

The Impacts of Social Support and Early Life Stress on Stress Reactivity in Children and
Adolescents

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Camelia E. Hostinar

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Adviser: Megan R. Gunnar, Ph.D.

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Abstract

The goal of the present study was to investigate the impacts of social support and early life stress on individual differences in HPA axis reactivity in children (ages 9-10) and adolescents (ages 15-16). The primary aims were: 1) to experimentally manipulate the provision of social support in the laboratory and examine its effect on levels of salivary cortisol in response to the Trier Social Stress Test for Children; 2) to investigate parenting quality variables that may moderate the social buffering effect based on coding of videotaped parent-child interactions; 3) to analyze the role of early life stress (orphanage-rearing versus birth family rearing) and current social network characteristics in predicting the cortisol response; and 4) to explore age and sex differences in stress reactivity and the social buffering of stress. A sample of 162 participants was recruited, roughly equally divided between the two age groups, experimental conditions (half were exposed to a parent support condition before the stress task, whereas half received support from a stranger), early life experience (adopted or non-adopted) and by gender. Analyses of cortisol stress responses revealed that in the non-adopted group parent support provided in the laboratory significantly dampened stress reactivity in children but not in adolescents when compared to the stranger support condition. Additionally, participants reared in orphanages showed atypical patterns of HPA reactivity and of responses to social support provided before the stressor. Implications and future directions are discussed.

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Preface

The investigation of social support as a moderator of life stress and a protective factor against physical and psychological illness is not new (Cobb, 1976; Taylor, 2007; Thoits, 1995). Epidemiologists have long acknowledged the crucial role of social and community ties in predicting longevity and overall health, above and beyond specific disease risk factors (Berkman & Syme, 1979). Many studies have used self-report checklists of life events, health outcomes, and social network characteristics to study the stress-buffering role of social support (for a review, see Cohen & Wills, 1985). More recent research has started to investigate the neurobiological mechanisms that may underlie this social buffering effect in adults by focusing on the role of conspecifics in reducing the reactivity of the hypothalamic-pituitary-adrenal (HPA) stress system (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Kirschbaum, Klauer, Filipp, & Hellhammer, 1995; Taylor et al., 2008). However, the scientific question of how social support buffers against stress has rarely been studied in late childhood and adolescence, with most prior work having focused on early childhood and adulthood. Variability in the effectiveness of social support in reducing stress responses has also not been previously studied in relation to early life stress experiences, an important factor in explaining individual differences in later stress reactivity across development. The goal of the present study was to investigate the impact of social support and early life stress (in the form of orphanage rearing) on individual differences in stress reactivity in children (approximately 9-10 years old) and adolescents (15-16 years old). The primary aims were to experimentally manipulate the provision of social support in the laboratory and to examine its effect on levels of salivary cortisol in response to the Trier Social Stress Test

for Children (TSST-C), to investigate parenting quality variables derived from videotaped parent-child interactions that may moderate the social buffering effect, to analyze the role of early life stress (orphanage-rearing versus birth family rearing) and current social network characteristics in predicting the cortisol response, and to explore age and sex differences in stress reactivity and the social buffering of stress. A sample of 162 participants was recruited (half were adopted from socially depriving institutions overseas during their early childhood, whereas half were non-adopted; the sample was also equally divided between children and adolescents and between the experimental condition featuring parent support before the laboratory stressors and the comparison condition recruiting support from a stranger). Chapter 1 examines developmental differences in the social buffering of the HPA axis between children and adolescents in the typically-developing group, as well as potential moderators of this protection against stress. Chapter 2 probes the role of early life stress in this phenomenon by examining differences between internationally adopted and non-adopted participants. Additionally, it tests specific predictors of variability in stress reactivity and in stress-buffering by parent support within the adopted group.

Chapter 1

Developmental Differences in the Stress-buffering Role of Parent Support for Children and Adolescents

Adolescence has been conceptualized as a period of dynamic reorganization of neurobehavioral systems subserving stress and emotion (Crone & Dahl, 2012; Dahl & Gunnar, 2009; Spear, 2000; Steinberg, 2005), which may underlie the increased prevalence of psychopathology and emotion dysregulation emerging during this developmental stage (Merikangas et al., 2010; Nelson, Leibenluft, McClure, & Pine, 2005). Much less is known about protective factors that could be empirically demonstrated to reduce the stress load for individuals undergoing this massive developmental transition. Social support may be one of these protective factors, given its widely recognized association with psychological well-being and physical health across the lifespan (Cohen, 2004; Holt-Lunstad, Smith, & Layton, 2010; Taylor, 2011; Thoits, 2011; Uchino, 2006; Uchino, Cacioppo, & Kiecolt-Glaser, 1996). One of the proposed explanatory mechanisms for these beneficial effects is that social support can buffer -i.e., reduce or block- the impact of stressful experiences (Cobb, 1976; Cohen & Wills, 1985; Ditzen & Heinrichs, 2013; Hostinar, Gunnar, & Sullivan, 2013). For instance, experimental studies with adults (e.g., Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Kirschbaum, Klauer, Filipp, & Hellhammer, 1995; Taylor et al., 2008) and with young children (reviewed in Gunnar & Donzella, 2002) have identified a dampening of the HPA axis response to stressors by social factors as one of the possible mechanisms underlying the benefits of social support. This reduction in acute stress responses with the presence or assistance of a conspecific has been termed the *social buffering of stress* and

it occurs pervasively across development (Hostinar, Gunnar, & Sullivan, 2013) and across species (Hennessy, Kaiser, & Sachser, 2009; Kikusui, Winslow, & Mori, 2006).

Despite the importance of this topic for public health, there is a surprising dearth of studies examining the stress-protective effects of social support in late childhood and adolescence, leaving the developmental changes in the social regulation of HPA stress reactivity extremely poorly understood in humans. The present study aims to address this significant gap in the literature and to examine whether parental support, a major protective factor against stress in infancy and early childhood, retains its potency into late childhood and adolescence. A further goal of the present study was to better characterize parental buffering phenomena in these less intensively studied age periods by examining potential moderators of these effects (e.g., support and conflict experienced with various members of one's social network, temperament). The current research focused on the reactivity of the HPA stress system given the widely documented links between glucocorticoids and numerous aspects of physical or mental health (McEwen, 2008). The HPA axis responds acutely to both psychological and physical challenges to the organism (Gunnar & Vazquez, 2006). The activation of the axis begins with neurons in the medial parvocellular region of the paraventricular nuclei of the hypothalamus (PVN) secreting corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP), which travel to the anterior pituitary and trigger the release of adrenocorticotrophic hormone (ACTH) into the general circulation (Cone, Low, Elmquist, & Cameron, 2003). ACTH subsequently binds to its receptors in the cortex of the adrenal glands, leading to the release of cortisol, which can be measured noninvasively in humans by collecting salivary samples.

Study hypotheses were theoretically informed by the prior literature on social buffering in early childhood or adulthood, on social changes during adolescence and on puberty-related alterations in the reactivity of the HPA axis, all of which are briefly surveyed below.

The Social Buffering of Stress across Development

Childhood. There is now a vast literature documenting the social regulation of the HPA axis by parents during early development (Gunnar, 2006; Gunnar & Donzella, 2002). Starting in infancy, characteristics of caregiving begin to be associated with aspects of cortisol reactivity. For instance, maternal sensitivity and cooperation while taking a 3-month-old out of the bath influence the infant's post-stressor cortisol recovery (Albers, Riksen-Walraven, Sweep, & de Weerth, 2008). Additionally, 6-month-olds whose mother provides tactile stimulation during a still-face paradigm (a distressing event for the infant during which the mother suddenly displays a still face) have lower cortisol reactivity and decreased salivary cortisol levels after this episode compared to infants who are not touched during the procedure (Feldman, Singer & Zagoory, 2010). Premature infants, who are frequently separated from their mothers for long periods of time after birth, greatly benefit from daily massage therapy, which lowers their salivary cortisol levels and promotes their growth (Field, 1995).

