Miller, Chen, & Zhou, 2007). Less cortisol variation over the day is indicative of hypocortisolism that is defined as the paradoxical suppression of the limbic-HPA axis under conditions of trauma and prolonged non-traumatic stress (Heim, Ehlert, & Hellhammer, 2000). A review of hypocortisolism in children concluded that lower basal cortisol levels and less diurnal variation in cortisol levels appeared to evolve

early in childhood and could possibly be a result of adverse early life conditions, chaotic or unpredictable schedules early in life, colic in infancy, institutional rearing, a neglectful environment or early trauma and neglect (Gunnar & Valquez, 2001). Hypocortisolism also has been found to be associated with a high stress sensitivity as indexed by Fries et al., (Fries, Hesse, Hellhammer, & Hellhammer, 2005). Finally, Susman (2006) theorizes that hypocortisolism is a vulnerability for aggressive behavior because of the persistent association between the two in the existing literature.

Recently, there has been a focus on relational aggression as a covert type of aggressive behavior prevalent in children and adolescents. Relational aggression is a unique behavior that occurs mainly during social interaction and in the establishment of close, intimate connections with others. The behavior inflicts harm through the purposeful manipulation of, and damage to, peer relationships and is achieved through covert strategies such as social exclusion and gossip. This behavior is associated with psychological adjustment problems and is also associated with internalizing behaviors, such as depressive symptoms, which can stabilize over time and have implications for future psychosocial adjustment (Crick & Bigbee, 1998; Crick, Grotpeter, & Bigbee, 2002; Crick & Zahn-Waxler, 2003; Goldstein & Tisak, 2004; Werner & Crick, 2004; Zahn-Waxler, Klimes-Dougan, & Slattery, 2000). Physiological indices of stress, specifically, cortisol, have not been considered a correlate of relational aggression but their inclusion is long overdue given the recent literature linking stress physiology, physical aggression and violence. In the current study, relational aggression is investigated as a type of aggressive behavior that may also be associated with vulnerabilities of the HPA axis, as has been described in the literature for physical

aggression and externalizing behavior. Relational aggression will be examined from within the biopsychosocial model that includes depressive symptoms and pubertal timing as moderators of its probable relationship with HPA axis functioning, namely cortisol.

The putative relation between cortisol and aggressive behavior is based on models proposing the association between externalizing behavior and HPA axis functioning (Raine, 2002; Raine, Brennan, Farrington, & Mednick, 1997; Stoff & Susman, 2005; Susman, 2006). Much of this research has focused on males and physical and verbal aggression with goals of instrumentality and physical dominance when intending to harm others (Block, 1983; Cairns, Cairns, Neckerman, Ferguson, & Gariepy, 1989). Recent research focuses on problems of regulation of the HPA axis as a concomitant of aggressive behavior (Pajer, Gardner, Rubin, Perel, & Neal, 2001; Popma et al., 2007; Popma et al., 2006). Specifically, the attenuation hypothesis suggests that lower, as opposed to higher basal cortisol levels is a correlate of aggressive behavior and is indicative of low HPA arousal (Susman, 2006). Extant findings suggest that aggressive behavior is associated with low levels of salivary cortisol (McBurnett & Lahey, 2000; McBurnett, Lahey, Capasso, & Loeber, 1996; McBurnett et al., 2005; Popma et al., 2007; Popma et al., 2006; Susman, Dorn, Inoff-Germain, Nottleman, & Chrousos, 1997). In one of the few studies involving girls, diminished morning plasma cotisol levels were prototypical of girls with conduct disorder in the absence of other psychiatric disorders (Pajer, Gardner, Rubin, Perel, & Neal, 2001). Atypical variations in diurnal rhythms of cortisol also are associated with aggressive behavior. Boys with disruptive behavior have less variation in their diurnal

rhythm of cortisol than boys without disruptive behavior (Popma et al., 2007). Thus, it was expected that less variation in diurnal cortisol rhythms would be associated with aggressive behavior, regardless of whether the aggression is overt or relational.

Social situations can influence HPA reactivity to stressful situations to a considerable degree (Dickerson & Kemeny, 2004; Hellhammer & Wade, 1993). Dickerson and Kemeny (2004) report that acute stressors with uncontrollable threats to the goal of maintaining the social self trigger reliable and substantial cortisol changes and that the magnitude of these responses depends on the intensity of the threat, its context, and the presence of vulnerability and protective factors (Dickerson & Kemeny, 2004; Gruenewald, Kemeny, Aziz, & Fahey, 2004; Seyle, 1975). In the few studies that have examined cortisol reactivity and its association with aggressive behavior in particular, a low or attenuated cortisol response has been indicated in children with externalizing behavior problems (Snoek, Van Goozen, Matthys, Buitelaan, & van Engeland, 2004), in boys with a high risk for substance abuse (Dawes et al., 1999) and in children with a higher aggression rating who also had low autonomic activity (Gordis, Granger, Susman, & Trickett, 2006). In contrast, a heightened cortisol response to stress was evident in boys who exhibited the most externalizing behavior and were anxious but a lowered cortisol response was evident in boys who exhibited the most externalizing behavior but were less anxious (Van Goozen et al., 1998). These discrepant findings are not surprising given the different social situations under which the HPA axis was stimulated.

The pubertal transition has not been adequately examined in relation to puberty yet puberty constitutes a period of rapid biological maturation as well as multiple

and diverse social role passages into new reference groups (Susman & Rogol, 2004). Puberty may be accompanied by an increase in aggressive behavior but even prior to puberty, the use of direct or overt aggression decreases, whereas the use of indirect or relationally aggressive acts either increase or remain stable (Cote, Vaillancourt, Barker, Nagin, & Tremblay, 2007). Puberty represents a particularly vulnerable time in which the adolescent who matures earlier or later, as compared to same-age peers (i.e. off-time adolescents) will be more vulnerable to the development of behavior problems and depression (Brooks-Gunn, Petersen, & Eichorn, 1985). In particular, being an early developer is particularly disadvantageous since earlier puberty disrupts the normal course of development and the adolescent may lack the cognitive and emotional maturity needed to solve challenges imposed by having an adult appearance too early in puberty (Caspi & Moffitt, 1991; Petersen & Taylor, 1980). There is evidence that early-maturing adolescents are more vulnerable to depressive symptoms and deviant peer pressure than late-maturer or on-time maturers (Ge, Brody, Conger, Simons, & Murry, 2002; Ge, Conger, & Elder, 1996, 2001a, 2001b; Ge et al., 2003; Warren & Brooks-Gunn, 1989). In addition, a recently published study found an association between early maturing adolescents and a strong preference for eveningness, which itself was associated with antisocial behavior (Susman et al., 2007). Conversely, later-maturing boys have been shown to exhibit more delinquency and be at risk for more psychopathology and depression in some studies (Graber, Seeley, Brooks-Gunn, & Lewinsohn, 2004; Michaud, Surio, & Deppen, 2006; Williams & Dunlop, 1999). Interactive effects of the vulnerabilities of the HPA axis component of the stress system,

along with the psychosocial vulnerabilities of timing of puberty and depression, have not been a focus of previous research on relational aggression and externalizing behavior.

The Current Study.

Based on an interactionist developmental framework (Magnusson & Cairns, 1996), HPA system vulnerabilities (cortisol) are integrated with timing of puberty, depressive symptoms and their interactions into a model of aggressive behavior (See Figure 2.1). This model was applied to the examination of relationally aggressive behavior with HPA axis functioning, pubertal timing and depressive symptoms.

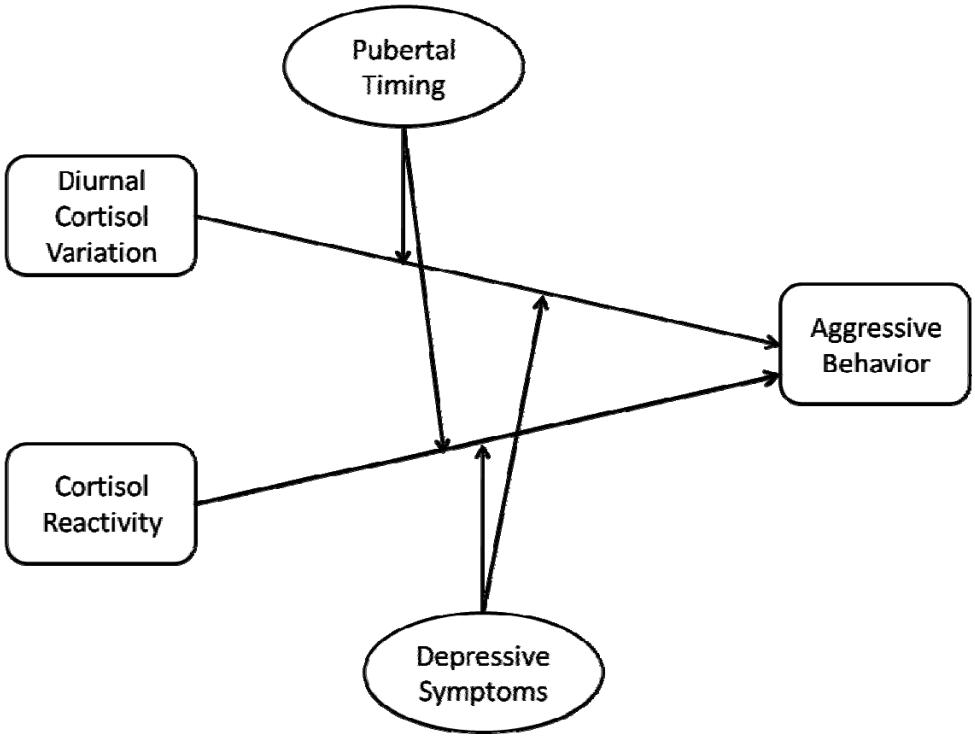

Figure 2.1 A conceptual model of HPA axis and psychosocial vulnerabities as interacting concomitants of aggressive behavior i.e. relational aggression and externalizing behavior

The results for relational aggression were compared with those of externalizing behavior, which has been widely examined in the existing literature. Based on the evidence offered here, it is argued that atypical HPA axis functioning, as characterized by a low diurnal variation in cortisol levels and an exaggerated or an attenuated cortisol response to a stressor will interact with the transitional stressor of timing of puberty. Such an interaction is proposed to place off-time adolescents at risk for relational aggression as well as for externalizing behavior. It is also argued that depressive symptoms will interact with atypical HPA axis functioning, as described above, such that depressed adolescents will exhibit more relational aggression. It was expected that externalizing behavior will also demonstrate a similar association. Specifically, it was hypothesized that: (1) off-time puberty in combination with a low diurnal cortisol variation and a heightened or attenuated response to a stressor would be related to relational aggression and externalizing behavior in young adolescents; (2) that adolescents who report more depressive symptoms and who have a low diurnal cortisol variation and a heightened or attenuated cortisol response to a stressor will also report greater use of relational aggression and more externalizing behavior; (3) that adolescents with off-time puberty in combination with high levels of depressive symptoms and low diurnal variation in cortisol levels or (4) that adolescents with off-time puberty in combination with high levels of depressive symptoms and a heightened or attenuated response to a stressor will exhibit more relational aggression and more externalizing behavior.

Methods

Participants

One hundred and thirty-five healthy children and adolescents and a parent or caregiver (89% mother, 9% father, 2% grandmother) participated in the current study. Girls were aged 8, 10 or 12 years (N=69, M=10.48 years, SD=1.56) and boys were aged 9, 11 and 13 years (N=66, M=11.49 years, SD=1.62). The age difference was designed so as to include boys and girls at similar stages of pubertal development since girls mature earlier than boys. Day-in-menstrual cycle was controlled as girls were assessed between days five and nine of their cycle. The racial/ethnic composition of the adolescents appears in Table 2.1, along with family socioeconomic status (SES) and other demographic characteristics of the sample. In brief, the sample is of interest as it is heterogeneous with regard to occupation and educational status and it was drawn from nonurban and non-suburban communities. Detailed study design, procedure and measurements have been previously reported (Susman et al., 2007) – see Appendix.

Measures

Diurnal Cortisol Variation. Participants were trained in a home saliva collection protocol before leaving a General Clinical Research Center (GCRC), where there were instructed to collect all samples on a non-school day within two weeks of the lab visit, when there were no other scheduled activities, such as sports, that would interfere with the collection protocol. Adolescents were also instructed to arise on the collection day at the same time of arising as on a school day and a parent was available

at home to supervise saliva collection. For each saliva collection, they were instructed to rinse their mouths with water before passively drooling into a 5 mL tube and to collect saliva up to the 4 mL mark on the tube for five minutes. The tube was then to be placed in the refrigerator and the time of collection recorded. Any activities that could affect cortisol levels (e.g., sports) or potential stressors (e.g. an argument with siblings) that occurred that day were noted.

Table 2.1 Demographic Characteristics of the sample for boys and girls

	Girls (N=69)		Boys (N=66)	
	Mean	SD	Mean	SD
Age (years, days)	10.48	1.56	11.49	1.62
Family SES	49.29	11.88	48.55	10.38
Racial Characteristics (%)				
White/Non-Hispanic	91		91	
Hispanic	4		3	
African American	4		3	
Asian American	1		3	
Tanner Stage				
1	25		10	
2	13		22	
3	23		17	
4	3		9	
5	2		2	
Refused	3		6	

Note. SES determined by Hollingshead (1975) scale.

Three samples of saliva were collected at 20-minute intervals on awakening and prior to breakfast or brushing teeth. Sample 1 was obtained immediately

on awakening, Sample 2 at 20 minutes post-wake time, and Sample 3 at 40 minutes post-wake time. Participants are requested not to eat or drink anything (except water) during the morning collection procedure. Sample 4 was collected prior to the midday meal, Sample 5 at 4.00 PM and Sample 6 at bedtime.

Diurnal cortisol variation was calculated by dividing the mean of the three morning cortisol levels by the bedtime cortisol level. Morning cortisol levels were the highest cortisol measurements whereas the bedtime sample (Mean time of collection=20:58 hours, SD=2:50) levels were the lowest (see Figure 2.2). Therefore these measures provided the most extremes in cortisol levels. The diurnal cortisol variation variable was then log-transformed for analysis in order to address issues of normality. The raw scores are reported in Table 2.2.

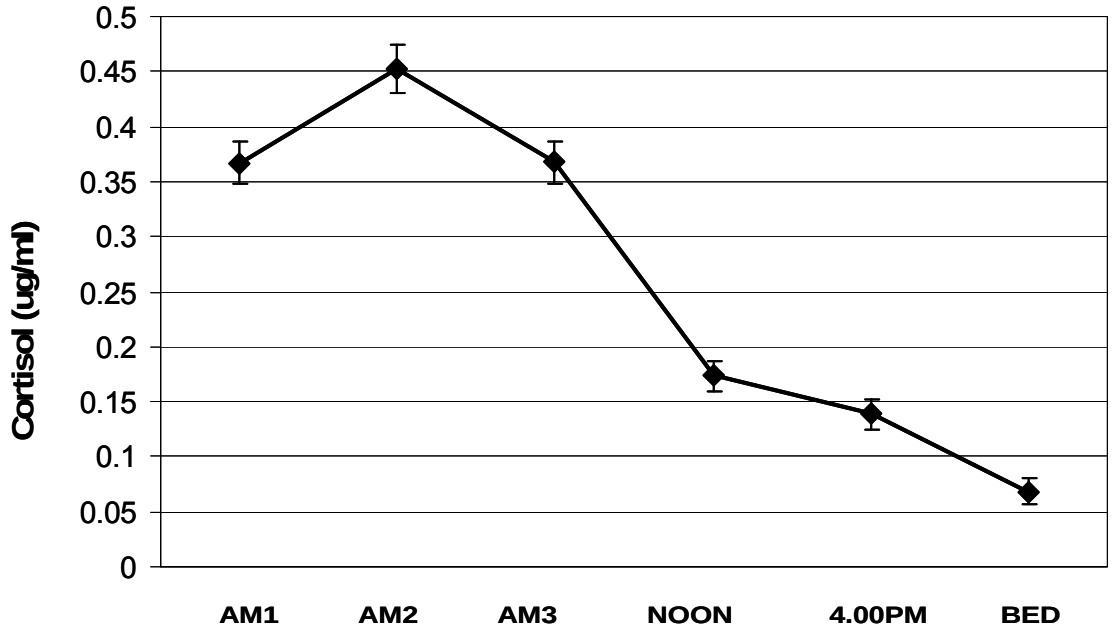

Figure 2.2 Mean cortisol levels across the day

Cortisol Reactivity. Stress reactivity was measured by the total increase over the baseline cortisol level during the GCRC lab visit. A total of five saliva samples was collected, two prior to the administration to the Trier Social Stress Test for Children (TSST-C) (Kirschbaum, Pirke, & Hellhammer, 1993) and three post-TSST-C. The TSST-C is a common method used to elicit an HPA stress response in a laboratory environment and includes both a cognitive and social evaluative challenge (Dickerson & Kemeny, 2004; Kirschbaum, Pirke, & Hellhammer, 1993; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). The TSST-C consists of a story completion task before two confederate judges. Subjects are told that their story would be evaluated in relation to the stories of other children their age, as well as a mental arithmetic task, which is the completion of an age-graded serial subtraction.

The first saliva sample was collected at Time T-20 minutes, the second, Sample 2, was collected at T-5 minutes; Sample 3, immediately post-TSST-C, T0; Sample 4 at T+10 minutes and Sample 5 at T+20 minutes (See Figure 3).

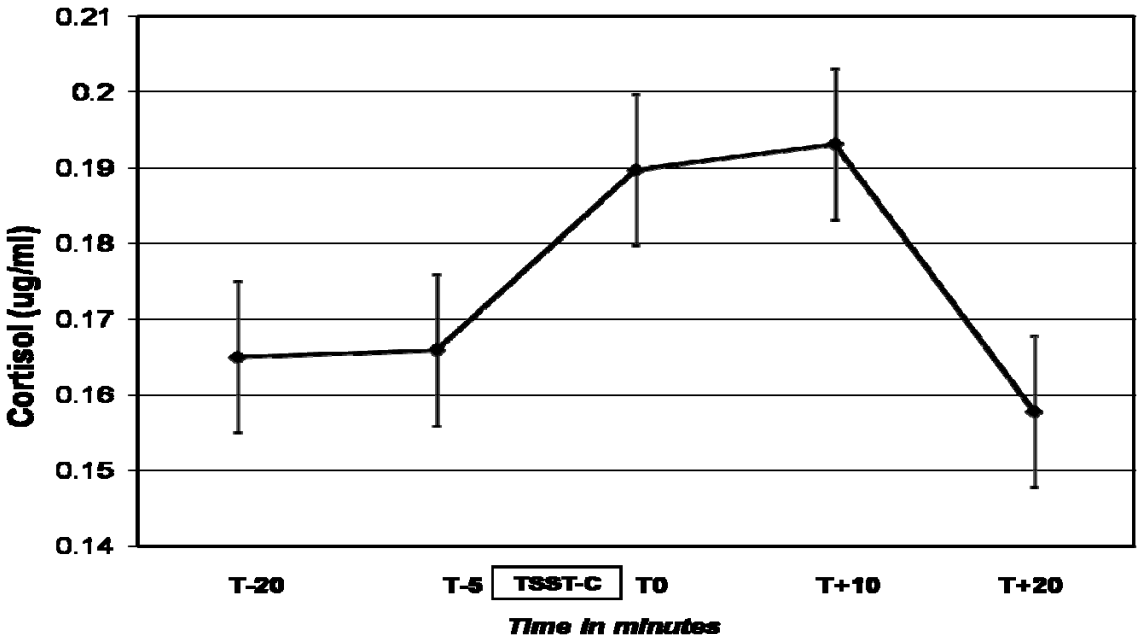

Figure 2.3 Mean laboratory cortisol profile, with standard errors, pre- and post- TSST-C

Samples were assayed for salivary cortisol, using a highly sensitive enzyme immunoassay (Salimetrics, State College, PA). Fifty microliters of saliva were used in duplicate determination, with a sensitivity range of 0.007 to 1.8 µg/dL (0.19 to 49.7 nmol/L). Intra- and inter-assay coefficients of sensitivity ranged between 5 and 10%. Cortisol reactivity was determined using area under the curve increase (Preussner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) in order to reflect change in cortisol levels post-TSST-C. Area under the curve increase (AUC*i*) allowed for the estimation of changes in cortisol subsequent to the stressor (m=sample measurement, t=time interval between measurements):

$$AUC_I = \left(\sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i) \cdot t_i}{2} \right) - \left(m_1 \cdot \sum_{i=1}^{n-1} t_i \right)$$

Timing of Puberty. This measure was used as an index of transitional stress and was calculated by comparing the pubertal stage of each subject to that of their peers of the same age and sex. Pubertal stage was assessed by a pediatric research nurse using Tanner criteria of genital and pubic hair stage for boys and breast and pubic hair stage for girls (Marshall & Tanner, 1969, 1970). After explaining the stages of puberty and showing the adolescent and parent pictures of each stage, adolescents and a parent then rated the adolescent stage of pubertal development. The nurse then did a physical exam to establish Tanner stage, which included breast palpation (Kaplowitz & Oberfield, 1999). If the adolescent did not consent to the physical exam (N=9), the adolescent's self-rating of pubertal development was substituted for the nurse rating. The correlation between the nurse rating and adolescent rating of breast (girls) or genital Tanner stage (boys) was $r=0.77$, $p<.01$. Breast and genital stage were used in the analysis given that these growth parameters are a direct reflection of gonadal hormone exposure. Pubertal timing was then calculated by regressing pubertal stage (genital or breast) on chronological age and calculating a residual timing score for each individual, which were then used in the analysis (Dorn, Susman, & Ponirakis, 2003). A residual score indicates the distance between the pubertal stage of the individual and the regression line for the whole sample. A negative residual indicates later timing of puberty (i.e. less development than expected) and a positive residual indicates earlier timing of puberty (more development than expected).

Relational Aggression. Relational aggression was assessed by the Children's Social Behavior Scale (CSBS) created by Crick and Grotpeter (1995).

Reliability was acceptable with a standardized Cronbach's alpha of 0.66. A higher score indicates a higher degree of self-reported relational aggression.

Externalizing Behavior: Child Behavior Checklist. The measure of antisocial behavior was the externalizing score on the Child Behavior Checklist (CBCl). The CBCl is a well standardized, norm-referenced scale of behavior problems completed by parents (Achenbach, 2001). Parents rate their child's behavior on a three-point scale (0=Never, 1=Sometimes, 2=Often) for 113 behavioral and emotional problems. Both the aggressive behavior and the rule breaking behavior scales contribute to the final external score. The score for externalizing behavior was used in the current analysis (α=0.98).

Children's Depression Inventory (CDI). Childhood and adolescent depressive symptoms was assessed by the Children's Depression Inventory (Kovacs, 1983). The CDI is a self-report, symptom-oriented scale that was designed for school-aged children and adolescents. For each item, the subjects are asked to choose one sentence that best describes them in the last two weeks in each of the 27 items. For example, subjects would pick one of the following sentences that best described how they felt in the past two weeks: 'I am sad once in a while; I am sad many times; I am sad all the time'. A higher total score from the summed items indicates more depressive symptoms. Reliability in this study was good with a standardized Cronbach's alpha of 0.86. CDI scores were log-transformed for analysis but raw scores are reported in Table 2.2.

A*nalysis Plan.* All analyses were carried out using SPSS 15.0 for Windows. The initial step in the analysis was to identify potential confounding influences on the relationship between vulnerabilities, timing of puberty and aggressive behavior. First, the effect of prescription and over-the-counter drugs on aggressive behavior was examined (Hibel, Granger, Kivlighan, & Blair, 2006). There were no significant mean group differences between those on versus off prescription drugs (e.g., CNS stimulants, psychotropic drugs, antihistamines). In addition, there were no correlations between taking over the counter or prescription drugs and timing of puberty, cortisol or depressive symptoms. Second, the relationship between the externalizing behavior and relational aggression variables and SES was examined but SES was not correlated with any of the antisocial variables or relational aggression. Chronological age was not included in the analysis as it was incorporated into the timing of puberty variable. Finally, the hypotheses were tested using hierarchical regression analysis with relational aggression and externalizing behavior as the outcomes. A *p* level of 0.1 was judged to be appropriate when assessing interactions, given the documented difficulty in detecting interactions in nonexperimental designs (McClelland & Judd, 1993) as well as the exploratory nature of our study. Nonsignificant interactions ($p>0.1$) were removed from the model, according to their hierarchy i.e. nonsignificant three-way then two-way interactions were removed from the model, until the most parsimonious model was identified.

Table 2.2 Descriptive statistics of the present sample used in analysis

	Combined Sample (N=135)		Girls (N=69)		Boys (N=66)	
	Mean	SD	Mean	SD	Mean	SD
Salivary Cortisol Awakening:Bedtime Cortisol Ratio	14.45	16.88	12.62	11.83	16.15	20.46
Cortisol Reactivity	1.23	1.20	1.27	1.50	1.18	0.76
Pubertal Timing	-0.01	0.99	-0.02	0.99	-0.00	0.99
Total CDI Score	3.81	4.03	3.90	4.33	3.71	3.73
Relational Aggression	7.95	2.95	7.91	3.20	7.98	2.70
CBCl External Score	4.26	5.26	4.59	5.46	3.93	5.08

Note. The untransformed sample means are displayed above. Log-transformed means for awakening:bedtime cortisol ratio and Total CDI score were used in the analysis.

Results

The demographic characteristics and Tanner stage distribution of the sample appear in Table 2.1.

Correlations. Pearson product-moment correlations were computed for child sex, pubertal timing, depressive symptoms, diurnal cortisol variation, cortisol response to a laboratory stressor, relational aggression and externalizing behavior (See Table 2.3). Depressive symptoms were significantly related to both relational aggression

and externalizing behavior and there was a moderate correlation between relational aggression and the diurnal cortisol variation.

Table 2.3. Pearson product-moment correlations for the variables used in analysis

	1	2	3	4	5	6	7
1. Sex	-	-0.009	-0.003	-0.147	0.015	-0.013	-0.063
2. Pubertal Timing		-	0.167[a]	-0.174[a]	-0.117	0.081	0.120
3. Total CDI Score			-	-0.107	0.065	0.203**	0.287**
4. Diurnal Cortisol Variation				-	.191*	.174[a]	-.027
5. Cortisol Response					-	-0.139	-0.023
6. Relational Aggression						-	0.032
7. Externalizing Behavior							-

Note. (**p<0.01,*p<0.05, [a]p<0.10)

Hypothesis testing. Hierarchical linear regression techniques were used to test the hypotheses. In each model, the biological vulnerabilities were entered first, followed by pubertal timing, then depressive symptoms and finally, the interaction terms. Sex differences were evaluated in each model by including sex as a moderator. When sex was not a significant moderator, it was removed from the model (See Tables 2.4 and 2.5). Significant interactions were probed further using the procedures proposed by Aiken & West (1991) with the slope of the relationship between the two interacting variables plotted at 1 SD above and below the mean, as well as at the mean of moderator.

Relational Aggression. The interaction between diurnal cortisol variation and pubertal timing was significant ($\beta = -0.33$, $p < .05$; $F = 2.87$, $R^2 = 0.07$, $p < .05$). At

one SD below the mean, as well as at the mean slope of pubertal timing, the simple slope of the relation between awakening to bedtime cortisol ratio and relational aggression was null, whereas at one SD above the mean, the relation was negative and significant, (B = -1.69, t = -2.47, p = .02). Relationally aggressive children and adolescents were characterized by a low variation between awakening and bedtime cortisol levels, indicating low diurnal cortisol variation in earlier timing adolescents who exhibit relationally aggressive behavior (See Figure 2.4). No sex differences were found in this relationship.

Externalizing Behavior. The main and interactive effects of timing of puberty, diurnal cortisol variation and cortisol response were not significant.

Hypothesis 2. It was examined whether adolescents who report more depressive symptoms and who have a lower diurnal cortisol variation and a heightened or attenuated cortisol response to a stressor will report greater use of relational aggression as well as more antisocial behavior. Diurnal cortisol variation, cortisol response, depressive symptoms and their interactions were regressed on: (1) relational aggression, (2) externalizing behavior.

Table 2.4 Hierarchical regression of relational aggression on diurnal cortisol variation, cortisol response, pubertal timing, depressive symptoms and their interactions.

Relational Aggression			
Model 1	β	F	R^2
Cortisol Variation	-.10		
Pubertal Timing	.35*		
Cortisol Variation * Pubertal Timing	-.33*		
		2.87*	.07
Model 2			
Cortisol Variation	-.29*		
Cortisol Response	.46[a]		
Cortisol Variation * Cortisol Response	-.50[a]		
		2.45[a]	.07
Model 3			
Cortisol Response	-.48**		
Depressive Symptoms	.31**		
Cortisol Response * Depressive Symptoms	.39*		
		4.84**	.08

Note. Only significant full models are shown above. **p<0.01, *p<0.05, [a]p<0.1

Relational Aggression. There was a significant interaction effect between diurnal cortisol variation and the cortisol response to the TSST-C (β = -0.50, p < .10; F = 2.46, R^2 = 0.07, p < .01). At one SD below the mean of cortisol response, the simple slope of the relation between diurnal cortisol variation and relational aggression was null. The mean slope of the relation between diurnal cortisol variation and relational aggression showed only trend significance (B = -1.12, t = -1.81, p = .07), while at one SD above the mean of cortisol response, the simple slope of the relation between diurnal cortisol variation and relational aggression was negative and significant (B = -2.82, t = -2.64, p = .01). Relationally aggressive children and adolescents were

characterized by an increased cortisol response to a laboratory stressor and a low diurnal cortisol variation (See Figure 2.5). No sex differences were found in this relationship.

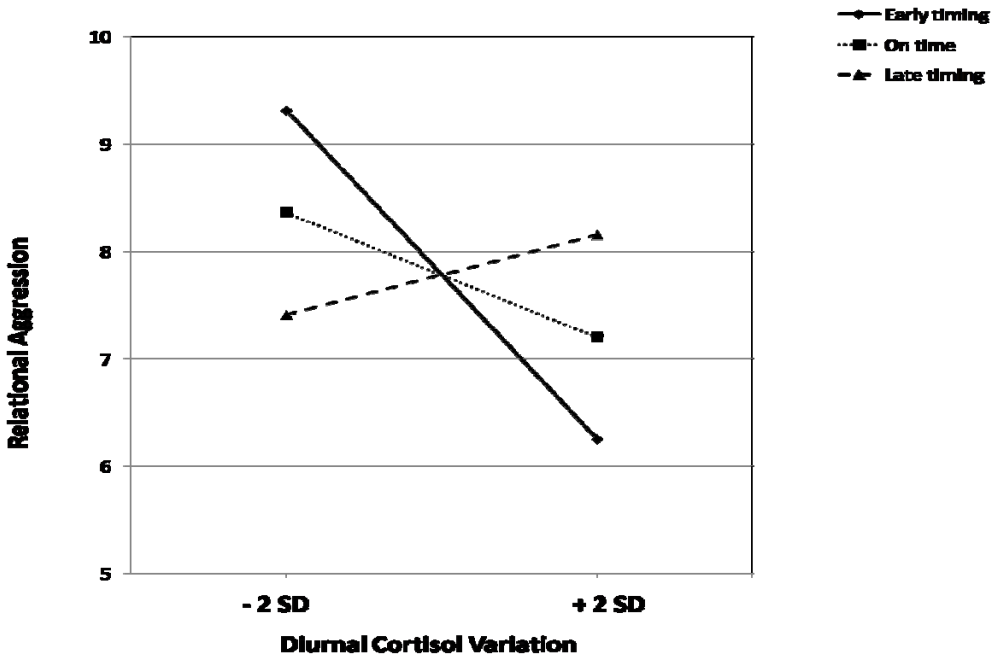

Figure 2.4 The interaction of diurnal cortisol variation,pubertal timing and relational aggression

Externalizing Behavior. The interaction between diurnal cortisol variation, cortisol response to the TSST-C and depressive symptoms was significant when externalizing behavior was examined ($\beta = -1.08$, $p < .05$; $F = 2.32$, $R^2 = 0.14$, $p < .05$). In girls reporting high levels of depressive symptoms, at one SD below the mean of cortisol response, the simple slope of the relation between diurnal cortisol variation and externalizing behavior was positive and significant ($B = 12.39$, $t = 2.71$, $p = .01$). Depressed girls with an attenuated response to a laboratory stressor but with a high

variation in their diurnal cortisol levels exhibited the most externalizing behavior problems.

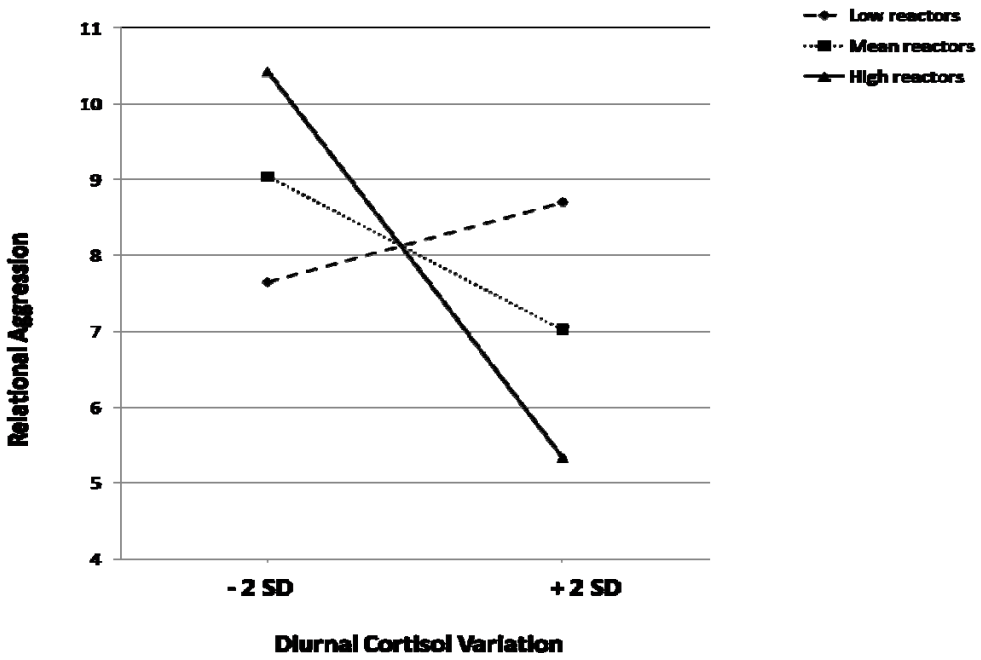

Figure 2.5 The interaction of diurnal cortisol variation,cortisol response to a laboratory stressor and relational aggression

Table 2.5 Hierarchical regression of relational aggression on diurnal cortisol variation, cortisol response, pubertal timing, depressive symptoms and their interactions.

Externalizing Behavior			
Model 1	β	F	R^2
Cortisol Variation	.36		
Cortisol Response	-.65		
Depressive Symptoms	.91*		
Cortisol Variation * Cortisol Response	.64		
Cortisol Response * Depressive Symptoms	1.08[a]		
Cortisol Variation * Depressive Symptoms	-.78[a]		
Cortisol Variation * Cortisol Response * Depressive Symptoms	-1.08*		
		2.32*	0.14
Model 2			
Cortisol Variation	-.04		
Depressive Symptoms	.24		
Pubertal Timing	-1.28		
Cortisol Variation * Pubertal Timing	.99[a]		
Cortisol Variation * Depressive Symptoms	.04		
Depressive Symptoms * Pubertal Timing	1.48*		
Cortisol Variation * Depressive Symptoms * Pubertal Timing	-1.12[a]		
		2.23*	0.13
Model 3			
Cortisol Response	.11		
Depressive Symptoms	.26**		
Pubertal Timing	.19		
Cortisol Response * Pubertal Timing	.55		
Cortisol Response * Depressive Symptoms	-.03		
Depressive Symptoms * Pubertal Timing	-.18		
Cortisol Response * Depressive Symptoms * Pubertal Timing	-.71[a]		
		2.48*	0.13

Note. Only significant full models are shown above. **p<0.01, *p<0.05, [a]p<0.1

Hypothesis 3. To examine whether off-time puberty in combination with high levels of depressive symptoms and low diurnal variation in cortisol levels also report greater use of relational aggression as well as more use of externalizing behavior, diurnal cortisol variation, pubertal timing, depressive symptoms and their interactions were regressed on: (1) relational aggression, (2) externalizing behavior.

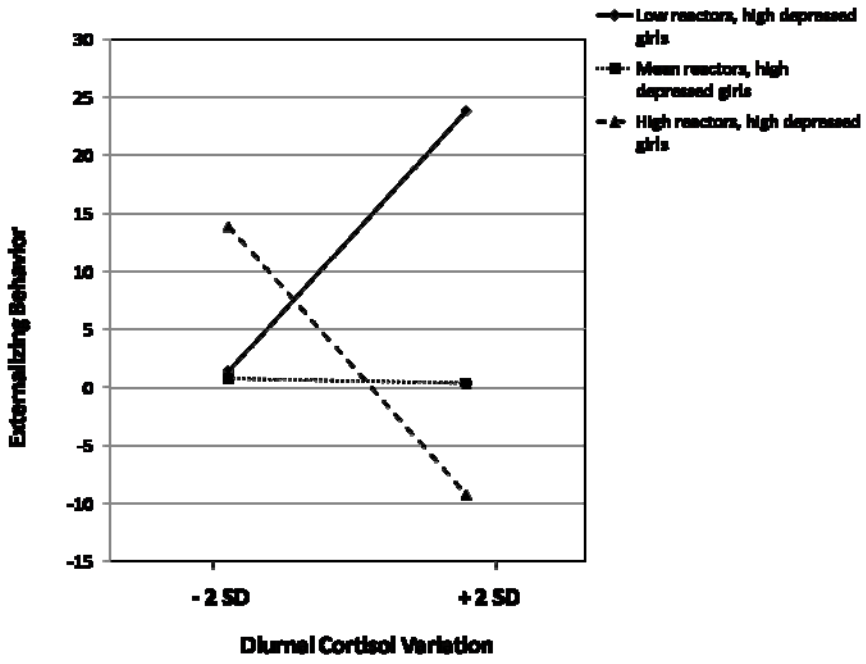

Figure 2.6 The interaction of diurnal cortisol variation and cortisol response to a stressor in depressed girls

Relational Aggression. The main and interactive effects of diurnal cortisol variation, timing of puberty and depressive symptoms were not significant.

Externalizing Behavior. The interaction between diurnal cortisol variation, timing of puberty and depressive symptoms was significant when externalizing behavior was examined ($\beta = -1.12$, $p < .10$; $F = 2.23$, $R^2 = 0.13$, $p < .05$). However, further post-

hoc probing using the Aiken and West procedures (Aiken & West, 1991) did not elucidate this three-way interaction.

Hypothesis 4. To examine whether off-time adolescents with high levels of depressive symptoms and a heightened or attenuated response to a stressor will exhibit more relational aggression and externalizing behavior, cortisol response, timing of puberty, depressive symptoms and their interactions were regressed on: (1) relational aggression, (2) externalizing behavior.

Relational Aggression. There was a significant interaction between the cortisol response to the TSST-C and depressive symptoms ($\beta = 0.39$, $p < .05$; $F = 4.84$, $R^2 = 0.11$, $p < .01$). At one SD below the mean of depressive symptoms, the simple slope of the relation between cortisol response and relational aggression was negative and significant ($B = -.19$, $t = -2.94$, $p = .004$). At the mean slope of depressive symptoms, the relation between cortisol response and relational aggression showed only trend significance ($B = -.08$, $t = -1.76$, $p = .08$), while at one SD above the mean of cortisol response, the simple slope of the relation between cortisol response and relational aggression was null. Relationally aggressive children and adolescents were characterized by an increased cortisol response to a laboratory stressor and low reported levels of depressive symptoms (See Figure 2.7). No sex differences were found in this relationship.

Externalizing Behavior. The interaction between cortisol response to the TSST-C, timing of puberty and depressive symptoms was significant when antisocial behavior was examined ($\beta = -1.83$, $p < .10$; $F = 2.48$, $R^2 = 0.13$, $p < .02$). Late

timing boys with high levels of depressive symptoms and a heightened response to a stressor exhibited the most externalizing behavior (B = .466, t = 2.24, p = .03). See Figure 2.8.

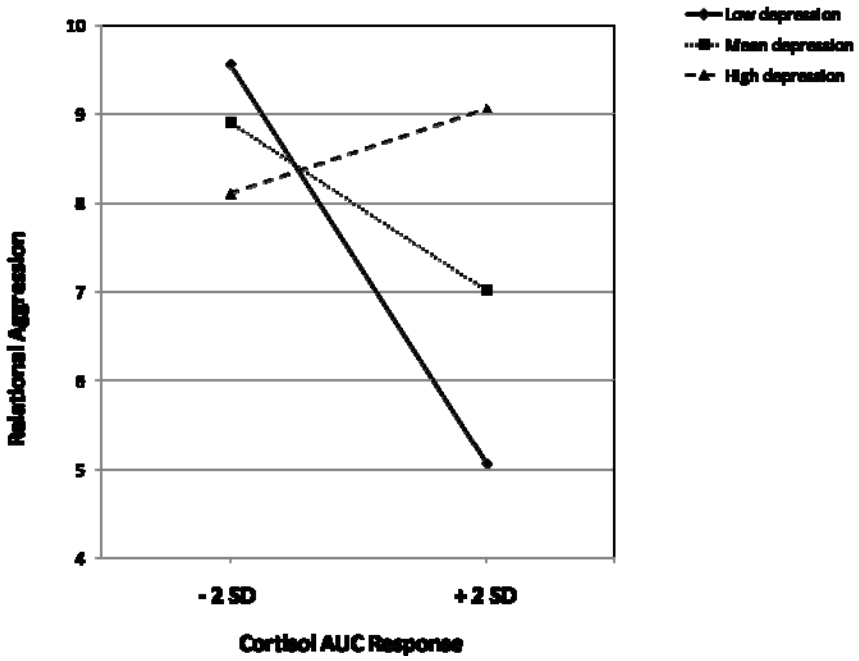

Figure 2.7 The interaction of cortisol response to a stressor and depression in relationally aggressive children

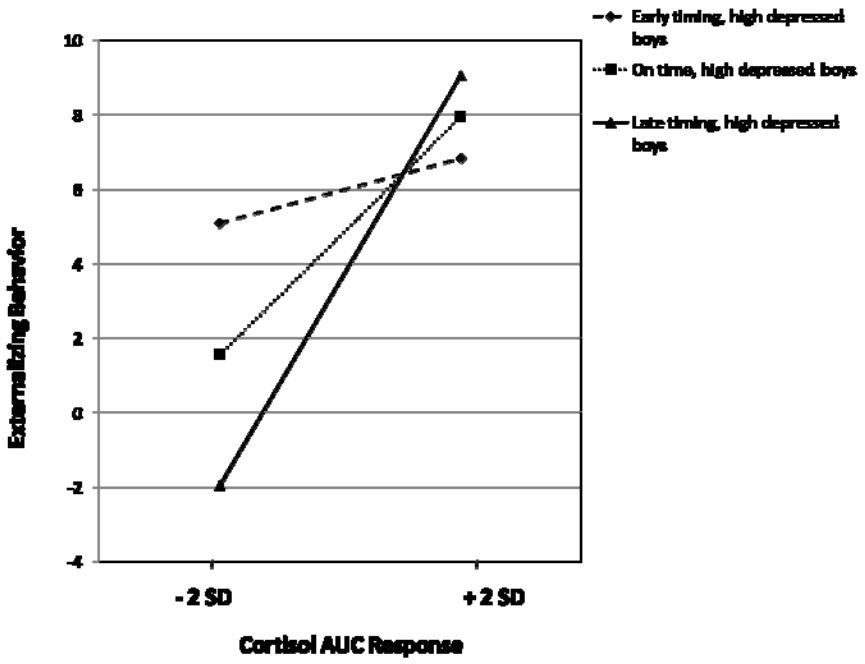

Figure 2.8 The interaction of cortisol response to a stressor, depression and pubertal timing in externalizing boys

Discussion

An interactionist developmental theoretical approach was used as a framework to develop hypotheses regarding HPA axis activity, pubertal timing, depression and aggressive behavior in young adolescents. Overall, the results showed that vulnerabilities of the HPA component of the stress system and their interaction with pubertal timing, in a model that included depressive symptoms were related to the use of more relational aggression and externalizing behavior. These findings are unique as they include the psychobiology of stress in the study of relational aggression and also compared the finding for relational aggression to those of the more commonly studied externalizing behavior. The findings also add to the current literature on HPA axis functioning and its relationship to relational aggression and externalizing behavior.

Four hypotheses were tested that were derived from the conceptual model (Figure 2.1) relating atypical HPA axis functioning to aggressive behavior. Support for the first hypothesis that a flattened diurnal rhythm of cortisol in earlier timing adolescents would predict more use of relational aggression was found. Early timing adolescents who had a low variation in cortisol levels across the day used more relational aggression than their counterparts. It appears that adolescents in whom these interacting vulnerabilities exist are those who are more likely to use relational aggression. According to Popma and colleagues (Popma et al., 2007), it is more disruptive boys who have a flatter diurnal rhythm when compared to normal controls. In addition, it was expected that earlier timing adolescents to exhibit more aggressive behavior as proposed by the transitional stress hypothesis (Angold, Costello, Erklani, & Worthman, 1999). Studies to date have examined vulnerabilities of the HPA axis and off-time pubertal development separately in their relation to physical aggression (Dorn, Susman, & Ponirakis, 2003; Fisher, 2001; Ge, Conger, & Elder, 1996; Popma et al., 2007; Susman, Dorn, Inoff-Germain, Nottleman, & Chrousos, 1997). However, in considering the interaction of these vulnerabilities in the use of relational aggression, the findings indicate that individuals in whom both exist are at a greater risk for the covert deviant behavior of relational aggression (Crick, 1995).

Early maturation seems to intensify the relationship between the diurnal variation of cortisol and relational aggression in our model. Specifically, early timing was associated with less diurnal variation in cortisol. Based on the theories proposed by Susman (2006) and Gunnar & Valquez (2001), atypical cortisol secretion may result from early exposure to environmental stressors. The social stress associated with

relational aggression suggests that concurrent stressors like depression may alter cortisol variations.

It was also expected that depressed adolescents with less diurnal cortisol variation as well as a higher cortisol response to a laboratory stressor would use more relational aggression and more externalizing behavior. The interaction between these two vulnerabilities was examined as it has been shown that both a flattened diurnal rhythm and heightened stress sensitivity are associated with depression as well as indicators of hypocortisolism (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Heim, Ehlert, & Hellhammer, 2000; Preussner, Hellhammer, & Kirschbaum, 1999; Smythe et al., 1997; Stone et al., 2001). The results show that the interaction between a lowered diurnal cortisol variation and a heightened response to a laboratory stressor was associated with the use of more relational aggression. Similar results were also evident for externalizing behavior but only in girls who reported higher levels of depressive symptoms. The most externalizing behavior was associated with two types of depressed girls – those with a high diurnal variation of cortisol but an attenuated response to a laboratory stressor and those with a low diurnal cortisol variation and a heightened response to the laboratory stressor. Evaluations of the separate roles of the diurnal rhythm of cortisol and heightened stress reactivity with aggressive and antisocial behavior have shown a low diurnal cortisol variation, low basal cortisol levels or an attenuated cortisol response to a stressor that was associated with aggressive and antisocial behavior (McBurnett, Lahey, Capasso, & Loeber, 1996; Pajer, Gardner, Rubin, Perel, & Neal, 2001; Popma et al., 2007; Susman, Dorn, Inoff-Germain, Nottleman, & Chrousos, 1997). Anomalous studies had found higher basal cortisol

levels in boys with an aggressive form of CD (van Bokhoven et al., 2005) and a heightened cortisol response in anxious externalizing boys (Van Goozen et al., 1998). In addition, males were the main participants in these earlier studies, although it has been shown that males and females respond differently to stressful situations (Stroud, Salovey, & Epel, 2002; Taylor et al., 2000). No evaluations of aggressive behavior and the interaction between the diurnal cortisol variation and cortisol response to a laboratory stressor have been reported and so the study presents a new and unique perspective on the influences of aggressive behavior in adolescence. Future studies are needed to shed light on the interaction between the diurnal cortisol variation and stress response and their association to relational aggression. These reports need to be replicated in both males and females as basal and reactive levels may be the result of different types of experiences.

Third, it was expected that off-time adolescents with higher levels of depression symptoms and a lower diurnal variation in cortisol levels would also report greater use of relational aggression and externalizing behavior. However the results were not significant for relational aggression. Further exploration of the significant interaction between timing of puberty and depression symptoms and externalizing behavior could not be examined using the post hoc methods suggested by Aiken and West (1991). The results are however provocative in that they demonstrate the salience of using an interactive framework when investigating the psychobiology of aggressive and antisocial behavior.

Finally, it was expected that off-time adolescents with high levels of depressive symptoms and a heightened cortisol response would exhibit more

relationally aggressive behavior and more externalizing behavior. This expectation was borne out for relationally aggressive adolescents with a heightened cortisol response regardless of their pubertal timing or sex. However, it was only true for those relationally aggressive adolescents who had reported low levels of depressive symptoms. These unexpected findings may be the result of the questionnaire methods used in the current study. The goals of relational aggression include the maintenance of position or rank within a social group and avoidance of punishment. Inherent in these goals may be a social desirability component. The subjects completed the CDI (Kovacs, 1983) with the interviewer present in the room and it is possible that these adolescents responded to the items based on how they wish to be perceived. On the other hand, the CDI items tend to be absolute and directed, e.g. I am sad all the time and the Child Social Behavior Scale (Crick & Grotpeter, 1995) is more conditional, e.g. “When I am mad at a person, I ignore or stop talking to them” In contrast, when the outcome was externalizing behavior, the significant interaction of these vulnerabilities held only for depressed, late timing boys with a heightened cortisol response to a stressor. Studies have shown that adolescents who are off-time, and in particular those who are late in their pubertal development, sometimes show delinquent behavior (Williams & Dunlop, 1999), have more evidence of psychopathology (Graber, Seeley, Brooks-Gunn, & Lewinsohn, 2004) and are more at risk for depression (Michaud, Surio, & Deppen, 2006). These findings therefore demonstrate a novel fusion of the literature on the association of both HPA axis functioning and psychosocial factors with aggressive behavior.

Overall, the results demonstrate the usefulness of using an interactionist framework when examining individual differences in relational aggression and externalizing behavior. Less variation in cortisol levels from morning to evening is only a moderate correlate of relational aggression and externalizing behavior. However, its interaction with other vulnerabilities, that is, heightened cortisol reactivity, pubertal timing and depressive symptoms, makes it especially potent when the use of relational aggression and externalizing behavior are considered. Gunnar & Vazquez (2001) noted that hypocortisolism can also be found in children reared in unpredictable environments, such as those in which they were maltreated, neglected or exposed to early trauma. It is therefore possible that these children are more at risk for the use of both relational aggression and externalizing behavior, especially if the vulnerabilities of early pubertal timing and depressive symptoms are present.

Previous literature has tended to focus primarily on overt aggression and externalizing behavior and the findings were mainly for males, with few exceptions (Pajer, Gardner, Rubin, Perel, & Neal, 2001). It is important to note that the current findings were significant in a non-clinical, community-based sample that included both boys and girls. Sex was not a significant moderator within the linear model when relational aggression was examined. These findings are consistent with a recent meta-analysis which, in suggesting a gender-similarities hypothesis, demonstrated that the magnitude of sex differences in psychological functioning may have been smaller than originally proposed (Hyde, 2005). In addition, relational aggression is generally recognized as a female-typical aggression. These findings demonstrate that use of relational aggression is associated with an interaction of biological and psychosocial

vulnerabilities in both boys and girls. On the other hand, sex differences were evident in the linear models that included externalizing behavior. However, these findings support the 'deviance hypothesis', where it was depressed off-time girls and boys that were the most externalizing (Angold, Costello, Erklani, & Worthman, 1999; Brooks-Gunn, Petersen, & Eichorn, 1985; Caspi & Moffitt, 1991; Petersen & Taylor, 1980). Nevertheless, vulnerabilities of the HPA axis also interacted with the psychosocial vulnerabilities of pubertal timing and depression to increase the likelihood of these adolescents demonstrating the use of more externalizing behavior.