Later in development, having a secure attachment figure present moderates the link between shy/fearful child temperament and cortisol reactivity around the age of 18 months; children who were both fearful and insecurely attached to the accompanying parent exhibited higher salivary cortisol levels in response to both inoculations and the Strange Situation procedure, which involves brief separations from and then reunions

with the attachment figure (Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996). The interaction between secure attachment relationships and child temperament in predicting HPA axis responses has been replicated by other studies as well (Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Spangler & Schieche, 1998). Importantly, these studies show that maternal presence dampens cortisol reactivity to threats even when the children exhibit fear behaviorally (Nachmias et al., 1996). Similar results are observed in naturalistic situations, with a recent study showing that securely attached toddlers who are accompanied by their mothers during the first few days of child care showed lower levels of salivary cortisol compared to children who are in insecure relationships but still accompanied by the mothers (Ahnert, Gunnar, Lamb, & Bartel, 2004). Secure attachments are considered to reflect a history of sensitive, responsive, and consistent caregiving (Ainsworth, Bell, & Stayton, 1974), whereby children learn that the parent is available and will respond to their needs. Furthermore, Nachmias et al. (1996) have suggested that securely attached infants have more coping resources, because they know they can rely on their caregivers to protect them from threat, and it is well-known from both the older infant and adult human studies that availability of coping resources predicts lower adrenocortical responses. However, it must be noted that extended separations (e.g., first few days in child care) produce salivary cortisol elevations 60 minutes from mothers' departure in all children (Ahnert et al., 2004), thus the social buffering effects of parent support early in development may be limited to briefer separations or to mild/moderate stressors. Relevant findings in childhood do not pertain only to maternal care, as father involvement during infancy has also been shown to be extremely important –e.g., it interacted with children's adrenocortical and autonomic

reactivity at age 7 to predict the presence of mental health symptoms at age 9 (Boyce et al., 2006). Thus, these parental support effects are crucial for understanding and promoting child health and well-being.

Adolescence. Few studies have examined the social buffering of stress reactivity in late childhood or adolescence. As an exception, a study by Seltzer, Ziegler, and Pollak (2010) showed that 7-12-year-old girls who had either direct contact or a phone conversation with their mothers after the TSST-C exhibited lower salivary cortisol levels post-stressor and also higher levels of urinary oxytocin. However, this study did not examine the process in boys and also seems to have tested a buffering effect on recovery from the stressor, which is a different aspect of the process compared to the pre-stressor support paradigm used in infant and adult studies showing diminished reactivity to a subsequent stressor. Secondly, the study cannot speak to developmental changes that may occur after the pubertal transition. In a more recent study using the same laboratory stressor, low-socioeconomic status African American adolescents aged 15-18 exhibited higher cortisol reactivity if their parents were rated higher in parental responsiveness during a home visit when the children were 4 years old (Hackman et al., 2013), which was interpreted as a sign of abnormally blunted reactivity in the low-parental responsiveness group. However, this experiment does not shed light on whether parents can serve as effective stress buffers for adolescents concurrently during this developmental period, even though it does make an important contribution by prospectively showing an enduring link between early life care and later reactivity. In another prospective longitudinal study, parents rated their own parenting style when the children were 6-13 years old and it was reported that higher levels of parental structure derived from these

self-ratings predicted lower cortisol reactivity to the Trier Social Stress Test (TSST) when adolescents were approximately 15-19 years old (this was true of both adolescents with bipolar disorder and the similarly aged comparison group, Ellenbogen & Hodgins, 2009). It would be useful to replicate these findings using observational measures of parenting.

A number of other correlational studies have attempted to link various aspects of social support and adolescent HPA activity without examining the question of social buffering directly or experimentally. Chaplin et al. (2012) reported that parenting behavior during a 10-minute laboratory parent-adolescent conflict discussion with their 10-16-year-olds predicted adolescents' blood pressure and anger in response to the discussion, but the task did not activate the HPA axis and included parents as an integral part of the stressor, thus buffering could not be tested. Bereaved youth between the ages of 10-29 exhibited constant and high cortisol output but no acute increase to a modified Trier Social Stress Test procedure conducted approximately 5 years after losing one of their parents (Dietz et al., 2013), potentially suggesting a blunting of typical stress reactivity. Other studies have found associations between positive family events and basal cortisol levels in adolescents (Dorn et al., 2009), or have shown moderating effects of maternal responsiveness measured in early adolescence (age 13) for the association between allostatic load at ages 9 and 13 and working memory later in adolescence (Doan & Evans, 2011), a finding that may be explained by HPA axis buffering, but this possibility was not tested directly given that the composite measure of allostatic load included multiple physiological indices (e.g. epinephrine and norepinephrine levels, body mass index, blood pressure, and cortisol levels). Lastly, other studies have examined

whether cortisol reactivity in early adolescence (age 12-13) predicted family problems 6 or 12 months later (it did not, Marceau, Dorn & Susman, 2013) or attempted to incorporate the role of peers in understanding stress buffering –e.g., 10-11-year-olds had lower levels of cortisol after negative events reported in their daily diaries if participants also reported that they were with their best friend at the time of the event (Adams, Santos, & Bukowski, 2011), but these correlational findings would be important to replicate with laboratory stressors.

Adulthood. In adults, one of the earliest investigations of social buffering reported that men but not women displayed a reduced salivary cortisol response to the TSST when preparing a speech with their romantic partner (Kirschbaum et al., 1995). A trend for an elevated cortisol response was found in women preparing with their male partner. The finding in men has been replicated and a more recent study showed an enhanced buffering effect of social support with intranasal administration of oxytocin (Heinrichs et al., 2003). In women, partner support coupled with a brief neck and shoulder massage had the same cortisol-dampening effect (Ditzen et al., 2007). Studies also show that women receiving support from a close female friend experience the same buffering effects of their autonomic stress responses (Fontana, Diegnan, Villeneuve, & Lepore, 1998; Uno, Uchino, & Smith, 2002), suggesting that the same may be true for buffering the HPA axis when recruiting female friends to provide support rather than typically male romantic partners. Indeed, sex differences seem to moderate the association between social support and HPA responses. For instance, when strangers provide support there is an interaction of sex and experimentally-induced closeness/familiarity in predicting the magnitude of cortisol responses, but in either case

stranger support does not seem to be successful in blocking stress responses in either males or females (Smith et al., 2009). This suggests that a certain level of intimacy or prior history may be required to instantiate social stress-buffering effects. Consistent with this interpretation, one study found that despite friendly and positive nonverbal signals from the two judges observing the speech performed during the TSST, the task still increased cortisol stress responses compared to a no-audience condition (Taylor et al., 2010). This increase was slightly higher but similar to the response observed in a condition where the judges exchanged negative glances and nonverbal feedback (Taylor et al., 2010). Thus, it seems to be difficult to remove the social-evaluative threat posed by strangers enlisted as judges, regardless of whether they are positively or negatively predisposed towards the participants. The mounting evidence that one's relationship history with the support provider plays a role in determining the efficacy of support raises questions about the sources of variability in these effects across individuals. These possible moderators are explored next.

Moderators of Social Buffering

Positive relationship experiences. Understanding factors that may modulate the effectiveness of social support in dampening stress responses has numerous clinical and health applications, but few studies have considered and empirically tested such potential sources of individual differences. For instance, researchers have not always considered the effect of the history or quality of the relationship when using an experimental provision of social support. This is an important oversight, since high perceived family support alone can lower blood pressure in response to stressful tasks (Broadwell & Light, 1999) even when family members are not present, whereas the closeness of a friendship

moderates social buffering effects on autonomic reactivity for women (Uno, Uchino, & Smith, 2002). Measuring perceived support from family and non-family is thus critical in understanding the breadth of the protection that social support may confer. Furthermore, some studies in adults suggest that one's relational history may impact anti-stress effects mediated by the HPA axis, not just those of the autonomic nervous system. For instance, Luecken (1998) found that college students reporting poorer quality relationships in their family of origin before age 16 had higher cortisol responses to a public speaking challenge and to watching a distressing video when tested in adulthood. Another experimental study found that adults with "Dismissing" attachment representations about their parents (i.e., minimizing the importance of their attachment during the Adult Attachment Interview, George, Kaplan, & Main, 1985) evinced the highest cortisol reactivity to the TSST, whereas those with "Unresolved" patterns of attachment towards their parents (i.e., disorganized cognitions that are often due to unresolved trauma or loss) exhibited a blunted or flat reactivity pattern (Pierrehumbert et al., 2012). However, another study failed to find a moderating effect of adult attachment classifications on cortisol reactivity (Ditzen et al., 2008). The difference may arise from the fact that the latter study derived their measure from a more limited attachment questionnaire, whereas the Adult Attachment Interview is more extensive and might have been less susceptible to self-report biases by using an indirect scoring method.

Negative Relationship Experiences. A positive relationship history is not the only aspect that influences social development and can lower stress reactivity, the converse is also true since negative interactions exert their own effects, often enhancing physiological stress responses (Seeman & McEwen, 1996). Reviews of the literature

show that risky families (i.e., replete with conflict, aggression, cold and harsh relationships) foster altered HPA or sympathetic nervous system reactivity and unhealthy biobehavioral profiles that include higher levels of physical and mental illness, as well as earlier mortality in the offspring (Repetti, Taylor, & Seeman, 2002). In addition, naturalistic observations by anthropologists have shown that children from rural Caribbean areas who were severely reprimanded by their parents showed elevated levels of glucocorticoids during the day followed by immunosuppression and a higher likelihood of subsequent respiratory illnesses (Flinn, 1999; Flinn & England, 1997). Laboratory studies also tend to show alterations in cortisol reactivity with exposure to negative family experiences. Specifically, toddlers exhibit blunted responses to challenging laboratory paradigms if they are exposed to interparental conflict in the home and maternal emotional unavailability (Sturge-Apple, Davies, Cicchetti, & Manning, 2012), a pattern that may be suggestive of down-regulation of the HPA axis subsequent to a period of chronic activation (Miller, Chen, & Zhou, 2007), which has sometimes been referred to as hypocortisolism (Gunnar & Vazquez, 2001). In adolescence (mean age 15), participants exhibited higher increases in cortisol if they self-reported more distress in response to a laboratory parent-child conflict discussion, but this effect was moderated by past or current internalizing symptoms such that these symptoms were associated with a blunted response pattern (Spies, Margolin, Susman, & Gordis, 2011). Thus both hyper- and hypo-responsivity of the HPA axis may be problematic for children and adolescents being involved in or exposed to family conflict.

Given the vast literature documenting links between family conflict and HPA axis functioning, it is theoretically plausible that these negative relationship experiences also

moderate stress-buffering effects such that family conflict may weaken the anti-stress properties of support from close family members. However, this hypothesis has yet to be empirically tested.

Temperament. Previous studies on social buffering in the first few years of life have shown that shy, behaviorally inhibited temperament interacts with the quality of parent-child attachment to predict HPA axis responses (Gunnar et al., 1996; Nachmias et al., 1996) such that children who are both insecurely attached and shy showed the most pronounced cortisol responses (as previously described), but there is some suggestion that when they are securely attached these behaviorally inhibited children might actually show more pronounced social buffering effects. Given that this fearful phenotype has been identified as a risk factor for the development of later anxiety disorders (Fox, Henderson, Nichols, Marshall, & Ghera, 2005; Lahat, Hong, & Fox, 2011), it would be critically important to examine whether the interaction between this temperament constellation and social support provision continues to occur in late childhood and adolescence, which would suggest a protective factor that can be harnessed in the prevention or treatment of many anxiety disorders.

In addition to these potential moderators of the social stress-buffering effect described above, puberty-related changes in stress reactivity and social transformations during adolescence may also modify the extent to which adolescents benefit from social support when confronted with stress. We briefly review the theoretical foundations for this possibility next.

Puberty and Social Development as Moderators of Social Stress-Buffering

Adolescence has been recognized as a period of dramatic changes in social, emotional and physical development. Many of these changes are driven by puberty. For instance, the brain and the HPA axis undergo important changes under the influence of gonadal and adrenal hormones during puberty, as shown by both animal models and human studies (Andersen & Teicher, 2008; Klein & Romeo, 2013; McCormick et al., 2010; Romeo, 2010). Basal cortisol levels have been shown to increase from childhood to adolescence (for a review, see Gunnar & Vazquez, 2006) and there is also some evidence that cortisol reactivity to both performance and peer rejection stressors may increase during this period (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009; Stroud et al., 2009; Sumter, Bokhorst, Miers, Van Pelt, & Westenberg, 2010). These changes are thought to be related to the stage of pubertal development, since adolescents in the later stages of puberty exhibit a lesser cortisol awakening response, steeper slopes in their diurnal cortisol rhythms and increased cortisol responses to self-reported worry or stress (Adam, 2006; Oskis, Loveday, Hucklebridge, Thorn, & Clow, 2009). Other studies have also found sex differences in puberty-related changes in HPA activity, with females but not males showing higher basal cortisol post-puberty (Netherton, Goodyer, Tamplin & Herbert, 2004; Stroud, Papandonatos, Williamson, & Dahl, 2011) as well as increasing total cortisol output following CRH challenge with pubertal development in females (Stroud et al., 2004; 2011). While a number of studies have not found sex differences in HPA reactivity with puberty (Gunnar et al., 2009; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Stroud et al., 2009; Sumter et al., 2010), others indicate that males tend to have higher HPA responses to performance stressors than females, especially later during puberty (Bouma, Riese, Ormel, Verhulst, & Oldehinkel,

2009; Klimes-Dougan et al., 2001; Zijlmans, Beijers, Mack, Pruessner, & de Weerth, 2013). This is the same pattern that has been observed in adults with respect to performance stressors (Kudielka, & Kirschbaum, 2005). It has been proposed that sexually dimorphic effects of gonadal hormones on select cortico-limbic brain regions (e.g., subgenual anterior cingulate cortex, insula) may explain higher levels of negative affect and of cortico-limbic neural activation to acute stress in adolescence among females, contrasted by stronger peripheral HPA and autonomic responses in males (Ordaz & Luna, 2012). This divergence is theorized to support a “fight-or-flight” pattern of responsivity in adult males and a “tend-and-befriend” pattern of coping in females, since it promotes mobilization of energy in males and it appears to be more conducive to social interactions to alleviate heightened distress in females (Ordaz & Luna, 2012; Taylor, 2006).

Some have argued that the heightened activity of the HPA axis during this period may play a role in the elevated levels of various types of psychopathology that have been observed during adolescence (Spear, 2000). Thus, exploring the extent to which parent support may buffer cortisol reactivity during this period may carry substantial implications for mental health prevention and treatment.

It must be noted that these pubertal changes are concomitant with massive transformations in the adolescents’ social world. For instance, it is known that adolescents report decreasing levels of emotional intimacy with parents and an increasing orientation towards and intimacy with peers across this period (Cauce, 1986; Harris, 1995; Hartup, 1996; Hunter & Youniss, 1982). Peer networks set the stage for romantic relationships (Collins, Welsh & Furman, 2009) and high levels of emotional support from

friends also spill over into other domains, being associated with positive school outcomes and high levels of self-competence (Cauce, 1986). Parents remain providers of support and nurturance throughout adolescence and young adulthood (Collins & Laursen, 2004; Furman & Buhrmester, 1992), however these relationships evolve and acquire different functions as peers gain salience (e.g., parents may provide more advice whereas peers are more likely to be relied on for intimate disclosure –Buhrmester & Furman, 1987; Hunter & Youniss, 1982). Furthermore, adolescents who are able to gain increasing autonomy while maintaining relatedness with parents seem to have ideal outcomes across multiple domains (Allen, Houser, Bell & O’Connor, 1994). Meta-analyses also show that multiple social contexts –family, peers, school and neighborhood- predict variability in positive adolescent outcomes, with no single context surpassing all others (Cook, Herman, Phillips, & Settersten, 2002).