There are limitations that arise in this study. Since social desirability and avoidance of punishment are inherent in the goals of persons who use relational aggression, there may have been an under-reporting of relational aggression. In addition, cortisol measurements were obtained on one day only and events occurring on the sampling day may have elevated or even lowered the cortisol levels at particular times of the day. However, two factors should be noted. First, the diurnal rhythm of cortisol has been shown to be stable across time and varied very little from an initial measurement (Smythe et al., 1997; Stone et al., 2001). The second is that a questionnaire was completed by the adolescents in which they reported the time of sample collection as well as if there were any noteworthy events that occurred on the day of sampling. No events were reported that might have significantly affected cortisol levels during the day. A second limitation recognizes the pubertal transition as a period where there is a rise in both gonadal hormones and adrenal androgens. The assessment of gonadal hormones as a further vulnerability of aggressive behavior is beyond the scope of the present study but provocative enough to stimulate its

investigation in future studies. Finally, the demographics of our sample limit our ability to generalize our findings to other racial/ethnic groups. The current study opens the door to replicate these findings in other racial/ethnic groups. Nonetheless, the study is innovative in that it tested hypotheses that considered the main and interactive effects of biological and psychosocial vulnerabilities associated with relational aggression, as opposed to the more often studied overt aggression.

The findings of this study parallel those on biopsychosocial correlates of overt forms of aggressive behavior and also add to the literature of both relational aggression and atypical HPA axis functioning. The cross-sectional nature of this study allows for the recognition of concurrent interactive associates of relational aggression. It is a cause for concern that a behavior considered harmless by many and mainly of the female domain could be associated with atypical HPA axis functioning at a vulnerable time of life when depressive symptoms start to rise. Further investigation is needed to examine the possible trajectories of physical and emotional health that might occur as a consequence of continued use of relational aggression and its continued association with atypical HPA axis functioning and other psychosocial vulnerabilities. It is especially important to continue to assess such a behavior within an interactionist framework.

Chapter 3

Paper 2

Relational aggression and externalizing behavior as predictors of diurnal cortisol variation across 12 months: The moderating influence of sex, pubertal timing and depression

Abstract

This report is based on a theoretical framework that considers dynamic integration of aggression, timing of puberty and externalizing behavior problems to examine the longitudinal effects of aggressive behavior on HPA axis activity, within the pubertal context. Specifically, the following hypotheses were tested: (1) higher levels of relational aggression will predict less diurnal cortisol variation, as moderated by off-time pubertal timing and depression across 12 months, especially in off-time, depressed girls and (2) higher levels of externalizing behavior will predict less diurnal cortisol variation, as moderated by off-time pubertal timing and depression across 12 months, especially in off-time, depressed boys. The sample consisted of 8- to-13-year-old boys and girls (N= 135) measured at three waves of measurement, every 6 months. Relational aggression predicted less diurnal cortisol variation over the 12 months in earlier timing, depressed adolescents. Externalizing behavior predicted a steadily decreasing diurnal cortisol variation over 12 months in early timing adolescents with lower levels of depression. Between-group differences indicate that girls who use relational aggression or externalizing behavior had less diurnal variation in cortisol levels over 12 months. These findings indicate the importance of employing an interactionist framework when investigating biobehavioral development.

Introduction

Aggressive behavior is a major public health concern and its development in childhood and adolescence has been found to lead to alcohol and substance use disorders (Alati et al., 2008; Gustavson et al., 2007; Tremblay, Mihic, Graham, & Jelley, 2007), domestic violence (Ehrensaft, Mofitt, & Caspi, 2004) and mental health disorders in adulthood (Eme, 2007; Farchiore et al., 2007; Lahey, Loeber, Burke, & Applegate, 2005). Furthermore, aggressive behavior, with its goals of instrumentality (using weapons with an intent to harm) and physical dominance, has been found to maintain moderate stability from childhood and adolescence into adulthood (Brame, Nagin, & Tremblay, 2001; Hofstra, Van der Ende, & Verhulst, 2000; Loeber, 1982; Nagin & Tremblay, 1999). The more recently studied relational aggression uses the social relationship as a vehicle of harm and employs covert strategies such as gossip and social exclusion. Relational aggression maintains some stability from preschool to adolescence (Bowie, 2007; Ostrov, 2008; Tomada & Schneider, 1997). Studies have found that use of this behavior also may increase from childhood to adolescence and into early adulthood (Cote, Vaillancourt, Barker, Nagin, & Tremblay, 2007; Murray-Close, Ostrov, & Crick, 2007). The identification of relational aggression in college samples has led to findings of its association with depression, alcohol and drug use problems (Storch, Bagner, Geffken, & Baumeister, 2004) and bulimic symptoms (Werner & Crick, 1999). The use of aggressive behavior in adolescence, regardless of type, is thus implicated in several adverse mental health outcomes in early adulthood.

Aggressive behavior has been consistently associated with perturbations of the hypothalamic-pituitary-adrenal (HPA) axis of the physiological stress system.

These studies have included cortisol, a steroid hormone that is easily measured in saliva (Kirschbaum & Hellhammer, 1989) as an indicator of HPA axis activity. Cortisol is the final hormone product of a cascade of physiological events within the HPA axis in response to intrinsic and extrinsic environmental disruptions. Cortisol also has diurnal variations and reacts to environmental challenges (Tsigos & Chrousos, 2002). Cortisol levels are highest in the morning on awakening, decreasing steadily across the morning and reaching the lowest levels toward the evening and night. The diurnal variation of cortisol is stable and reproducible (Selmaoui & Touitou, 2003), even across pubertal development (Knuttson et al., 1997; Matchcock, Dorn, & Susman, 2007). Individual differences in a small percentage of the population have been reported, with 10% (Stone et al., 2001) to 17% (Smythe et al., 1997) of participants exhibiting flattened diurnal rhythms, i.e. less cortisol variation across the day. Earlier studies on aggressive behavior and cortisol that were carried out in delinquent boys demonstrated that boys with lower levels of salivary cortisol exhibited more aggressive symptoms when compared to boys with higher cortisol levels (McBurnett & Lahey, 2000; McBurnett, Lahey, Capasso, & Loeber, 1996). In girls, morning plasma cortisol was lower in those with conduct disorder as compared to those without conduct disorder, in the absence of other psychiatric disorders (Pajer, Gardner, Rubin, Perel, & Neal, 2001). More recent studies have included examinations of the diurnal cortisol variation and physical as well as relational aggression (Blades, Dockray, & Susman, under review; Popma et al., 2007; Susman et al., 2007). These studies were carried out in both clinical and community-based samples and generally found that less variation in cortisol levels over the day was associated with disruptive disorder in boys (Popma et al., 2007) as well as

in children with more behavior problems (Susman et al., 2007). In early timing adolescents, a low diurnal cortisol variation and an exaggerated cortisol response to a stressor was identified in those who practiced relational aggression and in depressed girls who exhibited externalizing behavior (Blades, Dockray, & Susman, under review). HPA axis perturbations that include less variation in cortisol levels over the day and an exaggerated stressor response have been associated with both physical and relational aggression, and may be moderated by depression and pubertal timing over time.

The present study aimed to extend the literature by examining relational aggression and externalizing behavior, as predictors of diurnal variation in cortisol across 12 months. Since studies have shown that aggressive behavior is associated with low diurnal cortisol variation (Blades, Dockray, & Susman, under review; Popma et al., 2007; Susman et al., 2007), it was expected that the aggressive behavior and cortisol relationship to be moderated by sex, depression and pubertal timing (See Figure 3.1). Several studies have revealed that boys tend to prefer the use of physical and verbal aggression and girls prefer relational aggression in peer conflict (Crick, 1995, 1996, 1997; Ostrov & Keating, 2004; Werner & Crick, 2004; Werner & Nixon, 2005). However, sex differences in the effect of aggressive behavior on HPA axis activity have not been examined as extensively, even though boys and girls respond differently to stressors (Stroud, Salovey, & Epel, 2002; Uhart, Chong, Oswald, Lin, & Wand, 2006). In the same sample used in the current study, there were no sex differences in the cross-sectional association between relational aggression and cortisol (Blades, Dockray, & Susman, under review), even when depression and pubertal timing were considered as moderators of the relationship. On the other hand, sex differences were found when

externalizing behavior was the outcome. Late maturing, depressed boys with a heightened response to a stressor and depressed girls with an exaggerated stress response and a low diurnal cortisol variation were most externalizing. Possible longitudinal effects of aggressive behavior on diurnal cortisol variation within puberty were examined.

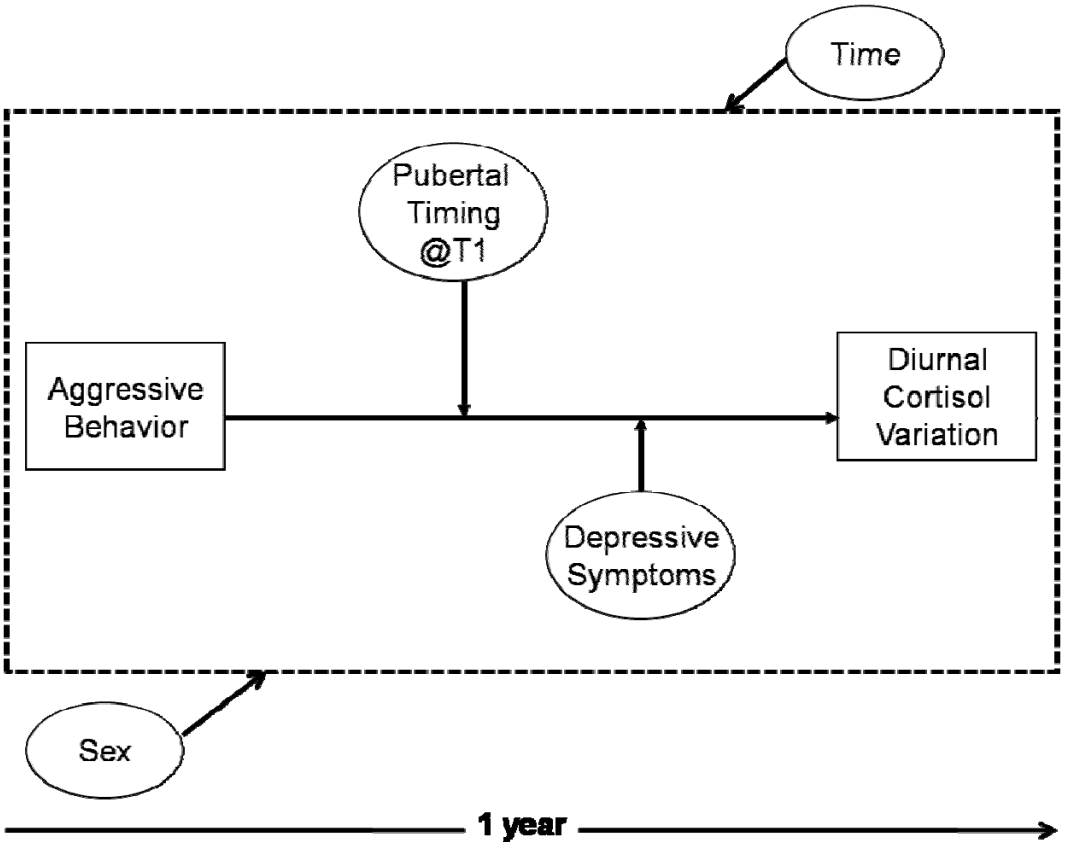

Figure 3.1 A conceptual model of sex, pubertal timing and depression as moderators of aggressive behavior and diurnal cortisol variation across 12 months

Puberty is a stressful transition and is a time when depression increases with increasing pubertal stage in healthy adolescents (Susman & Rogol, 2004). Furthermore, it is well known that when adolescents mature at a time not comparable to

their peers (i.e. off-time), they are more at risk for the development of depression and behavior problems (Brooks-Gunn, Petersen, & Eichorn, 1985). There is evidence that early-maturing adolescents are more vulnerable to deviant peer pressure and the development of depressive symptoms than later or on-time maturers (Ge, Brody, Conger, Simons, & Murry, 2002; Ge, Conger, & Elder, 2001a, 2001b; Warren & Brooks-Gunn, 1989). In contrast, later-maturing boys have been shown to exhibit more delinquency and to be at risk for more psychopathology and depression in some studies (Graber, Seeley, Brooks-Gunn, & Lewinsohn, 2004; Michaud, Surio, & Deppen, 2006; Williams & Dunlop, 1999). In addition, it has been found in a review of the depression and aggressive behavior literature that aggressive behavior shows a significant association with depression in children and adolescents. Furthermore, it was found that the rate of externalizing behavior is higher in depressed, as opposed to non-depressed adolescents (Angold & Costello, 1993). Relational aggression has also been continually associated with depression in children and adolescents. Several studies demonstrate that higher levels of depression are found in relationally aggressive children and adolescents (Crick & Grotpeter, 1995; Crick, Grotpeter, & Bigbee, 2002; Crick & Nelson, 2002; Werner & Crick, 1999). Therefore, it is possible that aggressive behavior, pubertal timing and depression might interact to increase the stress experienced by the adolescent. The confluence of these three factors may bring about frequent HPA arousal and have multiplicative, deleterious effects on overall HPA axis activity, in particular on the diurnal variation in cortisol over time.

Using a community-based sample, a theoretical framework is used that considers dynamic integration (Magnusson, 1999) across biological and behavioral

development in order to identify the longitudinal effects of aggressive behavior, both externalizing behavior and relational aggression, on the diurnal variation of cortisol in adolescents during the pubertal transition. In doing so, probable pathways may be elucidated that may lead to an understanding of the development of future psychopathology and substance use disorders.

The Current Study.

The present study aims to test the overall hypothesis that relational aggression and externalizing behavior will predict diurnal cortisol variation over the course of 12 months. Furthermore, it was expected that these associations will be moderated by sex, pubertal timing and depression (see Figure 3.1 for conceptual model). Since the literature shows that relational aggression and off-time pubertal timing are associated with internalizing problems and psychopathology especially in girls (Crick & Grotpeter, 1995; Crick & Zahn-Waxler, 2003; Ge, Conger, & Elder, 1996, 2001a), relational aggression was expected to predict less diurnal cortisol variation, especially in off-time, depressed girls. Conversely, given the wealth of aggressive and antisocial literature and HPA axis functioning in boys, it was expected that externalizing behavior will predict less diurnal cortisol variation in depressed, off-time boys. This is proposed given the literature demonstrating that off-time pubertal timing is associated with both externalizing and internalizing behavior problems in boys (Ge, Conger, & Elder, 2001b; Williams & Dunlop, 1999) and that aggressive behavior in boys is associated with low basal levels of cortisol (McBurnett & Lahey, 2000; Popma et al., 2006) and a lower diurnal cortisol variation (Popma et al., 2007; Susman et al., 2007). Specifically, it was hypothesized that:

(1) Higher levels of relational aggression will predict less diurnal cortisol variation, as moderated by off-time pubertal timing and depression across 12 months, especially in off-time, depressed girls;

(2) Higher levels of externalizing behavior will predict less diurnal cortisol variation, as moderated by off-time pubertal timing and depression across 12 months, especially in off-time, depressed boys.

Methods

Participants

135 adolescents (52% female) and a parent or caregiver (89% mother, 9% father, 2% grandmother) participated in a longitudinal community-based study that examined the physiology of puberty and antisocial behavior. At study entry, girls were ages 8, 10 or 12 years and boys were ages 9, 11 or 13 years (see Table 3.1). The age difference was designed to include boys and girls of similar stages of pubertal development because girls begin their pubertal development prior to boys. Days-in-menstrual cycle was controlled as girls were assessed between days five and nine of their cycle.

The inclusion criteria were (a) adolescents not on medications that would affect hormone levels (e.g. oral steroids) and were free from chronic health problems (e.g. diabetes, cancer) or serious mental health problems that would interfere with questionnaire completion and (b) children on psychotropic medications since this is an important group at risk for antisocial behavior. Family SES was heterogeneous at all

waves of measurement, as determined by the Hollingshead scale (Hollingshead, 1975). In brief, the sample is of interest as it is heterogeneous with regard to occupation and educational status and it was drawn from nonurban and non-suburban communities. Detailed study design, procedure and measurements (Susman et al., 2007) have been previously reported.

Table 3.1 Descriptive statistics for the sample at the three times of measurement

Variable	Time 1		Time 2		Time 3	
	Mean	SD	Mean	SD	Mean	SD
Male						
Age	11.52	1.61	12.03	1.70	12.64	1.63
Diurnal Cortisol Profile	9.96	2.39	7.31	2.53	6.94	3.47
Relational Aggression	7.98	2.69	7.18	2.32	7.52	2.67
Externalizing Behavior	4.61	5.45	4.00	4.57	3.49	4.29
Depressive Symptoms	3.71	3.73	2.81	2.84	2.80	2.81
Female						
Age	10.45	1.55	11.01	1.58	11.52	1.60
Diurnal Cortisol Profile	6.76	3.20	7.32	3.51	7.32	2.59
Relational Aggression	7.95	3.34	6.93	2.69	6.31	2.40
Externalizing Behavior	3.91	5.09	4.00	5.19	3.71	5.75
Depressive Symptoms	3.90	4.33	3.78	4.71	2.89	4.32

Subjects were interviewed at the General Clinical Research Center (GCRC) of a major research university. Visits were scheduled at 4.00 pm (+/- 1.5 hours), depending on the adolescent's schedule. Upon arrival at the GCRC, parents

and adolescents were briefed extensively on the study and given an opportunity to ask questions. They were then asked to sign consent or assent forms. Subjects were measured in three waves: at baseline (study entry – 0 months), 6 and 12 months later. The attrition rate was low with 96.3% and 94.1% of the original sample retained at the second and final wave of measurement, respectively. Of the participants, 91% of the sample was European American, 2% African American, 2% Asian American, 3% Latino and 2% described as Other/Mixed. Analyses indicated that those individuals who did not participate at waves 2 and 3 did not differ significantly from their peers on measures of relational aggression, externalizing behavior, pubertal timing at baseline, depressive symptoms or sex.

Measures

Diurnal Cortisol Variation. At each wave, participants and their parents/guardians were trained in a home saliva collection protocol before leaving a General Clinical Research Center (GCRC), where they were instructed to collect all samples on a non-school day within two weeks of the lab visit, when there were no other scheduled activities, such as sports, that would interfere with the collection protocol or cortisol levels. Adolescents were also instructed to arise on the collection day at the same time of arising as on a school day and a parent was available at home to supervise saliva collection. For each saliva collection, they were instructed to rinse their mouths with water before passively drooling into a 5 mL tube and to collect saliva up to the 4 mL mark on the tube for five minutes. The tube was then to be placed in a refrigerator and the time of collection recorded. Any activities or potential stressors (e.g. an argument with siblings) that occurred that day were noted in a daily diary. Saliva was

returned to the GCRC by the parents or a staff member from the study collected it from the home.

Three samples of saliva were collected at 20-minute intervals on awakening and prior to breakfast or brushing of teeth. Sample 1 was obtained immediately on awakening, Sample 2 at 20 minutes post-wake time, and Sample 3 at 40 minutes post-wake time. Participants were requested not to eat or drink anything (except water) during the morning collection procedure. Sample 4 was collected prior to the midday meal, Sample 5 at 4.00 PM and Sample 6 at bedtime.

Samples were assayed for salivary cortisol, using a highly sensitive enzyme immunoassay (Salimetrics, State College, PA). Fifty microliters of saliva were used in duplicate determination, with a sensitivity range of 0.007 to 1.8 μg/dL (0.19 to 49.7 nmol/L). Intra- and inter-assay coefficients of sensitivity ranged between 5 and 10%. The diurnal cortisol variation variable was calculated by dividing the mean of the three morning cortisol levels by the bedtime cortisol level. Morning cortisol levels were the highest cortisol measurements whereas the bedtime sample levels were the lowest (see Figure 3.2). The mean times of saliva collection in the morning were 7.45 am, 8.05 am and 8.25 am and the standard deviations were 0:50, 1:02 and 1.07 hours, respectively. For bedtime collection, the mean collection time was 20.58 pm and the standard deviation was 2:39 hours. There was a strong positive skew in the diurnal cortisol variation variable and so the diurnal cortisol variation variable was log-transformed for analysis in order to bring about normality in the distribution. The raw scores are reported in Table 3.1.

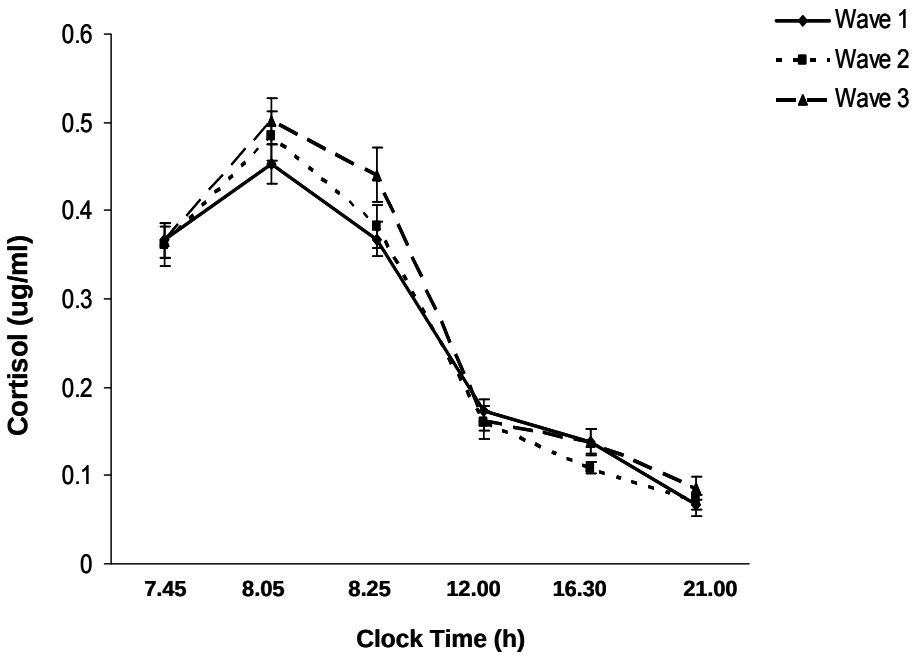

Figure 3.2 The mean diurnal cortisol profile for participants at each wave of measurement

Relational Aggression. Relational aggression was assessed by the Children's Social Behavior Scale (CSBS) created by Crick and Grotpeter (1995). Reliability was acceptable with standardized Cronbach's alphas of 0.66 at baseline (0 months), 0.66 (6 months) and 0.69 (12 months) respectively. A higher score indicates a higher degree of self-reported relational aggression.

Externalizing Behavior. Externalizing behavior problems were assessed using the Child Behavior Checklist (CBCL), a norm-referenced rating scale completed by parents (Achenbach, 2001). Parents rate their child's behavior on a three-point scale (0=Never, 1=Sometimes, 2=Often) for 113 behavioral and emotional problems. The externalizing behavior problem broad band scale was used in the current analysis and consists of the Aggressive Behavior (α=0.89, 0.86, and 0.87) and the Rule Breaking

Behavior (α=0.88, 0.65, and 0.54) subscales. Cronbach's alphas for each wave of measurement appear in parenthesis. Raw scores were used in the analyses.

Timing of Puberty. Pubertal timing measured at baseline was used in the present analysis. Pubertal stage was assessed by a pediatric research nurse using Tanner criteria of genital and pubic hair stage for boys and breast and pubic hair stage for girls (Marshall & Tanner, 1969, 1970). After explaining the stages of puberty and showing the adolescent and parent pictures of each stage, adolescents and a parent independently rated the adolescent stage of pubertal development. The nurse then did a physical exam to establish Tanner stage, which included breast palpation (Kaplowitz & Oberfield, 1999). If the adolescent did not consent to the physical exam (N=9 at baseline; N=9 at 6 months; N=13 at 12 months), the adolescent's self-rating of pubertal development was substituted for the nurse rating. The correlation between the nurse rating and adolescent rating of breast (girls) or genital Tanner stage (boys) was r=0.77, p<.01. Breast and genital stage were used in the analysis given that these growth parameters are a direct reflection of gonadal hormone exposure. Pubertal timing was then calculated by regressing pubertal stage (genital for boys or breast for girls) on chronological age and calculating a residual timing score for each individual, which was then used in the analysis (Dorn, Susman, & Ponirakis, 2003). A residual score indicates the distance between the pubertal stage of the individual and the regression line for adolescents in the same age and gender group. A negative residual indicates later timing of puberty (i.e. less development than expected) and a positive residual indicates earlier timing of puberty (more development than expected).

Children's Depression Inventory (CDI). Childhood and adolescent depressive symptoms was assessed by the Children's Depression Inventory (Kovacs, 1983). The CDI is a self-report, symptom-oriented scale that was designed for school-aged children and adolescents. For each item, the subjects are asked to choose one sentence that best describes them in the last two weeks in each of the 27 items. For example, subjects would pick one of the following sentences that best described how they felt in the past two weeks: 'I am sad once in a while; I am sad many times; I am sad all the time'. A higher total score from the summed items indicates more depressive symptoms. Reliability in this study was good with a standardized Cronbach's alpha of 0.86, 0.73, 0.71, respectively for each wave of measurement. There was a positive skew in the depressive symptoms variable, therefore CDI scores were log-transformed to achieve normality for analysis. Raw scores are reported in Table 3.1.

Analysis Plan. SAS 9.1 for Windows was the statistical program used in the present analysis. The data were checked for outliers. Nonnormality issues in the diurnal cortisol variation and depression variables had been addressed by applying the log-transformation. Outliers in the externalizing behavior variable were assigned a value of the Mean + 2SD prior to analysis (N=8). The hypotheses were assessed using a multilevel model (Singer, 1998) to assess individual- and group-level change. Boys were dummy-coded in the analysis. At level 1 of the multilevel model, we assessed the individual-level predictors of time (wave of measurement), aggressive behavior (relational aggression or externalizing behavior), depressive symptoms across the three times of measurement and pubertal timing at baseline. Interactions between these variables were also included at Level 1. At level 2 of the multilevel model, we assessed

group level change by sex on the effect that the time-varying covariates in level 1 (i.e. time, aggressive behavior, depressive symptoms) would have on the diurnal cortisol profile across the year.

Significance was judged at a *p* level of 0.05. Non-significant interaction terms were removed from the model. The most parsimonious models with the best fit according to the model fit statistics (AIC, -2log Likelihood) were accepted as the final models (see Table 3.2).

Results

Hypothesis testing. The results of the analyses are presented in Table 3.2. Significant interactions ($p<0.05$) were probed further using the procedures proposed by Aiken & West (1991) with the slope of the relationship between the interacting variables plotted at 1 SD above and below the mean, as well as at the mean of moderator(s). Only significant slopes are reported.

Hypothesis 1. It was proposed that higher levels of relational aggression will predict less diurnal cortisol variation, as moderated by pubertal timing and depression across 12 months. The interaction between relational aggression, depressive symptoms and pubertal timing at baseline was significant ($B=-0.09$, $F=7.94$, $p<0.01$).