Despite some prevailing notions of universally increased parent-adolescent conflict with puberty, it is not the case that all parent-child dyads experience negative interactions or that conflict increases linearly with age or pubertal status (Laursen & Collins, 1994). However, some parent-child dyads do experience heightened conflict (Collins & Laursen, 2004; Shanahan et al., 2007; Steinberg & Morris, 2001; Zeman & Shipman, 1997), particularly if the teenager experiences early puberty (Laursen & Collins, 1994). There is some evidence that the increase in the emotional distance between parents and adolescents and in the tendency to experience conflict coincides with the timing of puberty (Hill et al., 1985a, b; Steinberg, 1987, 1988). Another trend observed at the meta-analysis level is that the increases in conflict that do occur in early adolescence decline towards mid- to late-adolescence, despite the fact that the reported

intensity of conflict-related affect increases for adolescence across the period (Laursen, Coy, & Collins, 1998). The declining rate of conflict may be due to improvements in adolescent problem-solving and an increase in withdrawal from conflict as a coping strategy of mothers, fathers and adolescents themselves across the adolescent period (van Doorn, Branje, & Meeus, 2011). When intense conflict does occur, whether with parents or friends, it is associated with maladaptive outcomes for teenagers (Ehrlich, Dykas, & Cassidy, 2012), as well as with cortisol reactivity as described in previous sections.

Based on this prior work, it was hypothesized that the stress-buffering role of parent support would be diminished in 15-16-year-old adolescents compared to children, and that total perceived support from peers might show a moderating effect on cortisol reactivity in teenagers but not 9-10-year-olds. Lastly, conflict with either parents or peers might leave adolescents vulnerable to exacerbated stress responses, which may be able to be observed using the TSST-C. Based on the literature reviewed thus far, the next section summarizes the main predictions of the current research.

Study Hypotheses

The present project aimed to investigate the effect of social support on the cortisol response to the Trier Social Stress Test for Children (TSST-C) in 9-10-year-olds and 15-16-year-olds. These age groups were chosen to best highlight differences in social network changes between childhood and adolescence (Hunter & Youniss, 1982), but also contrasts in stress reactivity that are presumed to emerge between the pre- and post-pubertal period (Gunnar et al., 2009; Stroud et al., 2009). Furthermore, the project examined the role of individual differences in determining the magnitude of the stress-buffering effect (e.g., the observed quality of the parent-child interaction and of the

participant's perceived support from and conflict with their social network, as well as the role of shy/behaviorally inhibited temperament). The current study manipulated the provision of social support by the parent as an experimental variable (participants were randomly assigned to prepare for their public speaking task either with their parent or with a female stranger). Based on the prior evidence described above, we aimed to test the following six predictions: 1) parental support provision would dampen the cortisol response to the TSST-C in the experimental group compared to the control group in the entire sample; but 2) the parental buffering effect would be diminished in adolescents compared to children; additionally, 3) adolescents would show a stronger response to the social-evaluative task than children and higher basal cortisol levels; 4) the buffering effect would be moderated by the observed and participant-reported quality of the parent-child relationship in the experimental group; 5) social network characteristics (perceptions of overall support and conflict as well as those derived from specific close relationships) would moderate the magnitude of the cortisol response in both the experimental and the control condition; and 6) we predicted that shy/behaviorally-inhibited temperament would interact with parent support to predict cortisol reactivity such that children who are more shy will show stronger buffering effects than their extraverted counterparts in the parent support condition, but would show more exaggerated stress responses in the inactive support condition (i.e., receiving support from a stranger). Finally, the effect of sex and its interactions with condition and age were also explored throughout this report given previously mentioned sexually-dimorphic patterns of stress reactivity in humans, but no directional predictions were made given mixed results or insufficient prior work in the area.

Methods

Participants

A sample of 81 typically-developing participants were recruited, half of whom were children ($N = 40$, M age = 9.97 years, $SD = .52$, range 9.1 - 11.1 years old; 50% females) and half who were adolescents ($N = 41$, M age = 16.05, $SD = .39$, range 15.2 - 16.8; 48.8% females). All participants were raised by their birth families in a large Midwestern city. Subjects and their parents were recruited from the Institute of Child Development Participant Pool, a registry of residents who agreed to be contacted about developmental research studies. Children and adolescents were excluded if they met any of the following criteria: being diagnosed with an Autism Spectrum Disorder, Fetal Alcohol Syndrome, or any developmental disorder including but not limited to Pervasive Developmental Disorders. Participants were also excluded if they took steroid medications (due to their interference with cortisol assay results) or had any medical or psychological condition that their parents deemed would make participation challenging or too stressful for their child (e.g., reading delays such that completing questionnaires would be too difficult).

Parents reported a range of annual household income (from under \$35,000 for 3.7% of them to over \$200,000 for 11.1%), with a median yearly family income of \$100,000-\$125,000 in this sample. Only two parents did not report their household income. Parental education ranged from less than a high school degree to doctorate-level, with a median of 16 years of education for the parents attending the session as well as for their spouses.

Procedure

The following research protocol was approved by the Institutional Review Board at the University of Minnesota. Using a cross-sectional developmental design to compare individuals before and after the pubertal transition, 9-10 and 15-16 year-olds were recruited. The study had a balanced design, Age Group (2) x Sex (2) x Experimental Condition (2), with 10 subjects per cell in all but one cell where $N = 11$. Participants completed the modified Trier Social Stress Test for Children procedure (TSST-M, Yim, Quas, Cahill, & Hayakawa, 2010; an adaptation of the TSST-C by Buske-Kirschbaum et al., 1997). Previous work suggests that this laboratory procedure, which is comprised of a public speaking portion and a mental arithmetic task, is capable of eliciting a cortisol stress response in 9-12 year-olds, older adolescents, and adults (Gunnar, Talge & Herrera, 2009; Yim et al., 2010). The public speaking task required participants to introduce themselves to a hypothetical new classroom of students, to describe themselves and include at least one positive and one negative item about oneself in their speech, which was conspicuously videotaped. The mental arithmetic task required subtracting out loud by 7s from 758 for teenagers or subtracting by 3s starting from 307 for 9-10-year-olds.

Participants were accompanied by one of their parents to two laboratory sessions spaced up to one week apart, with all start times being scheduled between 3:30 - 4:30 pm in order to control for the diurnal variation in cortisol. Children and adolescents completed the consent process, read leisurely for 25 minutes, and were then instructed that they have 5 minutes to prepare a speech, which would be evaluated by three judges. Based on random assignment, half of the participants prepared the speech with a female stranger (the experimenter), while the other half prepared with their parent. In the Parent Support condition, the parent was instructed to help their child in any way they find

useful. More than 90% of parents attending the session were mothers. In the Stranger Support condition, the participant left the parent to prepare with the stranger in another room. The stranger stated that they were ready to help in any way participants found useful and followed their lead in providing assistance. The speech preparation and the speech itself were recorded by cameras visible to the participants. After 5 minutes of preparation, the experimenter escorted the participant to the experimental room unaccompanied by the parent in order to complete the speech and mental arithmetic task. The participant was alone in this room and was required to perform the social stress task in front of a two-way mirror and a conspicuously placed video camera, which lasted a total of 10 minutes. The participant was told that the experimenter and two other teachers (one male, one female) would watch them from the other side of the mirror and rate their speech performance and their arithmetic accuracy. Children and adolescents were also told that the speech would be shown to a classroom of students to rate how well they did compared to other participants in the study. This was accomplished using an audio recording of two teachers that sternly provided instructions for the speech. Replacing an audience of three judges with a two-way mirror has succeeded in elevating cortisol in 9-year-olds (Jansen et al., 2000). After completion of the TSST-C, all participants returned to the speech preparation room, where they relaxed with their parent for 10 minutes. Participants then completed questionnaires for the remainder of the time. Participants in the 9-10 years old age group completed questionnaires with the experimenter's assistance, which was provided whenever they encountered vocabulary they were not familiar with or questions they considered confusing. At the end of the session, all

participants and parents were debriefed about the protocol and given positive feedback on the speech and serial subtraction performance.