Table 3.2. SAS Proc MIXED results of the multilevel model investigating the effect of (a) relational aggression and (b) externalizing behavior on the change in the diurnal cortisol profile.

Model Effect	Estimate	df	F
(a) Relational Aggression			
Intercept	0.71***	1,296	
Sex	0.33*	1,276	4.51
Relational Aggression	0.04*	1,304	5.69
Pubertal Timing	-0.21	1,307	1.84
Depressive Symptoms	0.07	1,303	0.12
Sex x RA[b]	-0.05**	1,297	6.98
Timing[d] x RA	0.03	1,306	2.10
Depression x RA	-0.03	1,308	1.32
Depression x Timing	0.71**	1,286	8.25
RA x Timing x Depression	-0.09**	1,278	7.94
(b) Externalizing Behavior			
Intercept	0.96***	1,163	
Time	-0.06	1,115	0.72
Sex	0.08	1,115	1.10
Externalizing Behavior	0.03[a]	1,177	3.17
Pubertal Timing	-0.35*	1,140	5.02
Depressive Symptoms	-0.09	1,148	0.36
Time x EXT[c]	-0.003	1,231	0.02
Time x Timing[d]	0.16	1,141	2.53
Time x Depression	0.07	1,174	0.36
Timing x EXT	0.10**	1,212	6.87
Depression x EXT	-0.02	1,183	1.24
Sex x EXT	-0.03*	1,142	5.19
Depression x Timing	0.48*	1,142	4.46
Time x EXT x Timing	-0.06*	1,217	4.49

Time x EXT x Depression	-0.002	1,235	0.01
Time x Timing x Depression	-0.18	1,161	1.35
EXT x Timing x Depression	-0.12**	1,219	8.77
Time x EXT x Timing x Depression	0.08*	1,215	5.95

Note ***p<0.001, **p<0.01, *p<0.05, [a]p<0.1, [b]Relational Aggression, [c]Externalizing Behavior, [d]Pubertal Timing

Post hoc analyses of the interaction showed that for earlier timing adolescents with higher levels of depressive symptoms, the slope of the relation between diurnal cortisol variation and relational aggression was negative and significant (B=-0.02, t=-2.97, p<0.01). Earlier timing and depressed relationally aggressive children and adolescents were characterized by a low diurnal cortisol variation over the year of study (see Figure 3.3). This relationship showed no time or sex effects, demonstrating that the relationship between relational aggression and diurnal cortisol variation does not change in depressed, off-timing adolescents.

The interaction between sex and relational aggression was significant (B=-0.05, F=6.98, p<0.01). Post hoc analyses of the interaction showed that for girls with high levels of relational aggression, the slope of the relation between diurnal cortisol variation and relational aggression was negative and significant (B=-0.04, t=-2.73, p< 0.01).

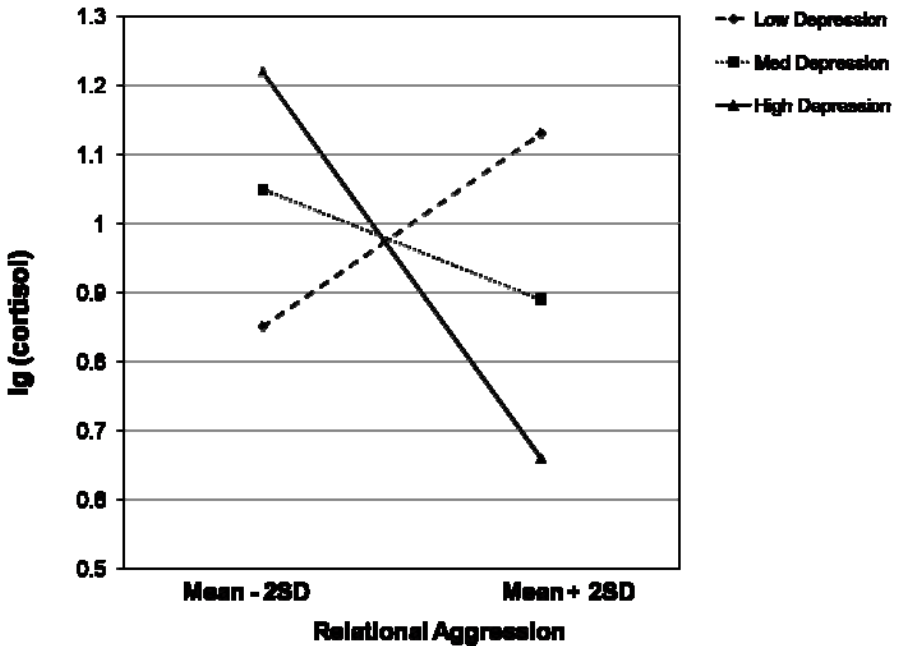

Figure 3.3 The interaction of relational aggression and depression as predictors of diurnal cortisol variation in early maturers over 12 months

Girls who report high levels of relational aggression have less diurnal cortisol variation across the 12 months, regardless of their pubertal timing or level of depression. For boys, the slope of the relation between relational aggression and diurnal cortisol variation was null. There were no time effects in this relationship for girls, indicating that the relationship between relational aggression and diurnal cortisol variation does not change over the 12 months (see Figure 3.4).

Hypothesis 2. It was proposed that higher levels of externalizing behavior will predict less diurnal cortisol variation, as moderated by off-time pubertal timing and depression across 12 months, especially in off-time, depressed boys. The interaction between time, externalizing behavior, depressive symptoms and pubertal timing was significant ($B=0.08$, $F=5.95$, $p<0.05$).

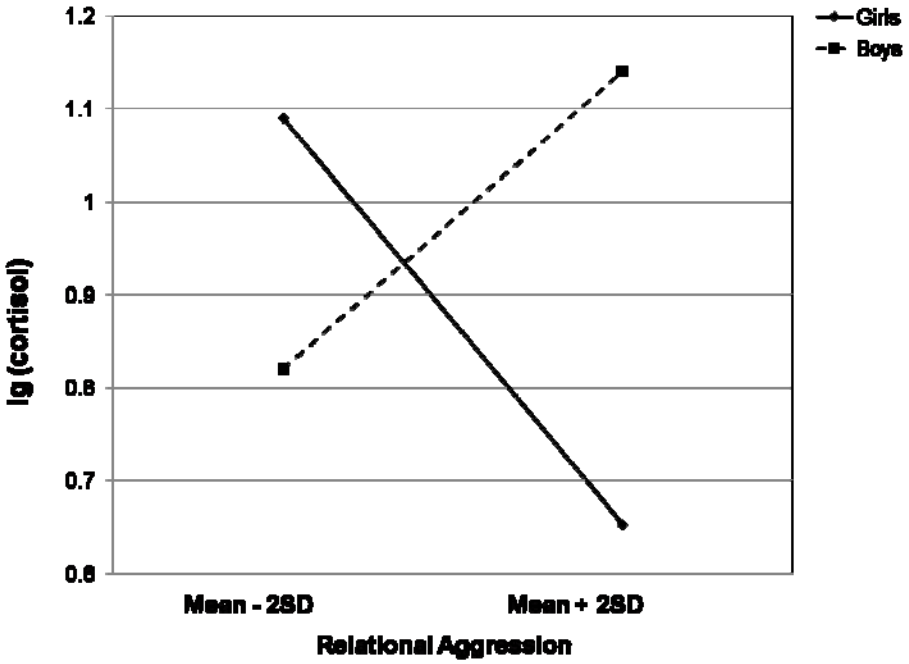

Figure 3.4 The interaction of sex and relational aggression as predictors of diurnal cortisol variation in girls and boys over 12 months

Post hoc analyses of the interaction showed that for earlier maturing adolescents with higher levels of externalizing behavior and lower levels of depressive symptoms, the slope of the relation between diurnal cortisol variation and time was negative and significant (B=-0.50, t=-2.11, $p<0.05$). Over time, earlier maturing adolescents with higher levels of externalizing behavior and lower levels of depressive symptoms have a steadily decreasing diurnal cortisol variation over the year of study in both boys and girls (see Figure 3.5).

The interaction between sex and externalizing behavior was significant (B=-0.03, F=5.19, $p<0.05$). Post hoc analyses of the interaction showed that for girls with higher levels of externalizing behavior, the slope of the relation between diurnal

cortisol variation and externalizing behavior was negative and significant (B=-0.02, t=-2.39, p< 0.05).

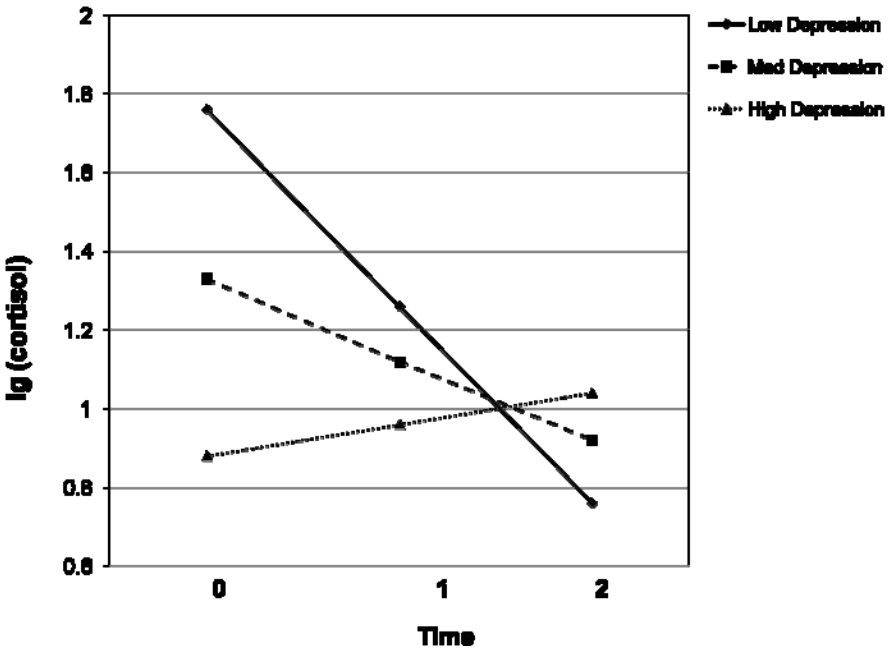

Figure 3.5 The interaction of time and depression in early maturing externalizers as predictors of diurnal cortisol variation over 12 months

Girls who exhibit higher levels of externalizing behavior have less diurnal cortisol variation over the 12 months, regardless of their pubertal timing or level of depression. For boys, the slope of the relation between relational aggression and diurnal cortisol variation was null. There were no time effects in this relationship for girls, indicating that the relationship between externalizing behavior and diurnal cortisol variation does not change over the 12 months (see Figure 3.6).

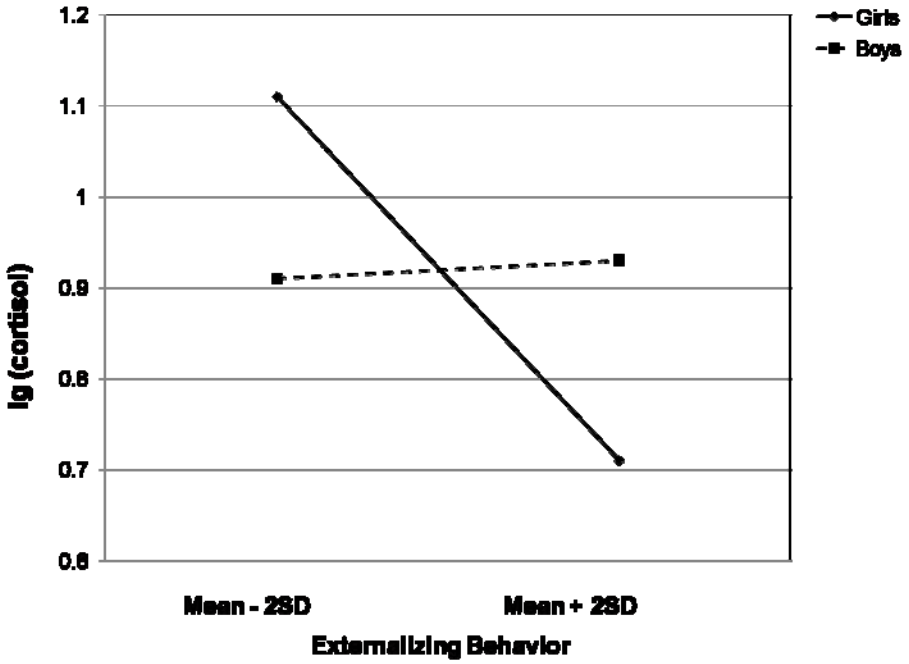

Figure 3.6 The interaction of sex and externalizing behavior as predictors of diurnal cortisol variation in girls and boys over 12 months

Discussion

The overall theoretical framework considered dynamic integration (Magnusson, 1999) across biological and behavioral development in order to identify the longitudinal effects of aggressive behavior on the diurnal variation of cortisol in adolescents during the pubertal transition. The results show that aggressive behavior, both relational aggression and externalizing behavior, predict less diurnal cortisol variation in girls when between-group differences by sex were considered. Less diurnal cortisol variation was predicted by early maturing, depressed adolescents who practiced higher levels of relational aggression. Decreasing diurnal cortisol variation was predicted in early maturing, externalizing adolescents with low levels of depressive

symptoms. These findings are unique as they demonstrate the longitudinal effects of aggressive behavior on HPA axis diurnal variations at puberty. For the first time, the findings identify relational aggression as a potent predictor of HPA axis activity. Previous studies have not included the longitudinal effects of relational aggression on HPA axis activity, although its association had been highlighted in a cross-sectional study (Blades, Dockray, & Susman, under review). The findings add to the current literature on HPA axis functioning, relational aggression and externalizing behavior.

Two hypotheses were tested with each predicting that relational aggression or externalizing behavior would predict less diurnal cortisol variation across 12 months, as moderated by pubertal timing and depression. Support was found for the first hypothesis that relational aggression predicts less diurnal cortisol variation across 12 months. Depressed, early timing adolescents who practiced higher levels of relational aggression had less diurnal variation in their cortisol levels across the 12 months. It had been predicted that the interaction of depression, pubertal timing and relational aggression would be more salient in off-time girls. However, sex was not found to be a significant moderator when pubertal timing and depression were involved. It is possible that off-time pubertal timing is a potent stressor in both boys and girls, especially if they are both relationally aggressive and depressed. Studies to date have demonstrated that both off-time boys (Graber, Seeley, Brooks-Gunn, & Lewinsohn, 2004; Michaud, Surio, & Deppen, 2006) and girls (Ge, Conger, & Elder, 1996, 2001a) have more behavior problems and depressive symptoms. Early maturers who practice relational aggression may do so to maintain their position in the peer group since their more adult appearance makes them different from their peers and might bring about

teasing and exclusion from that group. At the same time, a more adult appearance may also position the adolescent as a group leader who is responsible for maintaining all peer interactions within the group. This responsibility may be overwhelming for the somewhat cognitively immature adolescent. In turn, this responsibility may foster increases in relational aggression which the literature shows is strongly associated with depression (Crick, Bigbee, & Howes, 1996; Crick & Grotpeter, 1995; Werner & Crick, 2004) and can be a more potent stressor than academic stress (Crick & Nelson, 2002). As shown in the present analysis, the combination of these effects can bring about frequent HPA axis arousal over time and eventually result in a flat and steady diurnal rhythm (Miller, Chen, & Zhou, 2007), characterized by less diurnal variation in cortisol levels.

Relational aggression also predicts less diurnal variation in cortisol levels across the year when between-group differences by sex were considered. Girls in the sample who practice relational aggression have less diurnal cortisol variation across 12 months. Studies have shown that girls prefer the use of relationally aggressive behavior in peer conflict (Ostrov & Keating, 2004; Ostrov, Woods, Jansen, Casas, & Crick, 2004; Werner & Nixon, 2005), primarily to avoid punishment and maintain their position in the peer group (Delveaux & Daniels, 2000). At the same time, girls are more likely to form dyads and to withdraw from large peer groups (Benenson & Heath, 2006; Markovits, Benenson, & Dolenszky, 2001). Since girls' friendships tend to be mainly dyads or small peer groups, this may place increased salience on girls' friendships. The use of more relational aggression may reflect the instability and transience of peer friendships and the maintenance of the peer group may present as an ongoing stressor that may need

to be coped with, possibly on a daily basis. Ongoing stress from peer group dynamics can increase the likelihood of the development of HPA axis perturbations that continue to be predicted by use of relational aggression over the long term in girls.

It was also expected that higher levels of externalizing behavior would predict less diurnal cortisol variation, especially in off-time boys. It was found that externalizing behavior predicted a steadily decreasing diurnal cortisol variation across 12 months in early timing adolescents. Sex was not a significant moderator of externalizing behavior and diurnal cortisol variation, when pubertal timing and depression were included in the model. These findings are similar to those for relational aggression and once again lead us to propose that off-time pubertal timing is a salient stressor regardless of sex. Even though depression was a significant moderator of this relationship, the slope of the relation between externalizing behavior and diurnal cortisol variation was significant only at low levels of depression in early timing adolescents. Although it is possible that these adolescents under-reported their levels of depressive symptoms, it is also probable that depression does not need to be as high in the externalizing adolescent as it does in the relationally aggressive adolescent to exert a potent effect on HPA axis activity. It is also highly probable that some collinearity exists between externalizing behavior and depressive symptoms as comorbidity of these behaviors have been reported in the literature (Angold & Costello, 1993). It is of some concern however that whereas the passage of time did not change the effect of relational aggression on the diurnal cortisol variation, continuously higher levels of externalizing behavior in early maturing adolescents predict the consistent decline in diurnal cortisol variation over the 12 months. The decreasing diurnal cortisol variation

demonstrates the plasticity of the HPA axis and suggests that allostasis is constantly disrupted over the 12 months. Allostasis is the maintenance of stability through physiological change and frequent cycles of allostasis can lead to allostatic load, referring to the wear and tear on bodily systems (McEwen, 1998). This has implications for adolescent health since frequent HPA axis arousal can lead to the inhibition of growth hormone, sex steroids and thyroid secretions (Chrousos & Gold, 1992). Adequate levels of these hormones are essential for biological and behavioral development at such a crucial period of growth in the lifespan. These results highlight a need for behavioral interventions in early maturing, externalizing adolescents in order to prevent further escalation of externalizing behavior.

Conversely, between-group differences by sex indicate that the effects of externalizing behavior on diurnal cortisol variation predict less diurnal variation of cortisol levels in girls only. Externalizing behavior tends to be a non-normative strategy for girls in peer conflict (Werner & Crick, 2004) and is generally not the behavior of choice in situations that are aggression-provocative (Crick, Grotpeter, & Bigbee, 2002; Ostrov, Woods, Jansen, Casas, & Crick, 2004). Therefore, such behavior in girls may invite censure from authority figures such as parents and teachers while at the same time, externalizing behavior increases the possibility of social exclusion and thus, isolation. Social isolation is a potent activator of the HPA axis and results in the elevated secretion of cortisol (Cacioppo et al., 2000; Pressman et al., 2005). Although we do not have the capacity to examine this factor in the present study, future studies could examine how externalizing behavior and the social isolation that may result potentially affects HPA axis activity.

Overall, the use of the dynamic integration perspective (Magnusson, 1999) to examine the effect of aggressive behavior on diurnal cortisol variations has proven to be beneficial in highlighting the multiplicative effects of stressors in puberty. Although the extant literature has predominantly focused on physically aggressive and externalizing behavior, the effectiveness of relational aggression as a stressor has been demonstrated in a non-clinical community-based sample of boys and girls. It is alarming however, that both relational aggression and externalizing behavior could continue to predict less diurnal cortisol variation across the 12 months. Sher (2007) noted in a review that HPA axis abnormalities may contribute to the development of alcohol use disorders and low diurnal cortisol activity was associated with cannabis use (Huizinck, Ferdinand, Ormel, & Verhulst, 2006). The persistent prediction of less diurnal variation in cortisol levels across time may increase the risk that those adolescents who practice relational aggression and externalizing behavior will develop alcohol and substance use disorders later in adolescence and in early adulthood. Concurrently, HPA axis perturbations and early maturation may also increase susceptibility to overweight, obesity, hypertension and related diseases in later life (Hulanicka, Lipowicz, Koziel, & Kowalisko, 2007). Once again, these findings demonstrate the importance that should be placed on the development of effective intervention programs that target early maturers who exhibit relational aggression and externalizing behavior before and during puberty.

Limitations arise in the study as well. First, relational aggression tends to be under-reported due to social desirability, one of the objectives of relational aggression (Delveaux & Daniels, 2000). However, it has been established that it is

higher, as opposed to lower levels of relational aggression that predict a decreased variation in diurnal cortisol levels. Second, cortisol levels were measured on one day at baseline, 6 months later and 12 months later. However, marked stability has been demonstrated with time for these three measurements (see Figure 3.1), as has been demonstrated in other studies (Smythe et al., 1997; Stone et al., 2001). Third, a questionnaire completed by the participants each time they collected saliva at home did not indicate any untoward events that would have affected cortisol levels during the day. Fourth, the sample size was relatively modest across the three times of measurement post hoc analyses were carried out to calculate effect size. These analyses demonstrated a medium effect size (J. Cohen, 1989). Finally, a predominantly Caucasian sample was used and this homogenous sample reduces the generalizability of the results to other ethnic groups. A recent study in adolescents from different ethnic groups indicated group differences in diurnal cortisol profiles (DeSantis et al., 2007). Further research is needed on aggressive behavior and HPA axis activity in multiple ethnic groups so as to elucidate possible pathways leading to the development of health disparities.

In summary, the results from the current study highlight a need to carry out longitudinal studies on aggressive behavior and HPA axis functioning as there is a paucity of studies on the longitudinal physiological effects of aggressive behavior. It is especially important to develop studies that will investigate the use of dynamic integration of biology and behavior in predicting mental and physical health disorders in late adolescence and adulthood. Additionally, further longitudinal studies on the physiological effects of relational aggression are necessary as relational aggression

increases from childhood, through adolescence to adulthood, where it is associated with harmful behavior in the college environment. The singular effects of relational aggression and externalizing behavior on HPA axis activity were considered. Additional, pertinent information may be acquired if the combination of both relational and physical aggression were also considered when investigating longitudinal effects on HPA axis activity. Such studies can only increase our understanding of the development and stabilization of these behaviors and their association with health and disease.

Chapter 4

Paper 3

Sex, ethnicity and the interaction of cortisol reactivity and externalizing behavior problems and BMI z-score one year later

Abstract

An increased prevalence and ethnic disparities in overweight and obesity in adolescence has led to concerns for adolescent and adult health. Adolescent obesity is relatively stable and generally leads to adult overweight and obesity, as well as obesity-related diseases such as noninsulin dependent diabetes and heart disease. Furthermore, disparities in health are a major public health concern as morbidity and mortality tend to differ between ethnic groups, with the highest mortality rates in non-Hispanic Blacks. Five hundred and eighty-nine adolescents aged 8 to 12 at study entry and drawn from an Eastern and a West Coast sample were examined at baseline (0 months) and 1 year later. The study aimed to test the hypothesis that overweight and obesity is predicted by HPA axis activity in adolescents across 12 months, when moderated by externalizing behavior. Specifically it is hypothesized that there will be sex and ethnic differences in adolescents with externalizing behavior problems who have a higher total cortisol output (AUC*g*) or a higher cortisol increase (AUC*i*) at baseline (0 months) and a higher BMI z-score one year later. Sex and ethnic differences were identified. Non-Hispanic Black West Coast girls and Eastern girls who have a diminished cortisol output are heavier a year later, although the levels of externalizing behavior problems vary. However, only non-Hispanic Black girls with a heightened response to a stressor and higher levels of externalizing behavior were heavier a year later. These results suggest that precursors of ethnic disparities in the development of obesity and obesity-related disease are already present at a developmentally critical period in adolescence.

Introduction

Over the last three decades, the prevalence of overweight/obesity in children and adolescents has increased from 6.5% in 1976-1980 to 18.8% in 2003-2004 in children aged 6-11 years and from 5.0% to 17.4% in adolescents aged 12-19 years (Ogden, Flegal, Carroll, & Johnson, 2002). Disparities in overweight/obesity exist among racial/ethnic groups. By 2003-2004, the prevalence of overweight had increased by 7.5% in non-Hispanic white boys and 7.8% in non-Hispanic black boys. In comparison, the prevalence of overweight had only increased by 4.2% in Mexican American boys. The increase in overweight/obesity prevalence was higher in girls with non-Hispanic White and Mexican American girls increasing by 8% and 4.9% respectively. Non-Hispanic Black girls showed the greatest increase (12.2%) in overweight/obesity prevalence overall (Ogden et al., 2006). These statistics are alarming given the association of childhood overweight with the development of cardiovascular risk factors and early-onset Type 2 diabetes mellitus in youth and later adulthood (Freedman, Dietz, Srinivasan, & Berenson, 1999; Marcovecchio, Mohn, & Chiarelli, 2005; Must & Anderson, 2003). In addition, early-onset Type 2 diabetes mellitus is more aggressive than later-onset Type 2 diabetes mellitus and leads to the development of cardiovascular complications, reflected by a more adverse cardiovascular risk profile and cardiovascular death (Song & Hardisty, 2008). If these diseases develop in childhood and adolescence, and are exacerbated by overweight/obesity, they become precursors of early morbidity and mortality in adulthood. The top leading causes of death in the U.S. are heart disease and diabetes. Disparities in mortality rates exist, with blacks having the highest number of deaths per

100 000 in the population for heart disease and diabetes (CDC Fact Sheet 2004). It is important then to investigate the unique biobehavioral pathways that may lead to the development of overweight/obesity in adolescence. At the same time, it is also important to consider sex and ethnic differences that may elucidate the course of development of disparities in health and mortality (Morssink & Kumanyika, 2006; Yancey & Kumanyika, 2007) in adulthood.

Studies have implicated elevated cortisol levels in the development of obesity and obesity-related disease (Bjorntorp, 2001; Bjorntorp, Holm, & Rosmond, 1999). Cortisol is a steroid hormone that is produced from a sequence of physiological reactions, beginning in the hypothalamus, then the pituitary gland and finally the adrenal glands that secrete cortisol. The hypothalamic-pituitary-adrenal (HPA) axis is activated in response to environmental disruptions and is responsible for re-establishing allostatic conditions after a stressor is experienced (McEwen, 1998; Tsigos & Chrousos, 2002). However, if the stressor remains for an extended time period, the HPA axis is continually activated resulting in constantly elevated cortisol levels. This condition leads to the inhibition of other hormones necessary for growth, metabolism and reproduction and can lead to the development of a variety of disorders such as hypertension, artherosclerosis, osteoporosis and immune dysfunction (Chrousos, 2000; Chrousos & Gold, 1992). At the same time, the net effect of cortisol at the cellular level leads to the accumulation of fat in visceral depots in the body, resulting in weight gain (Bjorntorp, 1991, 1993).

Chronic elevations of cortisol also lead to diminished HPA activity and can influence the rhythm of cortisol that is normally highest in the morning on awakening

and decreasing to its lowest levels at night. Under chronically stressful conditions, diurnal cortisol variation decreases to a flat, steady rhythm. The chronic effects of stress on HPA axis activity can be seen in cases of traumatic stress as in war veterans and Holocaust survivors (Yehuda, Halligan, Grossman, Golier, & Wong, 2002) as well as in prolonged, non-traumatic stress such as in teachers with burnout and caregivers (Miller, Chen, & Zhou, 2007; Preussner, Hellhammer, & Kirschbaum, 1999). Although studies have demonstrated that body mass index and other anthropometric measurements of central obesity are associated with elevated cortisol secretions in response to perceived stress in adults (Bjorntorp, Holm, & Rosmond, 1999; Bjorntorp & Rosmond, 1999; Rosmond et al., 2003), no such studies have examined cortisol in the prediction of overweight in healthy children and adolescents. Therefore, the current study will examine the prediction of body mass index by cortisol reactivity in a multiethnic sample of adolescents.

The extant literature on externalizing behavior reveals its association with both HPA axis activity and overweight status in adolescents. Boys with disruptive disorder had less variation in cortisol levels across the day (Popma et al., 2007) as well as children with more behavior problems (Susman et al., 2007). In addition, both relational aggression and externalizing behavior were associated with less diurnal cortisol variation as well as a heightened response to a stressor (Blades, Dockray, & Susman, under review). These two types of aggressive behavior also predicted lower diurnal variation across one year (Blades, Rovine, & Susman, unpublished manuscript).

Studies show that overweight is associated with externalizing behavior problems (Judge & Jahns, 2007; Pine et al., 1996; ter Bogt et al., 2006) and that

aggressive behavior in adolescence predicts overweight in adulthood (Hasler et al., 2004). However, little research has been carried out to examine the role that a diminished cortisol output or elevated cortisol secretions in response to a stressor in externalizing youth can play in the development of overweight and obesity.

Maltreatment and neglect are additional life experiences also associated with both HPA axis activity and overweight status. Less variation in diurnal cortisol levels as well as lower basal levels were reported in children who have suffered abuse or neglect (Cicchetti & Rogosch, 2001; Gunnar & Valquez, 2001; Tarullo & Gunnar, 2006). Maltreatment and neglect have also been identified as risk factors for the development of overweight in adulthood, possibly due to the associated depression and atypical HPA axis activity observed in abused individuals (Johnson, Cohen, Kasen, & Brook, 2002; Noll, Zeller, Trickett, & Putnam, 2007). However, there is a paucity of research in the literature on the association of cortisol reactivity and the development of overweight in maltreated and neglected children. The present study aimed to examine the effect of neglect on the prediction of body mass index by cortisol levels in youth in a multiethnic sample of adolescents.

The overall aim of the present study was to examine sex and ethnic differences in the longitudinal prediction of body mass index by cortisol levels in externalizing youth (see Figure 4.1 for conceptual model). It was expected that there will be sex and ethnic differences since the literature has indicated that there are differences in the response to a stressor in boys and girls (Stroud, Salovey, & Epel, 2002; Uhart, Chong, Oswald, Lin, & Wand, 2006). Ethnic differences have been found in HPA axis activity (DeSantis et al., 2007; Yanovski, Yanovski, Cutler, Chrousos, &

Filmer, 1996; Yanovski et al., 1996) and prevalence of overweight (Ogden et al., 2006). Sex and ethnicity can have interactive, inter-locking, additive and possibly multiplicative effects on various aspects of the lives of persons of ethnic origin (Sanchez-Hucles, 1997). The unique stressors presented by the interactive effects of sex and ethnicity can in turn lead to the development of a hyper-aroused HPA axis. In addition, it was expected that externalizing behavior would act as a moderator of this relationship because of its association with both HPA axis activity and overweight (Blades, Dockray, & Susman, under review; Hasler et al., 2004; Popma et al., 2007; Susman et al., 2007).

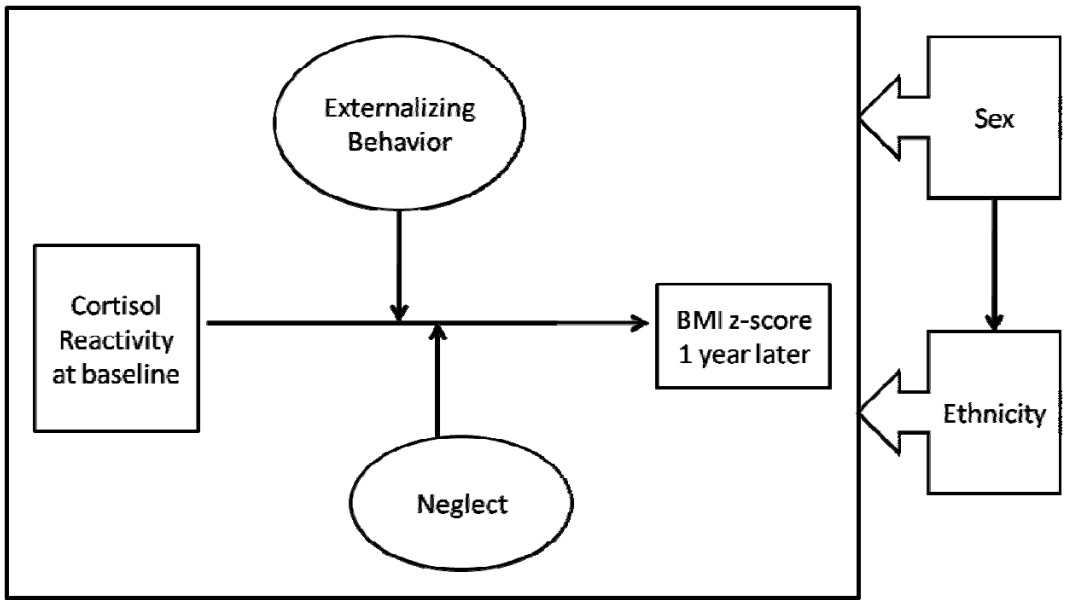

Figure 4.1 A conceptual model of sex and ethnic differences in the interaction of cortisol reactivity and externalizing behavior at baseline to predict BMI z-score one year later.

Specifically it was hypothesized that,

(1) Adolescents with a higher cortisol output will be heavier a year later, especially in those adolescents with higher levels of externalizing behavior problems, as moderated by sex and ethnicity. It is expected that:

a. Non-Hispanic Black girls with a higher cortisol output at baseline and higher levels of externalizing behavior problems will be heavier a year later, when compared to non-Hispanic Whites and Hispanic girls.

(2) Adolescents with more elevated cortisol levels following a stressor will be heavier a year later, especially in those adolescents with higher levels of externalizing behavior problems, as moderated by sex and ethnicity. It is expected that:

a. Non-Hispanic Black girls with more elevated cortisol levels following a stressor at baseline and with higher levels of externalizing behavior problems will be heavier a year later, when compared to non-Hispanic Whites and Hispanic girls.

(3) Neglected adolescents with a diminished cortisol output and attenuated levels of cortisol following a stressor at baseline will be heavier a year later, as moderated by sex and ethnicity.

a. Neglected non-Hispanic Black girls with a diminished cortisol output at baseline will be heavier a year later when compared to non-Hispanic Whites and Hispanic girls.
b. Neglected non-Hispanic Black girls with attenuated cortisol levels following a stressor will be heavier a year later when compared to non-Hispanic Whites and Hispanic girls.

Methods

Participants.

Participants were 589 children and adolescents enrolled from two study locations, the first from a small Eastern city and surrounding counties (Sample 1) and the second from a large West Coast metropolitan area (Sample 2). Participants in each sample were assessed at study entry (0 months) and one year later. In Sample 1, the third wave of measurement corresponded to a study duration of one year, whereas this time period corresponds to the second wave of measurement in Sample 2. These waves were chosen so that an adequate comparison of the samples could be made across 12 months.

Sample 1. One hundred and thirty-five children and adolescents (Mean age=10.98, SD=1.66) and a parent or caregiver (89% mother, 9% father, 2% relative) participated in a longitudinal community-based study in a small Eastern city and surrounding counties. The primary objective of the study for which the sample was drawn was to examine the physiology of puberty and antisocial behavior during the pubertal years. Detailed study design, procedure and measurements have been previously reported (Susman et al., 2007). Data from the first (baseline – 0 months) and third (12 months) waves are used in the current analyses. The attrition rate was low with 94.1% of the original sample retained at the third wave of measurement. T-tests carried out indicate that those individuals who were not measured one year later did not differ significantly from their participating peers on measures of body mass index (BMI), cortisol, externalizing behavior or sex (see Table 4.1 for demographic information).

Sample 2. Four hundred and fifty-four children and adolescents (Mean age=10.93, SD=1.16) and a parent or guardian (59% mother, 9% father, 14% foster parent, 14% relative, 2% other) participated in a longitudinal study of the effects of neglect on adolescent development. Neglected youth (N=301) were recruited from specific zip codes in the large West Coast metropolitan area. The adolescents were recent referrals to the Department of Child and Family Services (DCFS) for any type of neglect, as well as a comparison group. Types of neglect included general/severe neglect, physical abuse, sexual abuse, emotional abuse, caretaker incapacity and substantial risk and at-risk siblings. Comparison youth (N=153) were recruited from the same zip codes as the maltreated youth and screened for prior contact with the DCFS. Data from the first (baseline – 0 months) and second (12 months later) waves of measurement are used in the current analysis (see Table 4.1 for demographic information).

Procedures

C*ortisol Reactivity. Sample 1.* A total of five saliva samples was collected, two prior to the administration to the Trier Social Stress Test for Children (TSST-C) (Kirschbaum, Pirke, & Hellhammer, 1993) and three post-TSST-C. The TSST-C is a common method used to elicit an HPA stress response in a laboratory environment and includes both a cognitive and social evaluative challenge (Dickerson & Kemeny, 2004; Kirschbaum, Pirke, & Hellhammer, 1993; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). The TSST-C consists of a story completion task before two confederate judges. Subjects were also given a timed mental arithmetic task, which

consists of the completion of an age-graded serial subtraction. Subjects were told that their story would be evaluated in relation to the stories of other children their age.

The first saliva sample was collected after consent and assent forms were signed, 20 minutes before the TSST-C; Sample 2, the second saliva sample, was collected at 5 minutes before the TSST-C. Sample 3 was collected immediately post-TSST-C and Samples 4 and 5 were collected at 10 and 20 minutes post-TSST-C, respectively. The samples were frozen at -80 degrees Celsius until assayed for cortisol. Cortisol was assayed in all five samples using a highly sensitive enzyme immunoassay (Salimetrics, State College, PA). Fifty microliters of saliva were used in duplicate determination, with a sensitivity range of 0.007 to 1.8 µg/dL (0.19 to 49.7 nmol/L). Intra- and inter-assay coefficients of sensitivity ranged between 5 and 10%.

Sample 2. A total of six saliva samples was collected, two prior to the administration of the Trier Social Stress Test for Children (TSST-C) (Kirschbaum, Pirke, & Hellhammer, 1993) and four post-TSST-C. The first saliva sample was collected 45 minutes before the TSST-C; Sample 2, was collected at 10 minutes before the TSST-C, immediately following a 5 minute relaxation period which involved listening to soft music while viewing a picture of a beach scene. We collected Sample 3 immediately post-TSST-C and Samples 4, 5 and 6 were collected at 10, 20 and 30 minutes post-TSST-C respectively. The samples were transported on ice to Salimetrics, State College, PA and stored frozen at -80 degrees Celsius until assayed for cortisol. Cortisol was assayed in all six samples using a highly sensitive enzyme immunoassay (Salimetrics, State College, PA). Only Samples 2 to 6 were used in the calculation of cortisol reactivity.

Sample 1 reflected acclimation to a novel situation and so was significantly higher than Sample 2 ($t=3.01$, $p<0.01$).

Stress reactivity was measured by the total cortisol output and the total cortisol increase during the lab visit at 0 months in both studies. Cortisol reactivity was determined using area under the curve ground – AUC*g* and increase – AUC*i* (Preussner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) in order to reflect total cortisol output (AUC*g*) and change in cortisol levels (AUC*i*) subsequent to the stressor (See Figure 4.1). Cortisol measurements collected at the first wave of measurement are used in the present analyses. Area under the ground (AUC*g*) is represented by the equation (m=sample measurement; t=time interval between measurements):

$$AUC_G = \sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i) \cdot t_i}{2}$$

Area under the curve increase (AUC*i*) is represented by the equation:

$$AUC_I = \left(\sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i) \cdot t_i}{2} \right) - \left(m_1 \cdot \sum_{i=1}^{n-1} t_i \right)$$

Table 4.1 Demographic information for samples 1 and 2 at baseline (0 months)

	Sample 1		***Sample 2***	
	Male	Female	Male	Female
Age	11.49 (1.62)	10.48 (1.56)	11.00 (1.15)	10.87 (1.16)
Socioeconomic Status (SES)	49.29 (11.88)	48.55 (10.38)		
Yearly household income				
Less than $30 000			122	119
$30 000 - $60 000			55	56
More than $60 000			31	18
Ethnicity %				
Black	2	3	37	36
Latino/a	3	4	12	9
White	89	90	40	41
Asian	3	1		
Mixed/Biracial	3	2	11	14

Externalizing Behavior. Externalizing Behavior problems were assessed at baseline in both samples using the Child Behavior Checklist (CBCl), a norm-referenced rating scale completed by parents/guardians (Achenbach, 2001). Parents rate their child's behavior on a three point scale (0=Never, 1=Sometimes, 2=Often) for 113 behavioral and emotional problems. Raw scores from the externalizing behavior

problem subscale were used in the current analyses. Cronbach's alpha for both samples was good (Sample 1: α=0.99; Sample 2: α=0.92).

BMI z-score. BMI z-score is the z-score for the body mass index, controlled for age. Height and weight were measured three times in the lab at 1 year after baseline in sample 1. Height was measured twice and weight was measured three times in sample 2. Average height and weight, sex and chronological age (age in months) were used to estimate the BMI z-score, using a SAS program from the Centers for Disease Control (CDC) growth charts (http://www.cdc.gov/nccdphp/dnpa/growthcharts/resources/sas.htm).

A*nalysis Plan.* SPSS 15.0 for Windows was the statistical program used for the present analyses. The initial step in the analysis was to identify potential confounding influences on the relationship between cortisol, externalizing behavior and BMI z-score. First, the effect of prescription and over-the-counter drugs on cortisol reactivity, externalizing behavior and the BMI z-score was examined (Hibel, Granger, Kivlighan, & Blair, 2006). Subjects in Sample 2 who were on antidepressants (N=12) were more externalizing and had a higher BMI z-score one year later. Subjects in Sample 2 who were on central nervous system stimulants (N=26) were more externalizing. In addition, there were correlations between taking prescription drugs and cortisol AUC*i* ($r=0.19$, $p<0.05$) and BMI z-score ($r=-0.21$, $p<0.05$). These subjects on antidepressants and CNS stimulants were removed from further analyses (N=38) as they did not meet the criteria of the sample under study. The association between prescription drugs, cortisol and BMI z-score was not found in Sample 1. Chronological age was not included in the analysis as it was incorporated into the BMI z-score

variable. Subjects in Sample 1 were dummy-coded for analysis. Sample 1 contained few persons of non-Hispanic Black and Hispanic ethnic origin and there were no mean ethnic differences in cortisol reactivity, externalizing behavior or weight status (See Table 4.2 for descriptive information). Subjects in Sample 2 were coded as follows: 1 – non-Hispanic Blacks; 2 – non-Hispanic Whites; 3 – Hispanic; 4 – Mixed/biracial. Finally, using the dataset grouped according to child sex and ethnicity, the hypotheses were tested using hierarchical regression analysis with BMI z-score at 1 year later as the outcome. A *p* level of 0.1 was judged to be appropriate when assessing interactions, given the documented difficulty in detecting interactions in nonexperimental designs (McClelland & Judd, 1993) as well as the exploratory nature of our study.

Table 4.2. Descriptive Statistics for Samples 1 and 2 at baseline (0 months)

	Ethnicity	Sex	N	Cortisol AUCg	Cortisol AUCi	Externalizing Behavior	BMI z-score at1 year
Sample 1	Eastern	Male	66	9.60 (7.11)	0.89 (7.83)	4.59 (5.46)	0.67 (0.97)
		Female	69	10.07 (7.57)	0.31 (7.92)	3.93 (5.08)	0.55 (1.01)
Sample 2	Black	Male	78	6.67 (4.77)	0.18 (3.03)	9.42 (9.58)	0.84 (1.05)
		Female	71	7.36 (14.05)	0.03 (3.39)	10.45 (9.56)	1.31 (0.90)
	Hispanic	Male	85	7.78 (4.82)	0.57 (2.73)	9.89 (9.13)	1.06 (0.87)
		Female	80	6.89 (6.56)	1.14 (5.44)	7.72 (8.23)	1.10 (0.92)
	White	Male	25	9.71 (11.74)	0.29 (4.61)	9.91 (12.69)	0.97 (1.06)
		Female	18	7.97 (5.03)	1.16 (4.86)	9.18 (8.69)	1.11 (1.07)
	Mixed/ Biracial	Male	25	6.19 (3.03)	0.45 (2.01)	7.09 (7.43)	1.00 (1.01)
		Female	26	5.97 (4.80)	1.93 (4.44)	7.84 (8.01)	0.82 (0.97)

Results

Group Differences. T-tests reveal that on average, the West Coast metropolitan sample was more externalizing (t=-6.51, p<0.001) and had a higher BMI z-score a year later (t=-4.48, p<0.001) than the Eastern sample. On the other hand, the Pennsylvania sample had a higher total cortisol output (AUC*g*) at baseline than the West Coast sample (t=3.57, p<0.001).

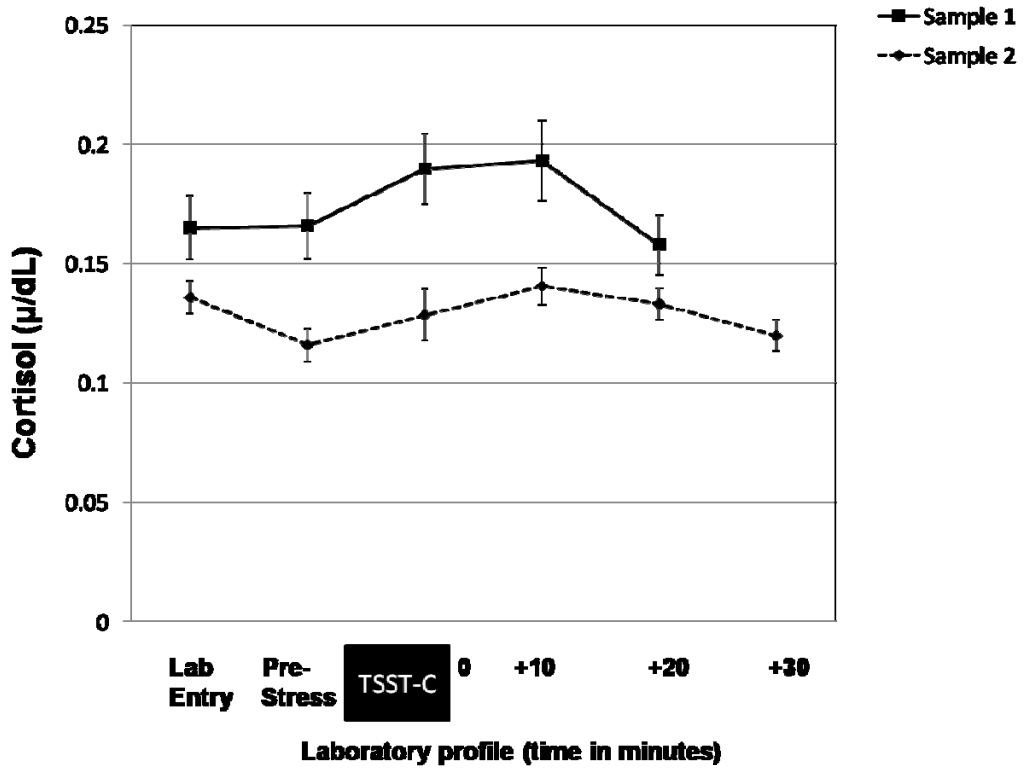

Figure 4.2 The mean laboratory cortisol profile pre- and post-Trier Social Stress Test for Children (TSST-C) in Samples 1 and 2

Correlations. Pearson product-moment correlations were computed for total cortisol output (AUC*g*), cortisol increase from baseline (AUC*i*) and externalizing behavior, measured at baseline and BMI z-score, measured a year later for both

samples (See Table 4.3). Total cortisol output (AUC*g*) at baseline correlated with BMI z-score a year later only in the Pennsylvania sample.

Table 4.3. Pearson-product moment correlations for each sample.

		Sample 2			
		AUC*g*	AUC*i*	Externalizing Behavior	BMI z-score at 1 year
Sample 1	AUC*g*		0.17**	-0.05	-0.09
	AUC*i*	0.35**		-0.05	0.06
	Externalizing Behavior	0.012	-0.05		-0.03
	BMI z-score At 1 year	-0.21*	0.02	0.03	

Note. **p<0.01, *p<0.05

Hypothesis testing. Hierarchical linear regression techniques were used to test the hypotheses. In each model, the cortisol variable (AUC*g* – total cortisol output or AUC*i* – total cortisol increase from baseline) was entered first, followed by externalizing behavior or neglect group and finally the interactions between the total cortisol output (AUC*g*) or total cortisol increase (AUC*i*) with externalizing behavior or neglect group to

predict BMI z-score one year later. Sex and ethnic differences were evaluated by grouping the samples by sex and ethnicity and estimating the linear model. Non-significant interactions ($p>0.1$) were removed from the models. Significant interactions were probed further using the procedures proposed by Aiken & West (1991) with the slope of the relationship between the two interacting variables plotted at 1 SD above and below the mean, as well as at the mean of the moderator. Only significant post hoc slopes are reported.

Hypothesis 1. It was expected that there would be sex and ethnic differences with externalizing behavior problems moderating the prediction of BMI z-score at one year from total cortisol output (AUC*g*) at baseline. The model was significant for the Eastern girls ($\beta=0.61$, $p<0.05$; $F=4.76$, $R^2=0.15$, $p<0.01$) and non-Hispanic Black girls ($\beta=-0.46$, $p<0.1$; $F=2.72$, $R^2=0.08$, $p<0.05$).

At one SD below the mean of externalizing behavior problems in the Eastern girls, the simple slope of the relation between the total cortisol output (AUC*g*) at baseline and the BMI z-score one year later was negative and significant ($B=-0.09$, $t=-3.68$, $p<0.01$). At the mean of externalizing behavior, the simple slope of the relation between cortisol AUCg and BMI z-score one year later was also negative and significant ($B=-0.03$, $t=-2.00$, $p<0.05$). A lower cortisol output at baseline predicted a higher BMI z-score one year later in low to medium externalizing behavior problems in Eastern girls (See Figure 4.3).

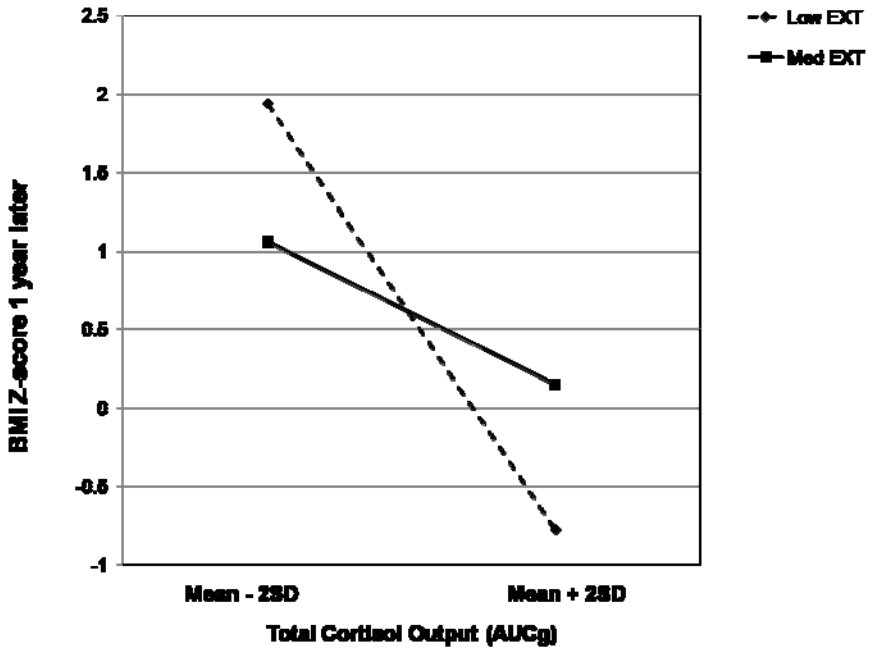

Figure 4.3 The interaction of total cortisol output and externalizing behavior at baseline as predictors of BMI z-score in Eastern girls one year later

The simple slope of the relation at the mean of externalizing behavior between the total cortisol output (AUC*g*) at baseline and the BMI z-score measured a year later was negative and significant (B=-0.05, t=-2.35, $p<0.05$) in non-Hispanic Black girls. At one SD above the mean of externalizing behavior, the simple slope of the relation between total cortisol output, measured at baseline and BMI z-score one year later was also negative and significant (B=-0.10, t=-2.03, $p<0.05$). A lower cortisol output at baseline also predicted a higher BMI z-score one year later, but in non-Hispanic Black girls who demonstrated medium to high levels of externalizing behavior (See Figure 4.4).