Salivary cortisol was collected four times during Session 1 (45, 65, 85, and 105 minutes from arrival to the laboratory). The first sample was collected immediately after the completion of the stress task, which was approximately 20 minutes since the end of the relaxation period, while the second, third and fourth samples were taken 20, 40, and 60 minutes after the end of the stress task. The last sample assessed recovery from the stress response, and the second sample was intended to capture the peak cortisol response. Two resting cortisol samples (45 and 65 minutes from arrival) were collected during Session 2. Prior work suggests that baseline cortisol samples may be ideally collected in this fashion: in the same laboratory conditions, at the same time of day but on a different day, and during rest (Lovallo, Farag, & Vincent, 2010). This also reduced the novelty of arriving to the laboratory and any anticipatory stress, since participants were told in advance that this session was intended to be a calm and relaxing baseline.

Measures

Salivary cortisol. Participants expelled saliva through a straw into pre-labeled vials. They were instructed to refrain from eating large, protein-filled meals and consuming caffeine or energy drinks two hours before arriving to the laboratory. After each session, the samples were stored in a laboratory freezer at -20°C until being shipped to the University of Trier, Germany for being assayed using a time-resolved fluorescence immunoassay (dissociation-enhanced lanthanide fluorescent immunoassay [DELFI]; intra-assay CV < 7%, interassay CV < 10%). All of the samples from a child were

included in the same assay batch, and the assay batches were balanced by group, age, and condition. Samples were assayed in duplicate and averaged.

Observational ratings of parental support. Speech preparation with the parent (for participants randomized to this condition) was rated in real time by the experimenter (E) using 5-point Likert items yielding two subscales: E-rated Parent Support (4 items measuring parental sensitivity, overall positive affect towards the child, effective and clear communication, and validation of the child's perspective; Cronbach's alpha = .92) and E-rated Parent Negativity (3 items measuring intrusiveness, criticism/hostility, and authoritarianism; scale Cronbach's alpha = .74). Additionally, parent behavior during the 5-minute speech preparation was double-coded from videotapes by two other independent coders (C; inter-rater reliability: $r(82) = .88$). This coding also used 5-point Likert scales and yielded a measure of C-rated Parent Support (6 items: encouragement, validation, assistance coping with stress/nervousness, positivity, sensitivity, and helpfulness; Cronbach's alpha = .71) and a measure of C-rated Parent Negativity (2 items: criticism and intrusiveness; Cronbach's alpha = .75). E-ratings and C-ratings were standardized and averaged to create two observational measures of Parent Support and Parent Negativity.

Daily diaries. The parent and the child each completed a daily diary on session days, containing information relevant to cortisol collection (all the information concerned the child participant): time of wake-up, time of breakfast and lunch, medication usage, caffeine consumption, distressing events experienced that day (e.g., arguments with siblings or parents), and number of hours of sleep during the previous night. Child report was used as a primary source of information and parents' data were used for imputation

when the child's information was missing, contradictory, or incomplete (e.g., vague or misspelled medication names).

Child Life Events Scale (Boyce et al., 1995). The parent selected any major life events that had occurred in their child's life in the 3 months prior to testing (from a list of 40 possible events –e.g., serious illness of parent, death of a grandparent, change in schools, problems with teachers, moving to a new home, etc.) and also rated the impact they thought each stressor had on the child. A total Life Events score was created by adding 1 for each event experienced by children/adolescents during this period ($M = 1.42$, $SD = 1.77$, range = 0-10). Parental ratings of each stressor's impact on the child were not used in the analysis to eliminate subjectivity and obtain a more standardized count of stressors across all participants.

Self-Assessment Manikin (Lang, 1980). Participants used 5-point Likert scales to rate how stressed they felt at five time points during the session: upon arrival to the laboratory, during speech preparation, while giving the speech, during the subtraction task, and at the time of completing the questionnaire (at least 30 minutes after the end of the stressor).

Male and Female Puberty Scales (Petersen, Crockett, Richards, & Boxer, 1988). This questionnaire assessed participants' physical changes indicative of puberty, including skin and body hair changes, age of first menstruation for women, etc. Both the parent and the child completed these questionnaires for increased accuracy. Puberty measures were used to verify that 9-10-year-olds were indeed pre-pubertal and 15-16-year-olds had begun puberty. The scale yields a score from between 1-4, with 1 signifying a lack of any signs of pubertal changes in a domain (e.g., body hair) and 4

representing completed changes. Participants were deemed to have reached puberty if they had average scores of at least 2.5 on the Petersen scale. All of the 9-10-year-olds were pre-pubertal and all but one teenager were pubertal according to self-report (this female scored a 2.4, equivalent to pubertal development being underway but nominally not meeting the cut-off; however, the mother's report indicated a score of 3.5, thus it is likely that the participant underestimated or was too embarrassed to endorse all the physical changes present).

Network of Relationships Inventory: Social Provisions Version (NRI-SPV) (Furman & Burhmester, 1985). The NRI-SPV is a well-validated 39-item questionnaire that was designed to be used with children and adolescents. It assesses ten relationship qualities including seven aspects of support (Companionship, Instrumental Aid, Intimate Disclosure, Nurturance, Affection, Reassurance of Worth, Reliable Alliance) and three other relationship features (Relative Power, Conflict, and Antagonism). Subjects rated these qualities using three Likert-type questions for each of eight specific relationships (whenever applicable): mother, father, sibling, a relative, the closest same-sex friend, the closest opposite-sex friend, a romantic partner, and any one extra individual. For instance, participants used 1-5 scales to rate "how much does this person like or love you" or "how often do you and this person get mad at or get in fights with each other." Principal Components Analysis with a Varimax rotation indicated that the seven aspects of support all loaded highly on a single factor (loadings between .68 and .85), whereas Conflict and Antagonism loaded highly on a second factor (loadings of .96 and .96). The Relative Power scale was not examined further as it only contained 3 questions and it loaded on a third factor by itself. Given the two Support and Conflict factors derived,

four composite measures were created by averaging across the 7 positive dimensions or the 2 negative relationship dimensions and then also averaging across relationships (mother, father, and sibling were averaged for family composites; opposite-sex or same-sex friends and romantic partners were averaged for peer composites; ratings about the distant relative were not included due to hypotheses pertaining to close family support, whereas the eighth extra person could not be included in any composites since some participants listed a family member and others listed extra peers). The four composite measures derived were Support from family ($M = 3.49$, $SD = .62$, range = 1.78 - 4.6; Cronbach's alpha = .93), Support from peers ($M = 3.18$, $SD = .69$, range = 1.85 - 4.76; Cronbach's alpha = .90), Conflict with family ($M = 2.35$, $SD = .84$, range = 1 - 4.5; Cronbach's alpha = .88) and Conflict with peers ($M = 1.63$, $SD = .62$; range = 1-3.28; Cronbach's alpha = .87). In addition, measures of support and conflict for each of six specific close relationships (mother, father, sibling, best same-sex friend, best opposite-sex friend, and boyfriend/girlfriend) were also explored throughout.

Early Adolescence Temperament Questionnaire–Revised, Parent Version (EATQ-R, Capaldi & Rothbart, 1992). Parents completed 5-point Likert items on how characteristic certain behavior patterns were for their child (e.g., “Feels shy about meeting new people”). Two temperament scales were of primary interest in this report: Shyness (5 items, Cronbach's alpha = .89) and Surgency (9 items, Cronbach's alpha = .71), which were highly correlated: $r(79) = -.49$, $p < .001$. Thus the mean scores for the two scales (surgency reverse-scored) were averaged to yield a measure of Shyness/Behavioral Inhibition ($M = 2.6$, $SD = .67$, range = 1.28 - 4.16).

Data Analysis Plan

Data preparation. Preliminary analyses were conducted to identify outliers in cortisol concentrations and values more than 3 SD from the mean were Winsorized and replaced with the value at the 99.7th percentile. Since cortisol measures displayed high skewness (between 3.9 -8.9) and high kurtosis (between 47.02 - 93.6), a log₁₀ transformation was applied to these concentrations to normalize their distributions and meet assumptions for statistical analyses.

Experimental Manipulation Check. A Sample (2) x Day (2) Repeated-Measures (RM) ANOVA with age and sex as fixed factors was conducted to analyze differences between four cortisol values: baseline and peak from Day 1 and baseline and peak from Day 2. This initial analysis explored whether the TSST-C served its purpose of eliciting a cortisol response above the Day 2 baseline in the entire sample. There were indeed significant effects of Day ($F(1, 77) = 15.24, p < .001$) and interaction of Day by Sample ($F(1, 77) = 8.82, p = .004$) such that the peak measure on Day 1 was significantly higher than the peak measure on Day 2 ($p < .001$). On Day 1 cortisol levels increased, with the peak sample being significantly higher than the first sample ($p = .037$), whereas on Day 2 cortisol levels were decreasing (as would be expected across the afternoon) with the second sample having lower levels than the first ($p = .034$). There were no significant interactions of Day x Sample with age or sex. The same cortisol patterns were observed across the two age groups and sex.

A RM ANOVA was then used to examine participants' 5 consecutive ratings of subjective stress on the Self-Assessment Manikin. This analysis also showed increased levels of stress during the speech and arithmetic tasks, with a significant within-subjects effect of Sample ($F(4, 320) = 112.82, p < .001$) such that subjective stress levels

increased leading up to and during the stress task and then decreased (see Figure 2). Pair-wise comparisons revealed that the stress ratings for speech preparation, speech and the arithmetic task was each higher than either stress upon arrival or stress after recovery (at least 30 minutes post-stressor), with all p values for these pair-wise comparisons being $< .001$.

Statistical Analyses

Hierarchical Linear Modeling (Raudenbush, 2001; Raudenbush & Bryk, 2002) was used to analyze the cortisol data, since it is ideal for modeling change over time, it accounts for the auto-correlated nature of multiple samples collected from the same individual and it allows for greater statistical power than testing the same effects using traditional RM ANOVA models (Raudenbush & Bryk, 2002) or using averages across the session. The analyses were implemented using the PROC MIXED procedure for linear mixed modeling in the SAS 9.2 Software (SAS Institute, Inc., 2009). The Level 1 model represented individual change in levels of cortisol as a function of time. We anticipated based on previous research (Lam, Dickerson, Zoccola, & Zaldivar, 2009; Smith et al., 2009) and the sampling times in this design that both a linear and quadratic term for time would be necessary to model cortisol reactivity. Indeed, the linear and quadratic time terms included in the equations below were both significant: $F(1, 217) = 8.09, p = .005$ and $F(1, 161) = 36.45, p < .001$. The Level 2 model explained between-subjects differences based on multiple independent variables: three dummy variables coding for Age group (child versus adolescent, with adolescent being the reference), Sex (female was the reference), and Condition (Stranger versus Parent support, with Parent being the reference), as well as their interactions. Several continuous variables and their

interactions with some of these categorical predictors were also entered at this level and tested in separate models: composite measures of social support from family, conflict with family, social support from peers, conflict with peers, and shyness/behavioral inhibition. The general hierarchical structure of the model tested was:

$$\text{Level 1: } \text{Log10 Cortisol}_{ij} = \beta_{1i} + \beta_{2i} \text{ time}_{ij} + \beta_{3i} \text{ time}_{ij}^2 + \text{Cortisol Covariates} + e_{ij}$$

$$\text{Level 2: } \beta_{1i} = \beta_1 + \beta_4 \text{Age}_i + \beta_7 \text{Sex}_i + \beta_{10} \text{Condition} + \dots + b_{1i}$$

$$\beta_{2i} = \beta_2 + \beta_5 \text{Age}_i + \beta_8 \text{Sex}_i + \beta_{11} \text{Condition} + \dots + b_{2i}$$

$$\beta_{3i} = \beta_3 + \beta_6 \text{Age}_i + \beta_9 \text{Sex}_i + \beta_{12} \text{Condition} + \dots + b_{3i}$$

All β coefficients represent fixed effects that were tested for significance, while b_{1i} , b_{2i} , and b_{3i} are random effects, and e_{ij} is the residual for subject i , at time j . All Level 2 predictors are not shown here for simplicity, but 2-way and 3-way interactions of age, sex and condition and other predictors were introduced at this level in separate analyses. Time was coded as 0, 1, 2, and 3 given that the samples were equally spaced. Estimated β coefficients are presented in tables, whereas their Type 3 F tests of fixed effects are reported in the text, as are specific β parameters resulting from planned contrasts to follow-up on significant interactions. Restricted maximum likelihood (REML) was used to estimate all models. Kenward-Roger adjusted degrees of freedom were used, as they are recommended for use with REML (Kenward & Roger, 1997). All figures show observed data, not estimated values.

The linear and quadratic slopes estimated through growth-curve modeling captured the dynamics of cortisol reactivity, namely the steepness of the increase in cortisol in response to the task (linear slope) and the downturn or deceleration in the curve (quadratic slope). An additional measure was derived to capture anticipatory stress

responses –i.e., cases in which participants’ levels were already elevated before the stressor task. This measure was calculated as a difference score between pre-speech levels (sample 1) and recovery levels (sample 4), in the same manner as Sumter et al. (2010) who found that anticipatory stress responses increase with puberty. These difference scores were also log₁₀-transformed after an initial linear transformation (adding 10 to all values to ensure that they are positive and can be log-transformed). Univariate analyses of variance (ANOVA) were then used to examine age, condition, and age x condition differences in anticipatory stress responses subsequent to analyzing the dynamics of reactivity.

Cortisol covariates. Cortisol is sensitive to numerous sleep, diet, medication, experiential, and demographic factors (Kudielka, Hellhammer, & Wüst, 2009). For this reason, participants and their parents completed questionnaires including information on some of the most important variables that could impact the HPA axis. An empirical method was used to select which factors to include as covariates in further analyses. Time since wake-up was a significant predictor of intercepts ($F(1, 79) = 4.13, p = .046$) and was included in all models to control for the fact that individuals were at different points in their diurnal rhythm, even though they were all tested at similar times in the afternoon. Then, the following variables were each introduced by itself in a separate model, testing its effects on the cortisol intercept, linear and quadratic slopes: number of hours slept the previous night, time of breakfast, time of lunch, number of distressing events experienced the day of testing, number of major life events in the previous 3 months, caffeine consumption, and medication usage (yes/no or coded according to Granger, Hibel, Fortunato, & Kapelewski, 2009, such that a score of 0 = “no effect on the HPA

axis”, 1 = “possible effect on the HPA axis”, or 2 = “known mechanism of action for effects on the HPA” was added for each medication to create a total medication score). Family income was the main demographic variable tested, given known associations between socioeconomic status and cortisol levels (Cohen, Doyle, & Baum, 2006; Evans & English, 2002; Lupien, King, Meaney, & McEwen, 2000), whereas sex and age were a part of the study design, thus they were included in all subsequent analyses. Analyses showed that hours slept the previous night had a significant effect on the intercept ($F(1, 89.9) = 4.93, p = .03$), whereas life events in the past 3 months had significant effects on the linear ($F(1, 214) = 10.15, p = .002$) and quadratic slopes ($F(1, 160) = 5.53, p = .02$). Family income also had significant effects on cortisol trajectories (linear slope: ($F(1, 209) = 6.85, p = .01$; quadratic slope: $F(1, 156) = 3.79, p = .053$). Both life events and lower income decreased the linear slope –i.e., they potentially blunted cortisol reactivity. These empirically-selected covariates were included in all models, whereas all others had non-significant effects and were excluded from further inquiry.

Results

Age, Sex, and Condition Differences in Stress Reactivity

The fixed effects of age, sex, condition and all their 2-way and 3-way interactions were tested as predictors of cortisol trajectories (including relevant cortisol covariates described in the Data Analysis plan). There was a significant effect of age group on the intercept ($F(1, 80.1) = 5.15, p = .03$), with adolescents having higher intercepts than children. No other age differences in slope parameters were observed.