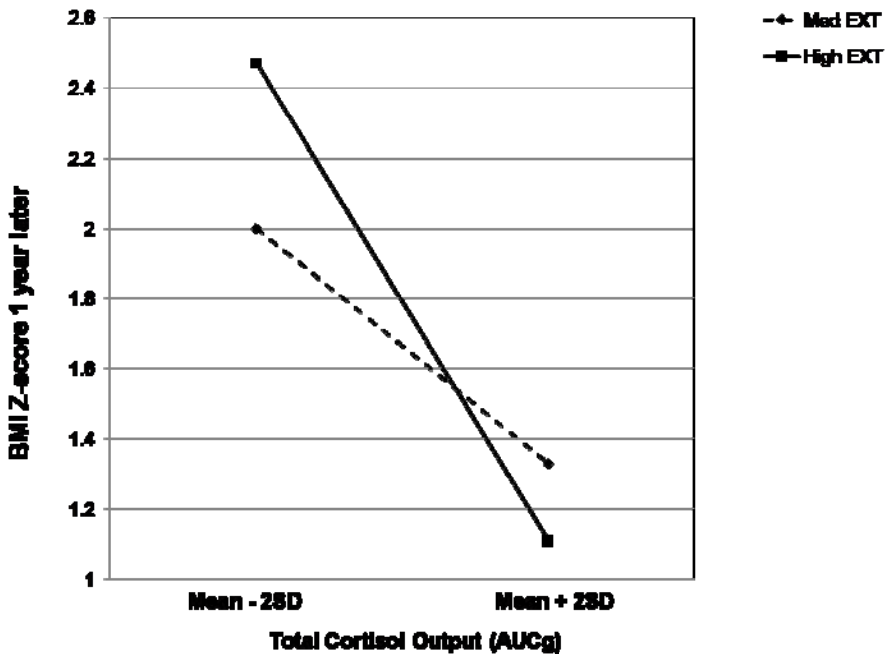

Figure 4.4 The interaction of total cortisol output and externalizing behavior at baseline as predictors of BMI z-score in non-Hispanic Black girls one year later

Hypothesis 2. It was expected that there would be sex and ethnic differences in the model where cortisol increase (AUC*i*) and externalizing behavior interact to predict BMI z-score 1 year later. The model was significant for non-Hispanic black girls only (β=-0.40, $p<0.05$; $F=3.60$, $R^2=0.12$, $p<0.05$).

At the mean of externalizing behavior, the simple slope of the relation between the cortisol increase over baseline (AUC*i*) and the BMI z-score measured a year later was positive and significant ($B=0.13$, $t=2.67$, $p<0.01$) in non-Hispanic black girls. At one SD above the mean of externalizing behavior, the simple slope of the relation between cortisol increase over baseline and BMI z-score one year later was a positive and significant ($B=0.26$, $t=3.14$, $p<0.05$). Higher cortisol reactivity predicted a

higher BMI z-score one year later in non-Hispanic black girls with medium to high levels of externalizing behavior (See Figure 4.5).

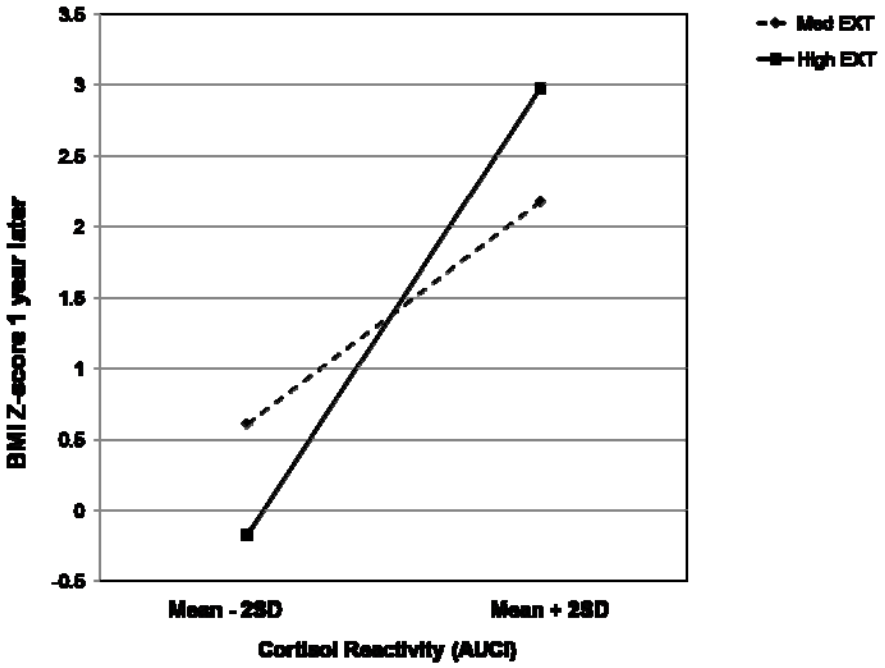

Figure 4.5 The interaction of cortisol reactivity and externalizing behavior at baseline as predictors of BMI z-score in non-Hispanic Black girls one year later

Hypothesis 3. It was expected that neglected adolescents with a diminished cortisol output and attenuated levels of cortisol following a stressor at baseline will be heavier a year later, as moderated by sex and ethnicity. However, neglect was not a significant moderator in the relationship between cortisol reactivity at baseline and weight status one year later.

Discussion

The present study aimed to examine sex and ethnic differences in the development of overweight from perturbations in HPA axis activity in externalizing

adolescents. This is the first study of its kind to examine the prediction of weight status by baseline cortisol levels in a multiethnic sample of adolescents. Previous studies of cortisol reactivity and weight status were carried out in adult samples. Overall the results show that both a diminished cortisol output and elevated cortisol secretions predicted a higher weight status one year later in externalizing non-Hispanic Black girls. Only a diminished cortisol output predicted a higher weight status in Eastern girls who exhibited low levels of externalizing behavior. There were no findings for boys in either sample.

It was proposed that adolescents with higher levels of externalizing behavior and a higher cortisol output at baseline would have a higher BMI z-score one year later and that sex and ethnic differences would moderate these relations. Sex and ethnic differences did exist but the model was significant only in Eastern girls and non-Hispanic Black girls. However, it was a lower and not higher total cortisol output at baseline, as hypothesized, that resulted in a higher BMI z-score a year later. Bjorntorp (2001) put forward the theory that it was important to examine the kinetics of the changing HPA axis and its relation to the development of obesity and chronic disease. It was expected that a higher cortisol output in response to a stressor would predict a higher weight status a year later. However, it is possible that there are other factors that may contribute to a diminished cortisol output. For example, in the non-Hispanic Black girls, it is the girls with the highest levels of externalizing behavior and the lowest total cortisol output that have the highest weight status a year later. As indicated previously, externalizing behavior is associated with diminished cortisol output across the day (Blades, Rovine, & Susman, unpublished data). Externalizing behavior is also

associated with less diurnal cortisol variation in boys with disruptive disorder and low cortisol levels in boys and girls referred for conduct disorder (Pajer, Gardner, Rubin, Perel, & Neal, 2001; Popma et al., 2007; Susman et al., 2007). A diminished diurnal cortisol output is likely to be associated with a diminished output in a laboratory setting when the non-Hispanic Black girls are exposed to the TSST-C. It is also possible that non-Hispanic Black girls from a large metropolitan area may be exposed to unsafe neighborhoods, racism/discrimination and social isolation. Social isolation also may be experienced by these girls because of the high levels of externalizing behavior that they exhibit (Crick & Bigbee, 1998; Ladd, 2006; Prinstein, Boergers, & Vernberg, 2001). Thereby, chronic emotional and social handicaps may cause frequent HPA axis arousal thereby leading to diminished output over time (Bjorntorp, 2001; Miller, Chen, & Zhou, 2007). Chronic HPA axis arousal in turn augments the development of overweight and obesity for the non-Hispanic Black girl. In contrast, it was the Eastern girls with the lowest levels of externalizing behavior and the lowest total cortisol output that were more overweight a year later. It is possible that less externalizing behavior may lead to more internalizing behavior or other forms of aggression, such as relational aggression, and this behavior has been related to diminished total cortisol output (Blades, Dockray, & Susman, under review; Blades, Rovine, & Susman, unpublished manuscript). Social isolation may also be a factor in these girls since loneliness is a potent HPA activator and results in elevated cortisol secretions (Cacioppo et al., 2000; Pressman et al., 2005). However, it is beyond the scope of the present study to investigate these possibilities.

Second, it was proposed that in adolescents with higher levels of externalizing behavior, a heightened cortisol response will be associated with a higher BMI z-score one year later and that sex and ethnic differences would exist in this longitudinal prediction. Sex and ethnic differences did exist in the model but was significant only for non-Hispanic Black girls. A heightened cortisol response to a stressor predicted a higher weight status one year later in non-Hispanic Black girls with higher levels of externalizing behavior. Even though these girls already show a diminished cortisol output, it appears that cortisol levels are elevated in response to a stressor. The data were examined once again to carry out a post hoc analysis of the interaction of total cortisol output (AUC*g*) and total cortisol increase (AUC*i*) to predict increased weight status in non-Hispanic Black girls. It was found that a diminished cortisol output and a heightened cortisol response predicted a higher weight status a year later. This atypical stress response suggests that a lack of self-regulation may exist in non-Hispanic Black girls in the large West Coast metropolis. A disadvantageous pattern of decision-making was found in subjects with the lowest cortisol levels (van Honk, Schutter, Hermans, & Putman, 2003). This pattern of bad decision making can extend to both conflict behavior and nutrition behavior (Anderson, Winett, & Wojcik, 2007; Constanzo & Woody, 1979). Overweight adolescents had significantly more externalizing problems as well as lower self-control, when compared to non-overweight adolescents (Judge & Jahns, 2007). In addition, adolescents under stress chose to eat more fatty foods, less fruit and vegetables and did more snacking, independent of gender, weight and ethnicity (Cartwright et al., 2003). In externalizing adolescents, eating may be determined by external factors, rather than the state of physiological hunger (Schachter, 1968). The

eating of non-nutritious fatty foods and snacks may be facilitated by living and going to school in a metropolitan area, where there is a greater concentration of cheap fast food outlets (Sturm, 2008). A combination of dietary behavior when under stress, especially when self-regulation and self-control is poor, as it tends to be in externalizing adolescents, may contribute to an increased weight status. Frequent stressors, both unpredictable and novel, as such as may be experienced by non-Hispanic female adolescents in a large metropolitan area, may also contribute to the hyper-arousal of the HPA axis and enhance the lack of self-regulation in the pathway to disadvantageous nutrition behavior.

Neglect was not a significant moderator in the prediction of BMI z-score a year later. Studies have shown that early adversity such as maltreatment and neglect are potent stressors that are associated with low basal cortisol levels and less diurnal cortisol variation in young children (Cicchetti & Rogosch, 2001; Gunnar & Valquez, 2001; Susman, 2006). Similar relationships between cortisol output at baseline and BMI z-score a year later were estimated in girls in the Eastern sample, where neglect is not reported or measured. It is therefore the case that regardless of their origin, HPA axis perturbations are a powerful predictor of a higher weight status a year later.

The literature shows that there are ethnic differences in HPA axis activity. In the few studies that were carried out in African Americans, it was found that African Americans have flatter diurnal rhythms, after adjusting for socioeconomic status (S. Cohen et al., 2006; DeSantis et al., 2007) as well as a more reactive HPA axis (Yanovski, Yanovski, Cutler, Chrousos, & Filmer, 1996; Yanovski et al., 1996), relative to non-Hispanic Whites. The present study has also demonstrated a heightened stress

response, relative to other ethnicities, that resulted in a higher weight status a year later in African American girls. This finding leads to implications for ethnic disparities in health with higher morbidity and mortality rates for Type 2 diabetes mellitus, both early and late onset and for ischemic and coronary heart disease in African Americans. A possible pathway for increased weight status and associated disease has been examined from a molecular level using HPA axis hormone products and associated activity. However, it is possible to investigate HPA axis activity and the development of overweight from the cellular level i.e. at the genetic level. The glucocorticoid receptor (GR) gene contains a polymorphism localized to the first intron and discoverable with the restriction enzyme, BC*l*I (Buemann et al., 1997; Weaver, Hitman, & Kopelman, 1992). This polymorphism is associated with abdominal obesity, insulin resistance and elevated blood pressure. In addition, it has implications for HPA axis regulation as this polymorphism was also associated with elevated cortisol secretion after lunch in homozygote men, in comparison with wild-type heterozygote men (Rosmond et al., 2000). It may be possible to propose that the frequency of homozygotes of this and other GR polymorphisms may be very high in the African American population after centuries of genetic selection brought on by slavery, admixture and discrimination.

There are limitations to the study that should be noted. First, the majority of adolescents in the urban sample were maltreated, which has been found to be associated with both perturbations of HPA axis activity (Cicchetti & Rogosch, 2001; Gunnar & Valquez, 2001) and overweight (Johnson, Cohen, Kasen, & Brook, 2002; Noll, Zeller, Trickett, & Putnam, 2007). However, analyses did not indicate that neglect was a moderator of the cortisol-weight status longitudinal relationship. Second, income

and pubertal timing may also have presented confounds in the prediction of weight status one year later. Post hoc analyses did reveal that both income and pubertal timing are potential moderators of the cortisol and weight status relationship. However, they served only to strengthen the direction of the relationship between cortisol at baseline and BMI z-score one year later. Third, parents and caregivers may have under-reported or over-reported externalizing behavior in both samples. However, the results were consistent between samples and in keeping with the literature on adults. Finally, the possibility of confounding arises when ethnicity and location are considered as cultural influences on the relationship between cortisol reactivity at baseline and weight status one year later, as moderated by externalizing behavior problems. Ethnicity refers to large groups of persons that may be classed according to cultural origin and culture may be determined as developed by a group over time in a particular geographical niche. The definitions of ethnicity and culture confer the possibility that regardless of ethnic origin according to race, persons living in the same location may have a shared system of beliefs, values and expectations, especially about behavioral sequences and meanings. However, post hoc analyses show that if location is substituted for ethnicity, the results are null, demonstrating that ethnicity is the stronger moderator of the cortisol-weight status relationship and is not confounded by location in the present sample.

Additional studies, both of a cross-sectional and a longitudinal nature, need to be carried out at the cellular and molecular level, using both the activity and physiological effects of the HPA and sympathetic-adrenal-medullary (SAM) axes when investigating ethnic disparities in health, especially obesity and obesity-related disease. Studies of this kind are necessary if a comprehensive picture of the pathogenesis of chronic

disease is to be fully understood. These studies should encompass measurements of HPA and SAM activity, as well as weight status in diverse ethnic groups over the developmental life span to determine the plasticity of physiological systems, the first appearance of obesity and obesity-related chronic disease, as well as additional factors that may interact with physiology to increase the risk and likelihood of morbidity and mortality. Additional studies are also necessary to investigate the effect of self-regulation in nutrition and conflict behavior on HPA axis activity in order to further elucidate eating behavior patterns in externalizing youth.

Chapter 5

Summary

Overall, the findings of these three studies indicate both cross-sectional and longitudinal associations between aggressive behavior and atypical HPA axis activity and the development of overweight/obesity. In addition, these findings are the first to include relational aggression in studies of aggressive behavior and HPA axis activity, as well as the first studies to investigate the association between HPA axis activity and overweight/obesity in children and adolescents. The findings also are unique as they are the first to use a dynamic integration model to examine sex and ethnic differences in biobehavioral development in community-based samples of children and adolescents. These studies advance the field given the increasing prevalence of overweight/obesity in children and adolescents and the ethnic differences that exist in obesity and related morbidity and mortality. In addition, overweight/obesity and an atypical stress response both are associated with the development of chronic disease. Therefore, it is important to identify possible mechanisms that may lead to the pathogenic processes of chronic disease early in development in order to offset these diseases.

In Paper 1, atypical HPA axis activity was measured by less diurnal cortisol variation and an exaggerated response to a stressor. The cross-sectional association of atypical HPA axis activity and relational aggression was moderated by pubertal timing and depressive symptoms. A major finding was that early maturers with a low diurnal cortisol variation as well as adolescents with both a low diurnal cortisol variation and an increased cortisol response to a laboratory stressor exhibited higher levels of relational aggression. No sex differences were found and the results were the same in boys and girls. Conversely, sex differences were found in those who exhibited

higher levels of externalizing behavior. Depressed girls with a low diurnal cortisol variation and either an attenuated or a heightened response to a stressor were most externalizing, whereas depressed later timing boys with a heightened cortisol response to a stressor exhibited higher levels of externalizing behavior. It is of interest to note that the diurnal cortisol variation was only moderately correlated with relational aggression and not correlated with externalizing behavior. However, when integrated into an interactionist framework and the timing of puberty was considered, the association between aggressive behavior and HPA axis activity was stronger. This finding highlighted the importance of using an interactionist framework when considering the integration in biological and behavioral processes at unique periods of development.

The findings also are the first to demonstrate the association of relational aggression with atypical HPA axis activity. An important aspect of the findings is that it highlights the lack of sex differences in the association of relational aggression with an atypical HPA axis activity as it is generally believed that relational aggression is a female-typical behavior that has implications only for future psychopathology (Crick, Ostrov, & Werner, 2006; Crick & Zahn-Waxler, 2003; Zahn-Waxler, Klimes-Dougan, & Slattery, 2000). Paper 1 opens the doorway to the recognition that the development of both physical and mental health disorders should be considered when examining the longitudinal, deleterious effects of relational aggression in both males and females.

In Paper 2, it was hypothesized that relational aggression as well as externalizing behavior predict less diurnal cortisol variation across 12 months. Pubertal timing and depressive symptoms were also hypothesized to moderate the prediction of less diurnal cortisol variation by relational aggression and externalizing behavior

interactions across 12 months. Paper 2 extended the findings of Paper 1 by using the same theoretical model but extended longitudinally questions about the links between aggressive behavior and diurnal cortisol variation. This design and analytical strategy allowed for the examination of the development of atypical HPA axis activity using relational aggression as well as externalizing behavior as predictors of diurnal variations of cortisol. At the same time, the use of multilevel modeling allowed for the examination of the between-group differences by sex at Level 2 of the model. Findings indicate that sex differences existed in the effect of aggressive behavior on diurnal cortisol variation over one year. Girls with higher levels of relational aggression or externalizing behavior problems exhibited a low diurnal cortisol variation. Findings also indicated that depressed, early maturing children and adolescents who practice higher levels of relational aggression had lower variation in their diurnal cortisol profiles. Early maturing, depressed children and adolescents exhibiting higher levels of externalizing behavior had decreasing diurnal cortisol variation over one year.

The findings described above have potential important implications for adolescent health. Frequent HPA axis arousal can lead to a low diurnal cortisol variation while at the same time, elevated cortisol secretion can lead to the inhibition of growth hormones, sex steroids and thyroid secretions (Chrousos & Gold, 1992). All three types of hormones are necessary for growth, reproduction and metabolism and ensure a healthy adolescent. In addition, the development of overweight/obesity is more than likely as elevated cortisol directs the accumulation of fat in visceral depots (Bjorntorp, 1996; Chrousos, 2000). Finally, the prediction of a low diurnal cortisol variation also has implications for the development of substance use disorders in early adulthood

(Huizinck, Ferdinand, Ormel, & Verhulst, 2006; Sher, 2007) as well as the development of chronic diseases such as hypertension and cardiovascular disease in later life (Hulanicka, Lipowicz, Koziel, & Kowalisko, 2007). An important public health message is that the origins and development of chronic diseases and psychopathology are present during such an important developmental phase for physical and cognitive growth.

Finally, in Paper 3, the prediction of weight status one year later by baseline cortisol reactivity and externalizing behavior was investigated. A unique opportunity arose enabling the inclusion of a sample of mixed ethnicity adolescents, some of whom were neglected. Both sex and ethnic differences were considered in the investigation. Externalizing behavior was a significant moderator of cortisol reactivity and weight status one year later but levels of externalizing behavior differed in sex and ethnic groups. A diminished cortisol output at baseline predicted a higher weight status one year later in Eastern girls with low to medium levels of externalizing behavior and non-Hispanic black girls with medium to high levels of externalizing behavior. It is possible that only low levels of externalizing behavior are required for the relationship between cortisol output and cortisol increase in response to a stressor at baseline and weight status to become apparent a year later in the Eastern girls. Findings also indicate that non-Hispanic Black girls with a higher cortisol response to a stressor and medium to higher levels of externalizing behavior are heavier a year later.

Neglect was not found to be a significant moderator of the cortisol reactivity and weight status relationship. The absence of findings was surprising given the literature that connects neglect in childhood to both HPA axis perturbations (Cicchetti & Rogosch, 2001; Gunnar & Valquez, 2001) and overweight/obesity

(Johnson, Cohen, Kasen, & Brook, 2002; Noll, Zeller, Trickett, & Putnam, 2007). These results are especially disquieting given the increase in the prevalence of overweight/obesity in adolescent girls. Non-Hispanic Black girls show the highest increase in overweight/obesity prevalence (12.2%), followed by 8% in non-Hispanic white girls (Ogden et al., 2006). Overweight/obesity in adolescence is associated with early-onset Type 2 diabetes and cardiovascular disease in adolescence and adulthood. The mortality rates for Type 2 diabetes mellitus in non-Hispanic Blacks is twice that of non-Hispanic whites (CDC Fact Sheet 2004). Conversely, even though mortality rates from cardiovascular disease in non-Hispanic Black and White females are comparable (39.8% vs. 39.6%), the mortality rate for non-Hispanic Black women is higher than the national rate, accounting for 368.1 per 100 000 deaths in the population (AHA Heart Facts for African Americans, 2005). Based on our findings, non-Hispanic black females appear to be at a distinct disadvantage for health especially with the identification of an atypical stress response in predicting future overweight. Sanchez-Hucles (1997) proposed that race and gender can have interactive, inter-locking, additive and possibly multiplicative effects on various aspects of the lives of women of ethnic origin (Sanchez-Hucles, 1997). This multiple jeopardy of ethnicity and sex can present unique stressors in the lives of non-Hispanic black adolescents, especially when discrimination is considered. It has been indicated that discrimination that combines racism and sexism in African American women was associated with increased stress reactions (King, 2005). Frequent stressors in the lives of non-Hispanic Black female adolescents may lead to frequent HPA arousal and the development of chronic disease through an atypical stress response and overweight/obesity.

These three studies highlight a necessity to carry out additional examinations of sex and ethnic differences in the association of aggressive behavior and the development of atypical HPA axis activity and overweight and obesity. It also highlights a need to include self-regulation as an additional process in these studies. Self-regulation can affect nutrition behavior, especially in externalizing youth (Anderson, Winett, & Wojcik, 2007; Judge & Jahns, 2007). Furthermore, investigations of additional moderators, specific to sex and ethnicity are necessary to further elucidate the pathways leading to chronic disease pathogenesis in adolescence and later in adulthood. As demonstrated in papers 1 and 2, the moderators of pubertal timing and depressive symptoms were significant when only Eastern adolescents were considered. However, when the Eastern sample was compared to the multiethnic West Coast sample, the moderator was significant at different levels of externalizing behavior in predicting future overweight from a diminished cortisol output. As mentioned above, perceptions of sexism and racism as stressors may be especially salient to African American women. More studies are needed to identify moderators particular to each ethnicity, while appreciating that ethnic similarities in biobehavioral associations also exist.

There is a paucity of research at the cellular and molecular levels of analysis that may involve differing mechanisms in leading to disease in different ethnic groups. Most of this research employs non-Hispanic White participants. Some exceptions do apply (S. Cohen et al., 2006; DeSantis et al., 2007; Yanovski, Yanovski, Cutler, Chrousos, & Filmer, 1996; Yanovski et al., 1996). The studies all emphasize a need to further investigate the unique effects that chronic stress may produce

physiologically within minority ethnic groups. For example, studies that have examined HPA axis characteristics and perturbations have done so in non-Hispanic Whites and Blacks mainly, as well as Hispanics. Further studies are needed in the literature that definitively investigate and implicate these unique physiological perturbations in the development of chronic disease in later life. The discovery of restriction enzymes such as *Bcl*I can be used as a potential marker in the genetic analysis of the glucocorticoid receptor (GC) gene (Buemann et al., 1997; Weaver, Hitman, & Kopelman, 1992). The GC receptor gene has been implicated in the stress response as well as in overweight/obesity development. Restriction fragment length polymorphism can be used to understand the genetic underpinnings of differences in disparities in susceptibility to chronic disease as well as ethnic disparities in mortality. Such an examination is not a question of racialized genetics. In the 19th and 20th centuries, the invention of the microscope allowed scientists to study characteristics of the pathogens that caused infectious disease and allowed for the effective targeting and elimination of these pathogens. Today, infectious disease, for the most part, is controllable. Of course, these efforts could not have been successful without the additional public health eradication using education and the promotion of sanitary conditions in public and private space. With similar advancements in genetics, analysis of genetic markers would allow for an effective complement of public health efforts in the attempted control of chronic, multifactorial disease. Public health efforts at levels of the population ensure that health and well-being is promoted. However, until there is sufficient understanding and targeting of chronic disease at the cellular and molecular levels in all ethnic groups, these public health efforts will fall short of the desired targets.

The most dramatic advances in identifying mechanisms involved in both aggressive behavior and obesity will come from not only studies at the genetic level but also at the behavioral and contextual levels. Current studies have identified unique behavioral components of the pathway leading to the development of HPA axis perturbations and overweight/obesity. Aggressive behavior was implicated in each study as playing the roles of both a predictor and a moderator of these developmental pathways. These findings have implications for the development of innovative behavioral interventions and approaches in childhood and adolescence. Behavioral interventions that target the reduction of externalizing behavior and relational aggression in childhood and adolescence, especially through self-regulation and decision making skills (Riggs, Sakuma, & Pentz, 2007) may reduce the deleterious effects on HPA axis activity and overweight/obesity. These interventions may also include alternative strategies for conflict resolution as well as teaching the recognition of true conflict in ambiguous situations. Once again, research on aggressive behavior in all ethnic groups is necessary and although this has been done to an extent, further study is necessary in order that the etiology of aggressive behavior may be understood cross-culturally, and so targeted effectively.

Limitations arise across the three studies. First, since the samples used in the first two studies were predominantly non-Hispanic White, the generalizability of these results to all ethnic groups is not possible. However, the similarity in the relationship between cortisol output and later weight status in Paper 3 in both non-Hispanic Black and predominantly White girls gives rise to the possibility that studies involving the investigation of HPA axis activity may give rise to similar associations in other ethnic

groups. However, this assertion will only be confirmed if the results of these papers are replicated in studies with a multiethnic sample. Second, the sample size in each study was modest. However, sample size was adequate to elucidate significant relationships between aggressive behavior, HPA axis activity and weight status. Third, Papers 2 and 3 were longitudinal studies that examined these relationships across a 12 month period. This time period is only a small fraction of time during the pubertal phase of the life span. Therefore, it would be of interest to investigate activity within the HPA axis over a longer course during adolescence. HPA axis activity may rebound if the stressor is controlled or removed (Miller, Chen, & Zhou, 2007). At the same time, it is important to monitor the longitudinal effects of HPA axis perturbations such as those found here, that is less diurnal cortisol variation and a heightened or attenuated response to a stressor and their effects on adolescent growth, metabolism and reproduction. These longitudinal investigations may further reveal the origin, development and susceptibility to chronic disease from adolescence to adulthood. Finally, recent studies have demonstrated the use of both measures of sympathetic-adrenal-medullary (SAM) and HPA axis activity in the investigation of the development of future psychopathology and disease (Bauer, Quas, & Boyce, 2002) and aggressive behavior (Gordis, Granger, Susman, & Trickett, 2006). The current studies used an integrative, as opposed to a multisystem approach, which has led to additional clarification in the development of an atypical stress response and overweight/obesity. Further studies should include measures of both HPA and SAM axis activity, both easily measured in saliva (Kirschbaum & Hellhammer, 1989; Nater et al., 2006) when investigating developmental trajectories of health and disease across adolescence.

Overall, the use of the dynamic integration perspective to investigate biobehavioral developmental pathways in adolescent health was useful. Through this perspective, relational aggression has been identified as a stressor that is potent enough to exert continuous and deleterious effects on HPA axis activity, resulting in less diurnal cortisol variation over 12 months. An atypical stress response, i.e. a diminished cortisol output and heightened cortisol reactivity, also was implicated in the prediction of a higher weight status 12 months later in Eastern and West Coast non-Hospanic Black girls. Additional studies are necessary to continue the examination of the unique interactions that may occur between aggressive behavior, i.e. relational aggression and externalizing behavior, an atypical stress response and the development of overweight/obesity. It is especially pertinent that the participants in these studies represent multiple ethnic groups. Finally, the use of genetic markers in studies of the development of the stress response and overweight/obesity are necessary to understand the susceptibility and development of chronic disease in childhood and adolescence.

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APPENDIX

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Morningness/Eveningness, Morning-to-Afternoon Cortisol Ratio, and Antisocial Behavior Problems During Puberty

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The relationship between morningness/eveningness (M/E) and morning-to-afternoon cortisol ratio, pubertal timing, and antisocial behavior was examined in 111 girls and boys ages 8 to 13 years. Cortisol levels showed a significant increase after awakening and declined thereafter ($p < .05$). Eveningness was related to a composite measure of antisocial behavior and rule-breaking and attention behavior problems and conduct disorder (CD) symptoms in boys and relational aggression in girls. In boys only, lower a.m. to p.m. cortisol ratio, indicating less circadian decrease in cortisol, was related to attention problems. Early pubertal timing was associated with boys' rule-breaking and attention behavior problems and CD symptoms and girls' relational aggression. The findings indicate that evening activity preference; extreme a.m. to p.m. cortisol ratios, in one case; and early pubertal timing were associated with antisocial behavior even in young adolescents, but the findings were stronger for boys than for girls.

Keywords: cortisol, morningness, eveningness, circadian, aggression

Current perspectives on antisocial behavior indicate that it begins early and is embedded in aggressive behavior, emotional dysregulation, genetic predispositions, disorganized families, peer interactions (Aguilar, Sroufe, Egeland, & Carlson, 2000; Moffitt, 1993), and biological processes (Stoff & Susman, 2005). Although an increasing number of studies with adults have considered biological processes in the ontogeny of antisocial behavior (see Raine, 2002), few studies have actually incorporated biomarkers and antisocial behavior simultaneously during puberty when such behavior is expected to rise in boys and girls. The aim of this report was to examine two vulnerabilities for antisocial behavior, morningness/eveningness (M/E) and the ratio of morning-to-afternoon cortisol (a.m. to p.m. ratio); the interaction of these vulnerabilities with timing of puberty; and antisocial behavior in young adolescents. M/E refers to individual differences in sleep–wake patterns and preferences for activity and alertness during the morning or evening and putatively has biological, psychosocial, and contextual components (Carskadon, Vieira, & Acebo, 1993). M/E also is proposed to have a genetic component, although the results of genetic studies are inconsistent (Robilliard et al., 2002). Overall, M/E is an understudied concept despite its potential role in antisocial behavior, family conflict, psychopathology, achievement, and sleep loss. Antisocial behavior herein refers to parent reports of externalizing behavior problems and oppositional and conduct disorder (CD) symptoms and adolescent reports of relational aggression.

The theoretical perspective upon which the study is based integrates multilevel processes consisting of biological and behavioral vulnerabilities, the timing of the pubertal transition, and antisocial behavior. The model was derived from integrating principles from developmental science (Magnusson & Cairns, 1996), biopsychosocial development (Lerner, 1998; Magnusson & Stattin, 1998; Susman & Rogol, 2004), and endocrinology (Bourguignon & Plant, 2000). These perspectives suggest that the ontogeny of behavior is a result of continuous interaction between individuals' biological processes and their social and nonsocial environments. The specific heuristic model guiding this report proposes, first, that psychological and biological risks (*vulnerabilities*) predispose individuals to an increase in behavior problems during puberty. Diathesis-stress models of psychopathology assert that most individuals have some level of predisposing risk, or diatheses, for psychosocial problems. The tendency to develop problems varies depending on the interaction between the degree to which risk factors are in place and the degree of stress experienced by the individual (Monroe & Simons, 1991; Richters & Weintraub, 1990). Applied to the case of pubertal development, the diathesis-stress model suggests that vulnerabilities existing prior to, or during, early puberty will interact with the transitional stressors of puberty to place individuals at risk for behavior problems (Caspi & Moffitt, 1991). Biological vulnerabilities are included here given

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the documented relationship between biological substances, that is, cortisol, and antisocial behavior (e.g., McBurnett et al., 2005; Pajer, Gardner, Rubin, Perel, & Neal, 2001). Second, *transitional stress* refers to the stress imposed by individualization and destandardization that accompanies periods of developmental change. Transitional stress with regard to puberty refers to the timing of physical maturational changes that may lead to an increase in behavior problems (Angold, Costello, Erkanli, & Worthman, 1999; Nottelmann, Inoff-Germain, Susman, & Chrousos, 1990). Periods of transition and the timing of transitions, such as puberty, bias vulnerable individuals toward behavior problems (Dorn & Chrousos, 1997). Hence, vulnerabilities are expected to interact with the transitional stress of puberty so as to be related to antisocial behavior.

Vulnerabilities: Morningness/Eveningness

M/E refers to individual differences in sleep-wake patterns and preferences for activity and alertness during the morning or evening (Carskadon et al., 1993). Eveningness is associated with poorer adjustment and school performance in older adolescents (Anderrson, 2001; Cofer et al., 1999; Giannotti, Cortesi, Sebastiani, & Ottaviano, 2002) and impulsiveness in boys (Caci, Robert, & Boyer, 2004). Eveningness preference also is related to loitering and intoxication (Andershed, 2005), attention problems, poor school achievement, injuries, and emotional instability compared with other chronotypes (Giannotti et al., 2002). These psychosocial problems are attributed to difficulties in interactions with families and schools created by the adolescent's being out of synchrony with cultural norms about the timing of activities.

Others have suggested eveningness as an endogenous characteristic that is linked to phase delay in sleep. At approximately age 10 years, some children begin to exhibit a later onset and offset of sleep (Carskadon, 1990; Ohayon, Carskadon, Guileminault, & Vitiello, 2004; Roenneberg et al., 2004), particularly if the day does not require a specific bedtime or time of awakening. This phase delay could be attributed exclusively to psychosocial or exogenous factors, such as increasing independence, relaxed parental restrictions, and involvement in late-night activities (Stattin & Kerr, 1999). The evidence in favor of M/E as an exogenous factor is its relationship with pubertal maturation. A positive and significant relationship between pubertal status and M/E was found in girls, with a similar, though nonsignificant, trend in boys (Carskadon et al., 1993). These findings were interpreted as supporting involvement of a biological factor in the adolescent phase-preference delay and M/E. If M/E has a biological component, as suggested by Carskadon and colleagues (1993), eveningness preference is expected to be present in children and young adolescents and will be related to behavior problems even before the phase delay that is expected to occur later in puberty. Thus, eveningness is proposed to be a vulnerability for antisocial behavior in the pre- and early-pubertal years as well as in later adolescence.

Cortisol

The biomarker cortisol is considered a strategic window on the stress system (Gunnar & Townsend, 2003), and individual differences in cortisol levels are a vulnerability for adjustment problems from the prenatal period (Susman, Schmeelk, Ponirakis, & Gariepy, 2001) to old age (de Bruin, Vieira, Rocha, & Viana, 2002). The hypothalamic–pituitary–adrenal (HPA) axis controlling the endocrine arm of the stress system is more activated in the morning hours than in the evening, and cortisol levels are typically close to their peak at morning awakening and rise further in the 20-min period after awakening in adults (Schmidt-Reinwald et al., 1999). Following the morning peak, cortisol decreases during the morning hours, plateaus in the afternoon and evening hours, and begins to rise in the early morning (Weitzman et al., 1971). However, the morning rise only recently was demonstrated in young adolescents. In a large-scale study that included 1,768 10–12-year-olds, the cortisol awakening response was present in 70.7% of children, but the increase was lower in children than in adults (Rosmalen et al., 2005). Given these findings and those with adults (Edwards, Evans, Hucklebridge, & Clow, 2001), it was expected that the cortisol awakening response would be apparent in young adolescents, with an initial rise postwakening followed by a decrease 40 min later, with the decline continuing over the morning hours resulting in a plateau in the afternoon and evening.

Circadian within-individual differences exist in patterns of cortisol secretion, and these variations are related to emotions and behavior. For instance, clinical depression is characterized by a flat circadian rhythm with high levels of cortisol secretion throughout the day (Gold, Goodwin, & Chrousos, 1988). Variations in normal circadian cortisol patterns also vary with contextual factors (Watamura, Donzella, Alwin, & Gunnar, 2002). Much of the previous research relating cortisol and psychological factors is based on one to three samples obtained on one occasion, usually in the morning or afternoon. Therefore, it is important to determine circadian patterns of cortisol secretion across the day as a marker of how variations in patterns of secretion relate to antisocial behavior. Accordingly, it is suggested that morning-to-afternoon cortisol ratio provides a more stable indication of the relationship between cortisol and antisocial behavior compared with levels obtained at any one time period during the day. A low morning-to-afternoon cortisol change is indicative of atypical patterns of circadian variation, given the normative pattern of higher levels in the morning tapering to a nadir in the afternoon. Overall, lower basal levels of cortisol are associated with antisocial behavior (McBurnett, Lahey, Rathouz, & Loeber, 2000; Pajer et al., 2001; Susman, Dorn, Inoff-Germain, Nottelmann, & Chrousos, 1997). With regard to circadian change, a low morning to late afternoon ratio is expected to be related to antisocial behavior in boys and girls.

Transitional Stress

Off-time pubertal development can generate high emotional arousal because being off time means being different from peers (Simmons & Blyth, 1987). Earlier timing of puberty is related to antisocial behavior in previous studies (e.g., Ge, Conger, & Elder, 1996; Ge, Brody, Conger, Simons, & Murry, 2002). In addition, the model described above proposes that the interaction between vulnerabilities and timing of puberty will be related to antisocial behavior. Caspi and Moffitt (1991) proposed that potentially disruptive transitions, like an early pubertal transition, produce personality continuity, not change. Thus, early timing of pubertal, hormone, and growth changes and the interaction of these changes with existing vulnerabilities is proposed to accentuate antisocial behavior problems during puberty. Specifically, the vulnerabilities of M/E and the ratio of a.m. to p.m. cortisol will be moderated by

the transitional stress of off-time puberty, given the common dependence on the hypothalamus for control of both the circadian rhythm and the timing of reproductive system changes at puberty. Thus, the hypothesis is that early timing, and the interaction between the vulnerabilities of eveningness, or a low ratio of change in cortisol across the day, will be related to antisocial behavior.

In summary, the following hypotheses were advanced. First, eveningness preference, compared with morning preference, will be related to antisocial behavior, externalizing behavior problems, oppositional and CD symptoms, and relational aggression. Second, a low a.m. to p.m. cortisol ratio, or less circadian change in cortisol, will be related to antisocial behavior. Third, pubertal timing will interact with M/E or cortisol ratios such that the adolescents with eveningness preference and lower a.m. to p.m. ratio, or eveningness preference and earlier onset of puberty, will exhibit more antisocial behavior than adolescents with morningness preference, higher a.m. to p.m. cortisol ratio, or later onset of puberty.

Method

Participants

Participants were 111 healthy children and adolescents and a parent or caregiver (90% mothers, 8% fathers, and 2% grandmothers) who were participants in a longitudinal study of puberty and behavior. Data from the first wave of measurement were used in the current analyses. Girls were ages 8, 10, or 12 years ($n = 55$, $M = 10.49$ years, $SD = 1.51$), and boys were ages 9, 11, or 13 years ($n = 56$, $M = 11.44$ years, $SD = 1.63$). The age difference was designed to include boys and girls at similar stages of pubertal development because girls mature earlier than boys. Eight (15%) of the girls had reached menarche. Day in menstrual cycle was controlled as the girls were assessed between Day 5 and 9 of the menstrual cycle. The racial/ethnic composition of the adolescents consisted of the following for boys and girls, respectively: White/non-Hispanic (89%, 89%), Hispanic (4%, 5%), African American (2%, 5%), Asian (4%, 1%), and Native American (1%, 0%). The family socioeconomic status (SES) and other demographic characteristics of the sample appear in Table 1.

Table 1
Means, Standard Deviations, and Percentage for Demographic Characteristics of the Sample

Variable	Girls ($n = 55$)	Boys ($n = 56$)
Age (years)		
8	17	
9		19
10	19	
11		19
12	19	
13		18
M (*SD*)	10.49 (1.51)	11.44 (1.63)
Tanner stage[a] (%)		
1	20	7
2	10	20
3	20	15
4	1	9
5	2	0
Refused	2	5
Family SES[b]		
M (*SD*)	46.2 (10.6)	47.2 (10.6)

Note. SES = socioeconomic status.
[a] Tanner stage was based on breast stage for girls and genital stage for boys. [b] SES was determined by the Hollingshead Scale.

Sample Recruitment

The aim was to select a sample that was heterogeneous in terms of occupational and educational status of the parents from a nonurban area. The recruitment strategy consisted of obtaining a list of names of children from designated ZIP codes from the American Student List (ASL), a commercial enterprise that provides lists of names of school-age children. The list of names was generated by ASL from ZIP codes supplied by the investigator. The ZIP codes were chosen from the county in which the research lab was located and adjacent counties that had easy accessibility to the lab. The list from ASL included the name, address, and phone number (in some cases) of children enrolled in the education system in the designated ZIP codes. Names were chosen at random from the list until all names were exhausted. A letter was mailed to the parents of the 966 children on the list. Either the parents called the lab to ask about the study or families were contacted via phone calls to inquire whether the adolescent was interested in participating in the study or, whether the adolescent was ineligible, if a sibling was interested in participating. Eligibility criteria were as follows: boys ages 9, 11, or 13 years; girls ages 8, 10, or 12 years; not on medications that would interfere with hormone levels (e.g., oral steroids); and free from chronic health problems (e.g., diabetes, cancer) or serious mental health problems that would interfere with completing the questionnaires. Children on psychotropic medications were included, as eliminating these children would have excluded an important group at risk for antisocial behavior. Eighty-five children were enrolled on the basis of responses from the parents of the children on the list. Of the remaining children, research staff were unable to contact 584 (e.g., returned letters, no published phone number), and 89 were ineligible on the basis of study criteria. Other reasons for nonparticipation included discomfort with research procedures ($n = 11$); family problems ($n = 3$), such as death in the family; lack of time ($n = 41$); not interested ($n = 39$); or multiple reasons ($n = 1$). The remaining participants were obtained from flyers distributed throughout the community and from telephone responses to e-mails distributed to staff at a large university. These efforts resulted in an additional 48 families contacting the research project; of these, 26 participated.

The sample was heterogeneous with regard to occupational status: 8% machine operators, semiskilled workers; 15% skilled craftsmen, clerical; 31% medium business, minor professional, technical; and 45% major business/professional, according to Hollingshead (1975) criteria. Similarly, the educational level of the sample was diverse, with 1% less than high school education; 24% high school graduate; 20% associate degree/specialized training; 26% bachelor's degree; and 29% postgraduate/masters. The sample had a disproportionate number of families (54%) employed at the university but was approximately representative of the number of citizens who were employed by the university and resided in the

counties from which the families were recruited. In brief, the sample was of interest because it was heterogeneous with regard to occupation and educational status and was drawn from nonurban or nonsuburban communities.

If the adolescent and a parent or guardian were interested in participating, the parent was administered a telephone screening interview by a pediatric nurse or a graduate student to establish the adolescent's eligibility for the study. The screening interview also asked questions about the child's overall health, behavior problems, use of prescribed and over-the-counter medications, and school performance.

Procedures

If the eligibility criteria for participation were met, a visit was scheduled for the prepubertal/pubertal adolescent and 1 parent or guardian at a General Clinical Research Center (GCRC) of a research university. The visits were scheduled at 4:00 p.m. (±1.5 hr) depending on the adolescent's school schedule. The study protocol was approved by the Institutional Review Board of a university and the advisory committee of the GCRC. All methods and procedures were executed in accordance with a written protocol. Upon arrival at the GCRC, the parent and adolescent were read an explanation of the study, given an opportunity to ask questions, and asked to signed a consent or assent form.

Measures

Vulnerabilities: Morningness/eveningness. Circadian preference was assessed using a scale designed for use with adolescents that consists of self-reported phase tendencies (Carskadon et al., 1993). Participants responded to 10 questions about their preferred time of day for activities (e.g., bedtime, waking time, recreational activities, and tests). Scores could range from 43, indicating extreme morning preference, to 10, indicating extreme evening preference. The full-scale reliability of M/E is reported to be good, $\alpha = .82$ (Carskadon & Acebo, 1992) and is related to sleep times in children and adolescents (Giannotti et al., 2002). In the current sample, the M/E scale Cronbach's alpha was .75.

Cortisol. Saliva offers a noninvasive route for the measurement of the unbound fraction of cortisol and is highly correlated with the circulating unbound fraction, $r = .54$ to $r = .97$, with changes in plasma levels reflected in salivary samples within minutes (Kirschbaum & Hellhammer, 1989). Upon completing the GCRC visit, each adolescent and parent was trained on the protocol for collection of saliva at home. Participants were instructed to collect all samples on a nonschool day, when the child did not have other scheduled activities, such as a sports activity, that would interfere with the collection protocol. The potentially confounding problem of time of arising on a nonschool day was avoided by instructing the adolescents to arise at the same time on the collection day as on a school day and record the time of day of rising. A nonschool day was chosen because collecting saliva in a school context presents multiple feasibility problems including the difficulty of collecting saliva at specific times in a school setting and the need for parental supervision with younger children to accurately collect saliva and avoid missing data. The current design avoided these problems because a parent was available to supervise saliva collection and the times of collection could be more closely adhered to in the home.

Three samples of saliva were collected at 20-min intervals upon awakening and prior to breakfast, teeth brushing, or eating: Sample 1 was obtained immediately on wakening, Sample 2 at 20 min postwake time, and Sample 3 at 40 min postwake time. Additional samples were collected at noon prior to the midday meal, at 4:00 p.m., and at bedtime. Participants were instructed to rinse their mouths with water before passively drooling into a 5-mL tube and to collect saliva to the 4-mL mark on the tube within 5 min and then put the tube in the refrigerator. Participants were requested not to eat or drink (except water) during the collection procedure. Participants and/or their parents recorded the time of collection for each sample and noted any activities or potential stressors (e.g., argument with siblings). Participants were instructed to collect the samples within 2 weeks of the completion of the lab visit.

Saliva samples were refrigerated (4 °C) after collection at home and later transferred to a −70 °C ultralow freezer until assay. All samples were assayed using a highly sensitive enzyme immunoassay specifically designed for use with saliva (Cat. no. 1-0102/1-0112; Salimetrics, State College, PA). The test requires only 25 μl of saliva (for singlet determinations) and has a range of sensitivity from 0.007 to 1.2 μg/dl and average intra- and interassay coefficients of variation of 5.34% and 9.86%. The standard curve was highly reproducible (mean $R^2 = .998$). In each assay, controls representing low and high salivary levels were included, and all samples and controls were assayed in duplicate and the average used in analyses. Outliers in the distribution ($n = 2$) were not discarded but were reassigned a value equaling two standard deviations above or below the mean following the procedure of Kertes and Gunnar (2004). The a.m. to p.m. cortisol ratio was calculated by dividing the mean of the three morning cortisol levels by the 4:00 p.m. cortisol level. The rationale for using the 4:00 p.m. cortisol ratio (mean time of collection = 4:48 p.m., $SD = 56$ min), as opposed to the bedtime cortisol ratio, was that there was a wider variation in the collection of the bedtime cortisol sample. Thus, the bedtime value may not reflect a true early nighttime circadian score.

Keep-by-bed diary. The adolescents kept a diary at their bedside to report day of the week when the saliva was collected, bedtime, awakening time, method of awakening (e.g., alarm clock), sleep quality, difficulty waking up, unusual events (e.g., fight with a parent), and saliva collection time. The purpose of the diary was to inform the analysis of factors that should be controlled in the models and to provide specific information on the time of saliva collection.

Transitional stress: Pubertal stage and timing. Pubertal stage was assessed by a master's degree-level pediatric research nurse, using Tanner criterion of genital and pubic hair stage for boys and breast and pubic hair stage for girls (Marshall & Tanner, 1969, 1970). The nurse first explained the five stages of puberty and showed the adolescent and parent pictures of the five Tanner stages. The parent and the adolescent, independently, assigned a 1 to 5 rating of the adolescent's stage of pubertal development. The nurse then conducted a physical exam, which included breast palpation as recommended by Kaplowitz, Oberfield, & the Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society (1999). Breast stage (girls) or genital stage (boys) was used in the analysis. If the adolescent did not consent to the physical exam ($n = 8$), the adolescent's self-rating of his or her pubertal stage was substituted for the nurse's

rating. The correlation between nurse's rating and adolescent's rating of Tanner stage (breast/genital) was $r = .76$, $p < .01$. Pubertal timing was then established by regressing pubertal stage on chronological age within boy and girl groups and calculating a residual for each individual (Dorn, Susman, & Ponirakis, 2003). The residuals then were used as an index of timing of puberty in the statistical analyses. A residual indicates how far an individual's value for pubertal development is above or below the regression line representing the expected value for his or her age; thus, a negative value indicates later timing (i.e., development less than expected), and a positive value indicates earlier timing. For the regression analysis, chronological age was calculated as days since birth date (as reported by the parent).

Antisocial behavior: Diagnostic Interview for Children (DISC–IV). The DISC–IV is a structured interview used to assess symptoms of psychiatric disorders in children and adolescents in accordance with *Diagnostic and Statistical Manual of Mental Disorders* criteria (American Psychiatric Association, 2000; Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000). The DISC is a commonly used and completely preconstructed computerized interview. The DISC has been reported to be reliable and accurate in the diagnosis of psychiatric syndromes and has been shown to be even more reliable in diagnoses than clinicians (Angold & Fisher, 1999). There are parallel versions for parents (DISC–P for 6- to 17-year-olds) and youth (DISC–Y for 9- to 17-year-olds). Parent reports were used in the current analysis, given the higher reported correlation of parent scores across time (Piacentini et al., 1999). As psychiatric disorders are infrequently diagnosed in a community sample, symptom counts of oppositional defiant disorder (ODD) and CD, were used in the current analysis. The number of adolescents receiving a diagnosis in the last year was: ODD, $n = 13$; CD, $n = 1$.

Child Behavior Checklist. Externalizing behavior problems over the last 6 months were assessed with the Child Behavior Checklist/4–18 (CBCL), a norm-referenced behavior rating scale completed by parents (Achenbach, 2001; Achenbach & Edelbrock, 1983). Parents rate their child's behavior on a 3-point scale for behavioral and emotional problems that occurred during the past 6 months. Three of the externalizing behavior problem subscales were used in the current analysis; Cronbach's alpha for these subscales in this sample were Rule-Breaking, .69; Attention Problems, .70; and Aggressive Behavior, .90. The T scores on the CBCL scales for this sample were comparable to the published norms (Achenbach, Verhulst, Baron, & Althaus, 1987): Rule-Breaking Behavior, 51.79, Attention Problems, 52.00, and Aggressive Behavior, 52.9.

Relational aggression. Relational aggression is behavior specifically intended to hurt another child's friendships or feelings of inclusion in a peer group and has been used as a self-report index of psychosocial maladjustment (e.g., Salmivalli, Kaukiainen, Kaistaniemi, & Lagerspetz, 1999; Austin & Joseph, 1996). The Relational Aggression Scale from Crick and Grotpeter's (1995) Children's Social Behavior Scale was used in this report (Cronbach's $\alpha = .67$). A higher score indicates a higher degree of self-reported relational aggression.

Composite score: Total antisocial behavior. A composite score of the three CBCL subscales scores, relational aggression scores, and ODD and CD symptoms scores also was calculated. The raw CBCL scores, DISC–R symptoms, and relational aggression were summed given the correlation among the scores. The Cronbach's alpha for the total antisocial behavior score was .74 for boys and .78 for girls. For both boys and girls, all subscales contributed comparably to the total score. The individual subscales also were analyzed separately on the basis of the rationale that the stress response is differentiated in adolescents with different types of problems. For instance, inattentive compared with hyperactive adolescents show a decrease in cortisol poststressor (King, Barkley, & Barrett, 1998; Randazzo, Dockray, & Susman, in press).

Socioeconomic status (SES). The participating parent reported on the education and occupation of the parent/caregivers in the household. The range of possible SES scores was from 8 to 66 (Hollingshead, 1975).

Results

Morning Cortisol

The three morning cortisol samples (Samples 1, 2, and 3) initially were examined to determine whether young adolescents showed the expected high, first morning cortisol with a further rise approximately 20 min postwakening. The three morning cortisol samples portrayed the hypothesized pattern of change across the first 40 min after awakening. A 2 (sex) $\times$ 6 (cortisol samples) repeated measures analysis of variance was done with cortisol as the repeated measure to assess change across the day. There were significant mean changes in cortisol level from awakening to bedtime, $F(5, 85) = 72.07$, $p < .001$. The marginal mean for each sample significantly differed from all others, $p < .05$, except that the first and third morning samples did not significantly differ from each other. Figure 1 shows the mean cortisol level for the three awakening, noon, 4:00 p.m., and bedtime samples.