There was also a significant interaction of age x sex on the quadratic slope ($F(1,149) = 4.06, p = .046$), with follow-up analyses indicating that 9-10 year old girls

tended to have more negative quadratic slopes –i.e., more quickly decelerating curves ($F(1,72.4) = 4.70, p = .03$) than boys, whereas among teenagers there were no sex differences in intercepts or slopes (p values $>.27$).

Importantly, there was a significant interaction of age group and condition in predicting cortisol trajectories (effect on the linear slope: $F(1,199) = 3.93, p = .049$; effect on the quadratic slope: $F(1,148) = 3.50, p = .06$, see Table 1 for results of F tests for all effects in the model).

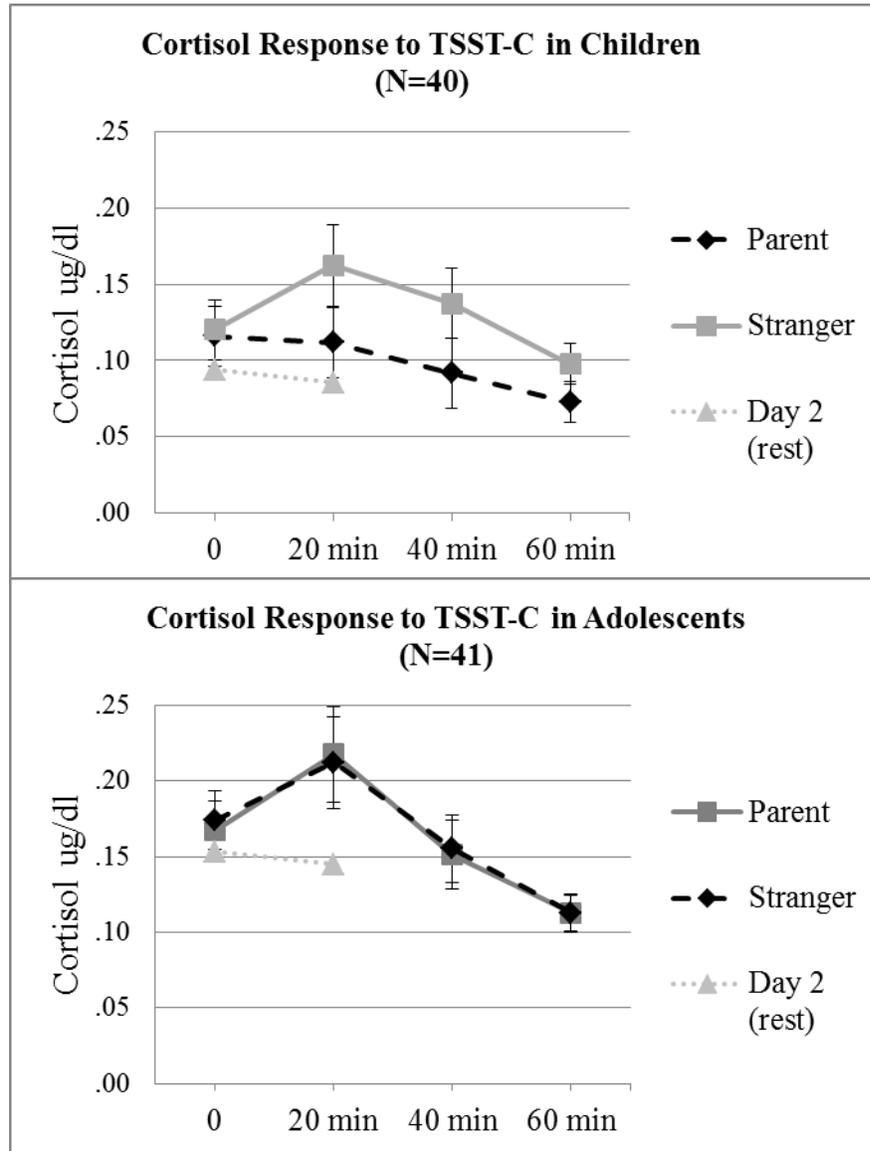
Table 1. Type 3 F tests for the model including age, condition, sex and their interactions as fixed effects. The following covariates were included in the model but not shown here for simplicity: effects of time since wake-up and hours of sleep on intercepts, effects of income and of life events on slopes. $\Delta p < .10$; $*p < .05$; $**p < .01$.

Effect	Num. df	Den. df	F	p
Linear	1	205	0.17	0.68
Quadratic	1	160	0.82	0.37
Age Group	1	80.1	5.15	0.03*
Age Group*Linear	1	200	0.01	0.91
Age Group*Quadratic	1	149	0.33	0.57
Sex	1	75.3	0.05	0.83
Sex*Linear	1	200	2.18	0.14
Sex*Quadratic	1	148	2.87	0.09 ^Δ
Condition	1	75.3	0.11	0.75
Condition*Linear	1	200	3.09	0.08 ^Δ
Condition*Quadratic	1	148	2.5	0.12

Age Group*Sex	1	75.9	1.55	0.22
Age Group*Sex*Linear	1	202	2.53	0.11
Age Group*Sex*Quadratic	1	149	4.06	0.046*
Condition*Age Group	1	75.7	0.21	0.65
Condition*Age Group*Linear	1	199	3.93	0.049*
Condition*Age Group*Quadratic	1	148	3.5	0.06 ^Δ
Condition*Sex	1	75.5	0.13	0.72
Condition*Sex*Linear	1	201	0	0.99
Condition*Sex*Quadratic	1	149	0.11	0.74
Condition*Age Group*Sex	1	75.8	0.15	0.70
Condition*Age Group*Sex*Linear	1	199	1.61	0.21
Condition*Age Group*Sex*Quadratic	1	148	1.67	0.20

Follow-up analyses within each age group revealed a significant main effect of condition on the linear ($F(1, 97.8) = 5.63, p = .02$) and quadratic slope ($F(1, 71.9) = 5.12, p = .03$) among 9-10-year-old children, with a significantly lower production of cortisol in the Parent compared to the Stranger condition (see Figure 1 for trajectories). As expected, children in the two conditions did not differ in the intercept, which should reflect their resting levels ($F(1, 36.3) = 0.02, p = .89$). Among adolescents, Parent support did not lower stress reactivity, with no differences in the intercept ($F(1, 37.5) = 0.13, p = .72$), linear ($F(1, 99.9) = 0.25, p = .62$) or quadratic slope ($F(1, 74.3) = 0.32, p = .57$) when compared to the Stranger support condition.

Figure 1. Observed cortisol responses by age group and condition (Stranger support or Parent support). The Trier Social Stress Test for Children (TSST-C) lasted 10 minutes and ended at the 0 minute mark on this graph. Error bars are SEMs.

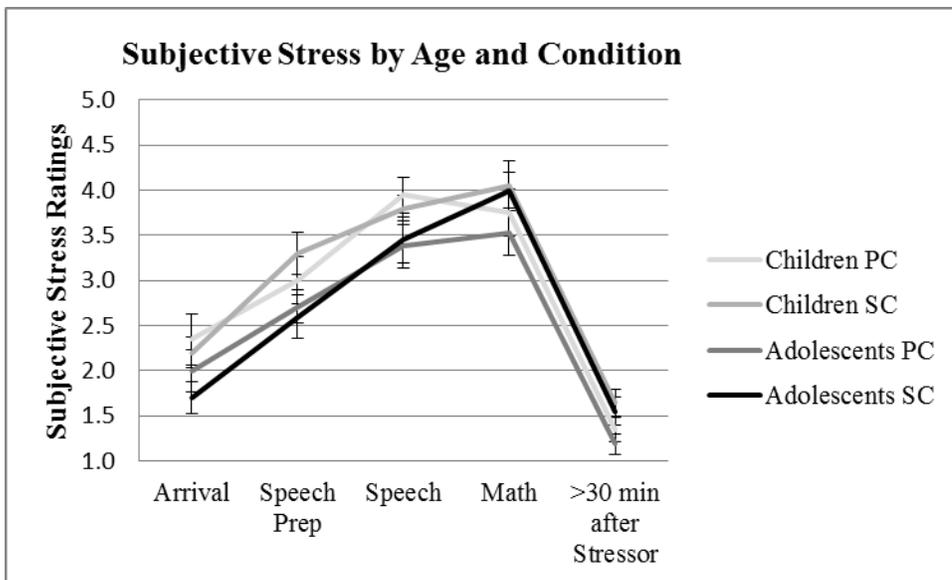


Given the goal of analyzing possible age or age by condition differences in anticipatory stress responses (see Data analysis plan), ANOVAs were used to test the main effects of age, condition, and age x condition on this measure, while controlling for the effects of sex and cortisol covariates (time since wake-up, hours of sleep, life events,

and income). There were no significant effects of age ($F(1, 72) = .89, p = .35$), condition ($F(1, 72) = .81, p = .37$) or age x condition ($F(1, 72) = .70, p = .41$). Figure 1 also highlights the fact that there were no such systematic differences in the anticipatory responses (i.e., starting level minus recovery level).