Descriptive Statistics: Antisocial Behavior

The means and standard deviations appear in Table 2 for M/E, a.m. to p.m. cortisol ratio, CBCL syndromes (attention problems, rule-breaking behavior, and aggressive behavior), DISC–IV (parent) ODD and CD symptoms, and relational aggression for boys and girls. There were no sex differences in the means for any of the variables.

The correlations between the measures for boys and girls appear in Table 3. There were few significant zero-order correlations between M/E and the indices of antisocial behavior for boys or girls. Correlations between a.m. to p.m. cortisol ratio and antisocial behavior were marginally significant. The antisocial indices were low to moderately high for boys ($r = .32$ to .68) and girls ($r = .35$ to .79). As anticipated, use of psychotropic medications was also correlated with antisocial behavior, with the antisocial adolescents reporting more use of medications.

Hypothesis Testing

The hypotheses were tested using hierarchical regression analysis with a separate model run for total antisocial behavior and each of the subscales. Chronological age, SES, and psychotropic medication use were entered in the first step, followed by M/E in the second step and a.m. to p.m. cortisol ratio in the third step, followed by pubertal timing, the interaction term between M/E and pubertal timing, and then the interaction term between a.m. to p.m.

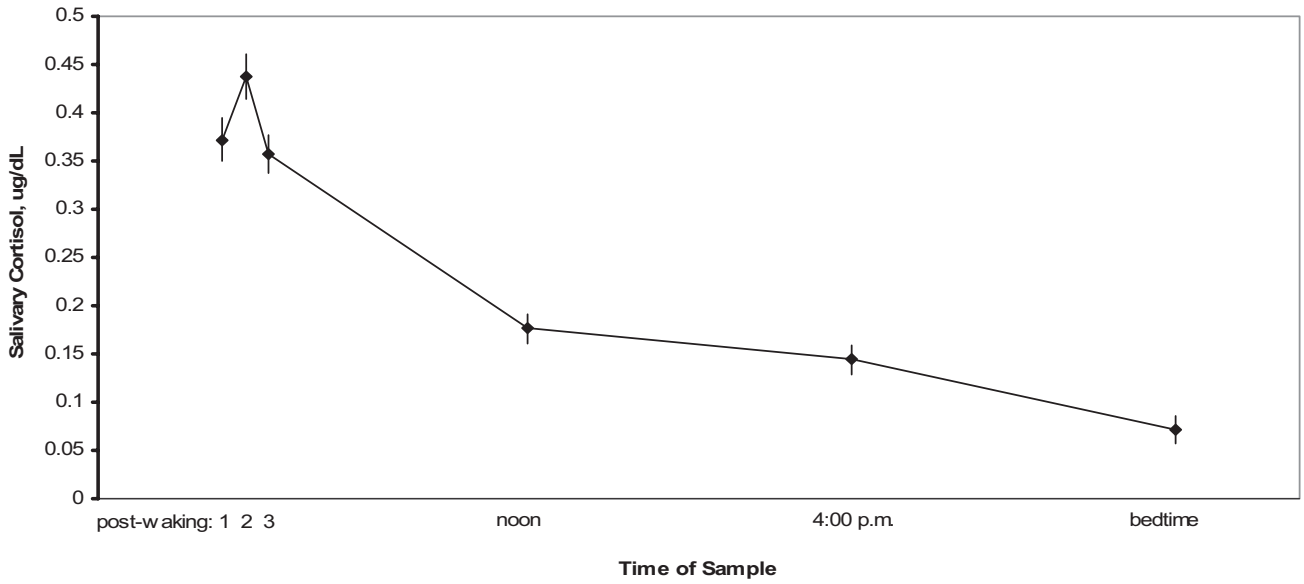

Figure 1. Means for three cortisol awakening response samples (postwakening:1, 2, 3), and noon, 4:00 p.m., and bedtime samples. The three postwakening samples were collected immediately upon waking, 20 min postwakening, and 40 min postwakening.

cortisol ratio and pubertal timing. The rationale for the blocks of variables was guided by the necessity of initially eliminating possible covariates and by our theoretical model. Age and the interaction of timing of puberty and M/E or a.m. to p.m. cortisol ratio were not associated with the antisocial criterion variables and so were dropped from the model with one exception. Even though SES was not significantly associated with antisocial behavior, including it improved the fit of the models; therefore, SES was included in the final models (see Table 4). In addition, medication use was associated with antisocial behavior but did not significantly change the significance of the relationship between vulnerabilities and antisocial behavior. The regression analyses were run separately for girls and boys because within-group patterns of association were expected to be different for boys and for girls as in previous studies (e.g., Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001; Susman et al., 1987). The betas, degrees of freedom, *F* statistics, and *p* values for all significant regression models appear in Table 4.

Table 2
Means and Standard Deviations for Morningness/Eveningness, Salivary Cortisol, Transitional Stress, and Antisocial Behaviors

Variable	*M*	*SD*
Vulnerabilities		
Morningness/eveningness score	29.50	5.19
a.m. to p.m. cortisol ratio	4.43	3.05
Transitional stress		
Tanner stage[a]	2.35	1.02
Timing of puberty (residuals)	0.05	1.03
Antisocial behavior	10.76	10.54
CBCL		
Attention problems	1.74	2.45
Rule-breaking behavior	0.81	1.40
Aggressive behavior	3.40	4.41
DISC-IV		
Conduct disorder symptoms	0.70	1.17
Oppositional defiant symptoms	2.93	2.44
Relational aggression	7.72	2.96

Note. CBCL = Child Behavior Checklist; DISC-IV = Diagnostic Interview Schedule for Children.
[a] Tanner stage was based on breast stage for girls and genital stage for boys.

Vulnerabilities

Total antisocial behavior. The total antisocial behavior score was negatively related to M/E for boys. This relationship indicates that eveningness preference is characteristic of antisocial boys.

CBCL rule-breaking behavior. M/E was negatively related to rule-breaking behavior for boys but not for girls. The negative correlation indicates that eveningness was associated with rule-breaking behavior problems.

CBCL attention problems. The relationship between rule-breaking behavior and M/E is paralleled by the relationship between M/E and the CBCL syndrome attention problems. Eveningness was associated with a greater number of attention behavior problems for boys but not for girls.

CBCL aggressive behavior. There was no significant relationship between M/E and the CBCL aggression problems for boys or girls.

DISC–IV ODD and CD symptoms. M/E was not related to oppositional problems. M/E was negatively related to parent reports of number of DISC–IV CD symptoms for boys but not for girls. CD symptoms were associated with eveningness in boys.

Relational aggression. As opposed to the findings for rule-breaking behavior, attention behavior problems, and CD symptoms, eveningness was related to relational aggression in girls but not in boys. This relationship was in the hypothesized direction; an

Table 3
Correlations Between Morningness/Eveningness, a.m. to p.m. Cortisol Ratio, Measures of Antisocial Behavior, and Psychotropic Drug Use

Variable	1	2	3	4	5	6	7	8	9	10	11
1. M/E	—	.15	−.16	−.01	−.27	−.14	−.1	−.35*	−.23	−.21	.18
2. a.m. to p.m. cortisol ratio	−.24	—		−.23	−.25	−.14	−.03	−.17	.18	−.21	.09
3. Total antisocial behavior CBCL	−.16	.09	—	.63**	.70**	.84**	.66**	.71**	.18	.34*	.34*
4. Attention problems	−.24	.18	.63*	—	.58**	.79**	.48**	.42**	.08	−.05	.29*
5. Rule-breaking behavior	−.13	.05	.70**	.52**	—	.77**	.38**	.20	.24	−.11	.32*
6. Aggressive behavior DISC-IV	−.01	−.04	.84**	.59**	.68**	—	.50**	.42**	.19	−.12	.31*
7. CD symptoms	.18	.28	.66**	.32**	.56**	.56**	—	.35**	.07	.03	.32*
8. ODD symptoms	−.31	.19	.71*	.16	.35**	.39**	.44**	—	.08	.03	.22
9. Relational aggression	−.27	.27	.18	.05	−.09	−.14	.20	.36**	—	.22	.18
10. Timing of puberty	−.17	−.29	.34**	−.24	.28*	.33*	.18	.31*	.09	—	−.18
11. Psychotropic drug use	.18	−.16	−.34**	−.29*	−.32*	−.31*	−.32*	.22	.05	−.18	—

Note. The correlations below the diagonal are for boys, and those above the diagonal are for girls. Lower scores on the M/E scale denote eveningness preference whereas higher scores denote morningness preference. M/E = morningness/eveningness; CBCL = Child Behavior Checklist; DISC-IV = Diagnostic Interview Schedule for Children; CD = Conduct disorder; ODD = oppositional defiant disorder.
* $p = .05$. ** $p = .01$.

eveningness preference was related to relational aggression in girls.

a.m. to p.m. cortisol ratio. Rule-breaking and aggressive behavior problems were not related to the a.m. to p.m. cortisol ratio in boys or girls.

Attention problems. The a.m. to p.m. cortisol ratio was negatively related to attention problems for boys but not for girls. The negative beta indicated that attention problems were related to a low a.m. to p.m. cortisol ratio, indicating that a smaller decrease in cortisol from awakening to 4:00 p.m. was characteristic of boys with attention problems.

DISC–IV ODD and CD. The a.m. to p.m. cortisol ratio was not related to ODD and CD symptoms in boys or girls.

Transitional stress. To test the hypothesis that timing of puberty interacts with vulnerabilities (M/E or a.m. to p.m. cortisol ratio), pubertal timing was established by regressing stage of puberty onto chronological age within age and sex to calculate the residuals for timing of puberty independent of age. Timing of puberty did not interact with M/E or a.m. to p.m. cortisol ratio. However, there was a notable direct effect for timing of puberty and antisocial behavior. For boys, earlier timing of puberty was related to a higher number of parent-reported rule-breaking and attention behavior problems and symptoms of CD. For girls, pubertal timing was related to self-reports of relational aggression. As expected, earlier as opposed to later timing was related to more antisocial behavior in both boys and girls. The betas, degrees of freedom, F statistics, and p values for the significant models are presented in Table 4.

Power Analysis

Given that the sample size was small, the power to detect significant relationships in the models was estimated using procedures described by Murphy and Myors (2004). The estimates of power for the final step of each regression model were rule-breaking problems, 0.72; attention problems, 0.69; CD symptoms, 0.58; and relational aggression, 0.81.

Discussion

The findings suggest that two less examined aspects of development, M/E and, to a lesser extent, circadian cortisol change, can tentatively be added to the assemblage of dimensions linked to antisocial behavior in young adolescents. The hypotheses linking M/E as a vulnerability for antisocial behavior and the transitional stress of timing of puberty were supported in most instances. Overall, the findings are consistent with those from studies of older adolescents and adults. The findings for circadian variations will be discussed followed by the findings for each of the components of the theoretical model.

The current findings are representative of only a few identified studies showing that morning basal cortisol levels in adolescents exhibit the hypothesized rise 20 min after awakening, with a decline at 40 min after awakening (see also Rosmalen et al., 2005). This pattern is concordant with the findings for adults in whom there is a stereotypical cortisol rise after awakening followed by a decrease at 40 min (Edwards et al., 2001). However, the awakening increase is lower in children than in adults (Rosmalen et al., 2005), suggesting a maturational component to the morning rise. The question could be raised as to whether the postural shift from supine to standing may induce a cortisol secretory episode that would be reflected in the 20-min sample. Recent evidence shows that the upright position does not contribute to the awakening cortisol response (Hucklebridge, Mellins, Evans, & Clow, 2002). The postwakening rise at 20 min and decline at 40 min likely reflect the normative entrained circadian variation in mammalian cortisol. Boyce and Ellis (2005) suggested that identification of the typical morning rise is important as it allows for estimating individual differences in circadian changes as a novel marker of adjustment problems in children.

Morningness/Eveningness

Preference for evening activities and sleep problems are vulnerabilities for antisocial behavior in older adolescents and adults (Cofer et al., 1999; Andersson, 2001). As is true in these older

Table 4
Hierarchical Regression of Antisocial Behaviors on SES, Psychotropic Drug Use, Morningness/Eveningness, a.m to p.m. Cortisol Ratio, and Timing of Puberty

	Rule-breaking behavior (boys only)						Attention problems (boys only)					
Variable	*B*	*SE*	β	*F*	R^2	ΔR^2	*B*	*SE*	β	*F*	R^2	ΔR^2
Step 1												
Family SES	−0.21	0.24	−.14				−0.02	0.04	−.26			
Psychotrope use	−2.40	1.11	−.44*				−3.74	1.86	−.51**			
Full model				3.02	.11	.11				3.92*	.17	.17
Step 2												
Family SES	−0.21	0.24	−.14				−0.02	0.04	−.24			
Psychotrope use	−2.30	1.12	−.42*				−3.74	1.86	−.51**			
M/E score	−0.03	0.04	−.20*				−0.14	0.07	−.36*			
Full model				3.50	.15	.04				3.71	.26	.09*
Step 3												
Family SES	−0.21	0.24	−.14				−0.02	0.04	−.21			
Psychotrope use	−2.31	1.09	−.42*				−3.74	1.82	−.51**			
M/E score	−0.04	0.04	−.20*				−0.13	0.06	−.33*			
a.m. to p.m. cortisol ratio	2.55	2.43	.11				−0.09	−0.07	−.34*			
Full model										3.53	.32	.06*
Step 4												
Family SES	−0.18	0.25	−.13				−0.02	0.04	.19			
Psychotrope use	−2.01	1.10	−.42*				−3.02	1.91	−.51**			
M/E score	−0.03	0.04	−.26**				−0.13	0.07	−.37*			
a.m. to p.m. cortisol ratio	2.28	2.49	.10				−0.08	0.06	−.35*			
Timing of puberty	0.32	0.22	.31*				0.13	0.38	.33*			
Full model				6.07*	.24	.09*				5.81	.37*	.05*

Note. SES = socioeconomic status.
* $p < .05$. ** $p < .01$.

samples, eveningness was related to our composite index of antisocial behavior, rule-breaking and attention behavior problems, and CD symptoms in young adolescent boys and relational aggression in girls. The findings were stronger for boys than for girls, which was also the case in older adolescents (Andershed, 2005) and young adults (Adan & Natale, 2002). The Andershed (2005) study also showed that 15-year-old boys changed more in the direction of eveningness than did girls over 18 months. Eveningness in boys may contribute to the known sex differences in aggression favoring boys later in life as has been extensively documented (Archer, 2004). In these young adolescents, the pattern of relationships cannot be attributed to the greater overall frequency of antisocial behavior problems in boys as there were no mean sex differences in any of the antisocial variables.

What is novel about the current findings is that the relationship between eveningness and antisocial behavior was apparent in pre- and early-pubertal adolescents. In older adolescents, eveningness has been attributed to social contextual influences (Stattin & Kerr, 1999), suggesting that phase-delay preference for evening activities is an exogenous, later-adolescence characteristic given adolescents' increasing independence and interest in late-night social activities, TV viewing, Internet use, peer relationships, and other social-contextual factors (Giannotti et al., 2002; Stattin & Kerr, 1999). In support of M/E as an exogenous characteristic, Stattin and Kerr (2001) suggested that preference for evening activities is part of an adolescent's values that would lead him or her into deviant behavior with peers. They showed that adolescents with self- (e.g., hanging out with peers, pleasure seeking and the here and now) versus other-focused (e.g., home-centered, family activities) values were more likely to be evening types. Hence, it was proposed that eveningness preference may become a more salient risk during adolescence when deviant adolescents engage in peer activities that foster eveningness. Given that the current sample was in the childhood and early adolescence period of development, it is not likely that they had yet adopted a late-night set of peer activities. An alternative interpretation is that youth who are more antisocial may report an evening preference because of their tendency to be antisocial in many domains including exaggerating their deviant or eveningness preference.

In contrast to an exogenous interpretation, Cofer et al. (1999) proposed an alternative view suggesting that individual differences in normative patterns of sleep/wake emerge in infancy, are stable over time, and are a driving, endogenous force in late-night activities. Consistent with Cofer et al. (1999), the current findings show that the relationship between eveningness preference and antisocial behavior indeed is evident in children and younger adolescents in the 8- to 13-year age range and suggest that eveningness preference emerges prior to adolescent-contextual influences, thus representing an endogenous temperamental dimension that is stable from childhood onward. In support of this interpretation, Andersson (2001) proposed that eveningness has a biological component that emerges early in life and therefore cannot adequately be explained by psychosocial and contextual factors alone (see also Carskadon et al., 1993). However, with advancing age, eveningness likely contributes to lack of sleep and attention regulation and lack of control. In turn, problems with regulation and control become associated with antisocial behavior, especially early substance use (Wong, Brower, Fitzgerald, & Zucker, 2004).

Conduct disorder (boys only)						Relational aggression (girls only)						Total antisocial behavior (boys only)					
B	*SE*	β	*F*	R^2	ΔR^2	*B*	*SE*	β	*F*	R^2	ΔR^2	*B*	*SE*	β	*F*	R^2	ΔR^2
−0.03	0.02	.06				−0.03	0.02	−.11				−0.08	0.09	−.09			
−2.34	1.01	−.45*				−2.71	2.60	−.09				−0.61	0.52	−.13			
			2.89	.24	.24				2.01	.03	.03				1.77	.04	.04
−0.03	0.02	.06				−0.03	0.02	−.11				−0.08	0.09	−.09			
−2.34	1.01	−.45*				−2.71	2.60	−.09				−0.61	0.52	−.13			
−0.02	0.04	−.39*				−0.15	0.09	−.22*				−0.49	0.22	.27*			
			7.90	.30	.06*				3.96	.10	.07*				4.05*	.14	.10*
−0.03	0.02	.06				−0.03	0.02	−.10				−0.08	0.09	−.09			
−2.34	1.01	−.42*				−2.71	2.60	−.09				−0.61	0.52	−.13			
−0.02	0.04	−.51**				−0.15	0.09	−.24*				−0.49	0.22	−.28**			
3.39	4.48	.12				−10.64	28.19	−.06				29.51	17.86	.18			
			7.96	.33	.03										2.7*	.15	.01
−0.03	0.02	.06				−0.03	0.02	−.09									
−0.02	0.02	−.42*				−2.71	2.60	−.09									
−0.02	0.04	−.51*				−2.51	2.50	−.28*									
−2.96	3.97	−.11				−13.25	27.61	−.07									
0.27	0.21	.25*				0.51	0.50	.38*									
			7.43	.39	.06*				3.65	.29	.19*						

In brief, the findings are consistent with those in older adolescents and adults as they show that preference for evening activities and sleep onset (Cofer et al., 1999; Andersson, 2001) are related to externalizing behavior problems. Overall, our findings suggest innovative hypotheses to be tested so as to more confidently establish eveningness as a predictable risk for antisocial behavior.

Cortisol

A small change from a.m. to p.m. cortisol characterized only boys with attention behavior problems. A pattern of low change in cortisol secretion during the day is interpreted as reflecting an attenuated sensitization of the HPA axis to circadian rhythms. Few studies have assessed circadian cortisol rhythms in children with externalizing behavior problems as the emphasis has tended to be on stressful experiences and accentuated responsivity of the HPA axis (e.g., Essex, Klein, Cho, & Kalin, 2002). However, lower cortisol in antisocial individuals has been noted (e.g., Pajer et al., 2001). The current finding should be interpreted with caution as attention problems only, in boys, were related to cortisol a.m. to p.m. ratio, and this relationship could be a chance finding.

Transitional Stress

Earlier timing of puberty was related to self-reports of CD symptoms in boys and relational aggression in girls. Many studies have shown that the timing of the pubertal transition, primarily earlier physical maturation, represents a significant risk for externalizing problems (Ge, Best, Conger, & Simons, 1996; Ge et al., 2002; Graber, Lewisohn, Seeley, & Brooks-Gunn, 1997; Susman, Dorn, & Chrousos, 1991; Caspi & Moffitt, 1991; Hayward, 2003; Stattin & Magnusson, 1990). For instance, early maturing girls displayed more norm-violating behaviors, delinquency, use of drugs, school problems, early sexual activities (Caspi & Moffitt, 1991; Magnusson, Stattin, & Allen, 1986), behavior problems (Ge, Conger et al., 1996), poor adjustment (Graber, Brooks-Gunn, Paikoff, & Warren, 1994), and other problems (Caspi, Lynam, Moffitt, & Silva, 1993; Dick, Rose, Kaprio, & Viken, 2000; Stattin & Magnusson, 1990). In boys, early timing is associated with early intoxication and alcohol consumption (Wichstrom, 2001). In this study, earlier timing of puberty was related to externalizing behavior in boys and relational aggression in girls, but pubertal timing did not interact with vulnerabilities. The absence of an interaction may reflect the young age of the adolescents who do not yet have the independence of interacting with deviant peers in late-night activities.

Earlier timing was related to relational aggression only in girls. Reports of relational aggression favoring girls began to appear in the literature in the 1990s in both preschool (Crick, Casas, & Mosher, 1997) and grade-school children (Crick & Grotpeter, 1995). In more recent studies, sex differences in relational aggression have not always emerged (e.g., Henington, Hughes, Cavell, & Thompson, 1998; Prinstein, Boergers, & Vernberg, 2001). Briefly, a broad interpretation of the findings is that the constructs were not particularly salient to explaining antisocial behaviors in girls, but vulnerabilities did demonstrate associations for boys even though the rates of behaviors did not differ by sex.

The findings contribute to the literature on timing of puberty and antisocial behavior in two ways. First, earlier timing of puberty was found to be related to antisocial behavior even in pre- and

early-pubertal adolescents; past studies focused on timing of puberty and adjustment during later puberty. Second, early timing of puberty was found to be a risk for antisocial behavior in both young boys and girls who showed a gender-stereotyped pattern of relationships (Xie, Farmer, & Cairns, 2003). The associations for timing of puberty for boys extended to physical, externalizing behavior and for girls to relational aggression.

Limitations

M/E preference was assessed only by a self-report questionnaire completed by the adolescents, and no observational data were available to confirm the adolescents' reports of bedtime activity. In addition, cortisol assessments were obtained on only 1 day, and events that occurred on that day may have altered circadian variations in cortisol levels. However, on the at-home sleep diary, none of the adolescents reported events that are known effectors of the cortisol stress response. Finally, the sample is small and multiple analyses were done. However, many of the analyses were completed to rule out possible confounding influences on antisocial behavior (e.g., age, medication use, SES). With regard to medication use and SES, medication use was associated with antisocial behavior as one would intuitively expect. But when controlled for in the analyses, medication use did not alter the findings. In contrast, SES did not have an independent relationship with antisocial behavior, but when it was included in our regression models, the variance accounted for between M/E and antisocial behavior was increased in almost all cases. Therefore, an additional caution is warranted in interpreting these complex results.

With regard to sample size, power estimates were good for the models and above what is expected in behavioral research (Mone, Mueller, & Mauland, 1996; Murphy & Myors, 2004; Sedlmeier & Gigerenzer, 1989). These estimates were based on the final five-variable model as opposed to the larger model with all possible covariates included, which could have led to a possible inflation in power. A final limitation is that it was not possible to validate that the samples were obtained at the time reported by the adolescents. Kudielka, Broderick, and Kirschbaum (2003) showed that there is a more marked cortisol spike in the morning in individuals who follow directions as verified by a compliance cap that time-dates each sample. It follows that accuracy of reporting time and antisocial behavior problems may be confounded in our sample. If it is the case that antisocial adolescents were inaccurately reporting the morning time of sampling, then the morning cortisol level should be lower in antisocial adolescents. To rule out this possible confound, we divided the sample into groups based on amount of antisocial behavior to determine whether the adolescents reporting the most antisocial behavior were more likely to misreport and thus not show a morning cortisol rise. Adolescents at $+1$ and $+2$ standard deviations above the mean were compared on antisocial behavior with those <1 standard deviation above the mean. There were no significant mean differences in morning cortisol levels between the groups, suggesting that the antisocial adolescents were accurately reporting their saliva collection times. Nonetheless, we recommend compliance checks in future studies.

In spite of seemingly adequate power, when a Bonferroni correction was applied, all of the betas became nonsignificant. However, applying the Bonferroni correction factor has disadvantages. First, as the chance of making a Type I error is reduced by applying a more stringent probability level, the chance of making a Type II error is increased. In this new biobehavioral research arena, it is especially important to balance the probability of Type I and Type II errors. Keeping in mind the advantages and disadvantages of using the Bonferonni correction factor, as cited by Perneger (1998), we simply described the rationale for what was done and discussed the possible interpretations of each result while acknowledging that Type I error findings may exist. Finally, the literature on the psychobiology of antisocial behavior is complex, and design decisions that were made at the beginning of this longitudinal study may not be the choices we would make today in this rapidly evolving field. As a consequence, caution is merited in interpreting the findings. Even in the face of limitations, there was a consistent pattern of findings suggesting that eveningness shares a robust relationship with antisocial behavior.

Implications

If eveningness is related to antisocial behavior, can early interventions reduce a preference for eveningness and subsequent antisocial behavior? First, preventive education for parents to recognize characteristics of eveningness and its potential related behavior problems may be important for initiating activities to reduce eveningness or strategies to deal with its consequences. A childhood pattern of eveningness preference, especially in boys, may signal the need for parental vigilance with regard to night-time activities and bedtime. Second, it may be easier to deal constructively with eveningness-related problems within the family during early adolescence to prevent further escalation in the later adolescent years when interaction with deviant peers is more likely. Parents should also be alerted that eveningness and related problem behavior is not just confined to childhood and early adolescence.

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Professional Presentations (selected)

- **Blades, K.T**., Susman, E.J., Granger, D.A. Sex differences in the asymmetry between physiological systems in antisocial and relationally aggressive adolescents. *Poster presented at the biennial meeting of the Society for Research on Adolescence, Chicago, IL, 2008.*
- **Blades, K. T.** Organizer and future chair of paper symposium on Biopsychosocial considerations on relational aggression and related aggressive and antisocial behavior. *Symposium presented at the biennial meeting of the Society for Research on Adolescence, Chicago, IL, 2008.*
- **Blades, K.T.**, Dockray, S., Susman, E.J. Cortisol, timing of puberty, depressive symptoms and relational aggression in early adolescence. *Paper presented at the biennial meeting of the Society for Research on Adolescence, Chicago, IL, 2008.*
- Susman, E.J., Granger, D.A., **Blades, K.T.**, Dockray, S. (August 2007). Alpha-amylase, timing of puberty and depression in adolescents. *Paper presented at a symposium at meeting of the International Society for Psychoneuroendocrinology, Madison, WI, 2007.*
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