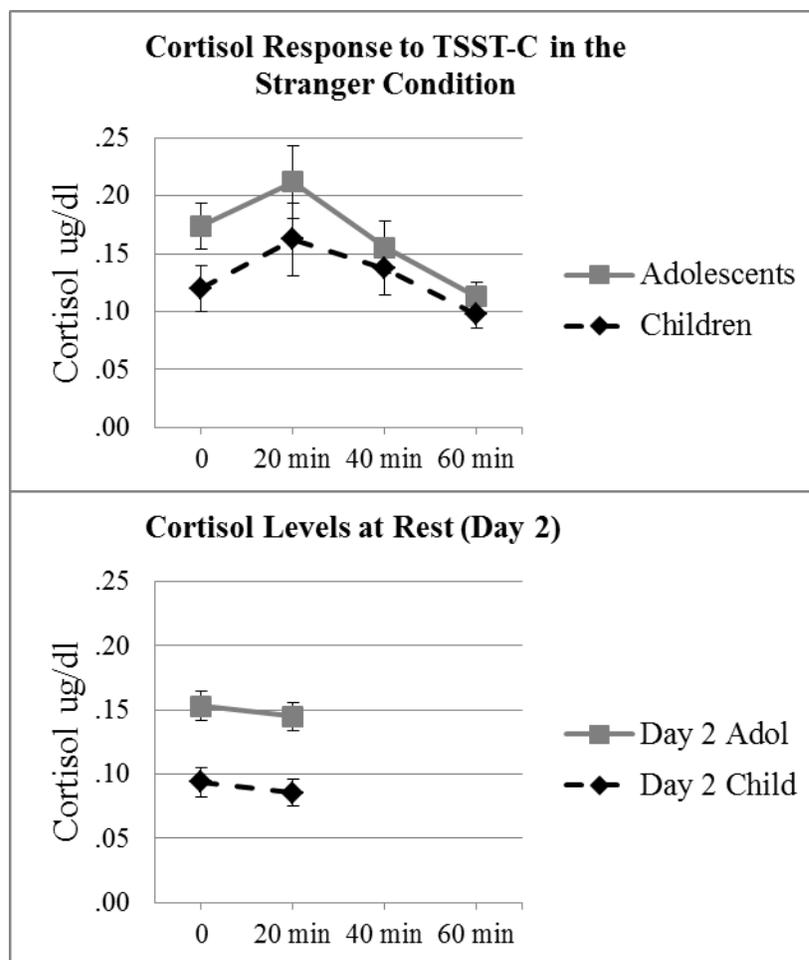
The age x condition differences were only observed using hormonal profiles of reactivity. When using growth-curve modeling to capture participants' changes in self-reported ratings of stress they experienced across the session (Figure 2) using the paper-and-pencil Self-Assessment Manikin, there were no effects of age, condition, sex or any of their 2-way or 3-way interactions on intercepts, linear or quadratic terms for these curves (p values between .08 and .96). The only non-significant effect with $p < .10$ was that of age group on the intercept ($F(1, 135) = 3.05, p = .08$).

Figure 2. Self-reported stress using 5-point Likert items sampled at five time points across the session. PC =Parent Condition; SC = Stranger Condition. Error bars are SEMs.



Given the aim of examining developmental differences in stress reactivity, we further probed the age group x condition interaction by examining the effect of age within the Stranger condition, which was the unbuffered stress response scenario across all participants. The two age groups did not differ significantly on the intercept ($F(1, 29.8) = 3.34, p = .08$), linear ($F(1, 108) = 1.50, p = .22$) or quadratic slope ($F(1, 76) = .61, p = .44$). However, an examination of Day 2 concentrations using a Day (2) x Sample (2) RM ANOVA revealed higher cortisol levels at rest in adolescents compared to children ($F(1, 79) = 15.67, p < .001$, see second panel in Figure 3).

Figure 3. Observed cortisol levels in the Stranger condition by age group.



Observed Support and Stress Buffering

The composite observational measure of support provided by the parent during speech preparation (for the 41 participants in the experimental condition) was not significantly correlated with the participant's own ratings of support generally received from that same parent ($r(39) = .19, p = .23$), whereas the measures of daily negative interactions reported by the participant and those captured during the laboratory observation also not being significantly related ($r(39) = .12, p = .46$). Given the fact that participant-reported and observer ratings captured different aspects of the relationships, they were analyzed separately.

The moderating role of the two composite observational ratings of parent support and parent negativity on the stress-buffering effect was tested first. These observational measures were only available for participants randomized to the Parent Support condition. In addition to age group, sex, age x sex and the cortisol covariates previously included, observed parent support and its interaction with age was introduced as a main effect on the intercept, linear and quadratic slopes. There were no significant effects of parent support or of the interaction of parent support by age group. Observed parent negativity in the laboratory did not have any main or interaction effects on cortisol growth curve parameters (p values ranging from .36 to .96) during the TSST-C testing day. When examining group differences in observed support/conflict, ANOVAs revealed no significant differences in age, sex, or age x sex in terms of either observed support ($F(1, 37) = 3.13, p = .09$; $F(1, 37) = .004, p = .95$; and $F(1, 37) = .98, p = .33$) or observed negativity ($F(1, 37) = .93, p = .34$; $F(1, 37) = .09, p = .77$; and $F(1, 37) = .10, p = .75$).

Perceived Support, Conflict and Stress Responses

Family measures of support were highly correlated with each other and peer measures also had smaller but significant associations among them (see Table 2).

Participants who on average perceived more support from family members also reported more support from peers.

Table 2. Bivariate correlations between measures of perceived support from close family members and peers. $N = 81$ for mother and father; $N = 79$ for sibling and same-sex friend; $N = 62$ for opposite-sex friend, and $N = 27$ for boyfriend/girlfriend.

	1	2	3	4	5	6	7	8
1. Support mother	1							
2. Support father	.74**	1						
3. Support sibling	.60**	.66**	1					
4. Support boyfriend/girlfriend	-.25	-.27	-.19	1				
5. Support same-sex friend	.13	.12	.25*	.27	1			
6. Support opposite-sex friend	-.04	.12	.18	.23	.36**	1		
7. Average family support	.89**	.86**	.84**	-.19	.18	.13	1	
8. Average peer support	.14	.17	.33**	.57**	.81**	.77**	.26*	1

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

Turning to conflict measures, similar patterns were observed to those noted above with support, such that measures of conflict with family members were correlated with each other. Participants who experienced conflict with family tended to also report conflict with peers (see Table 3).

Table 3. Bivariate correlations between measures of self-reported conflict with close family members and peers. $N = 81$ for mother and father; $N = 79$ for sibling and same-sex friend; $N = 62$ for opposite-sex friend and $N = 26$ for boyfriend/girlfriend.

	1	2	3	4	5	6	7	8
1. Conflict mother	1							
2. Conflict father	.70**	1						
3. Conflict sibling	.39**	.43**	1					
4. Conflict boyfriend/girlfriend	.10	-.19	.11	1				
5. Conflict same-sex friend	.35**	.40**	.31**	.39	1			
6. Conflict opposite-sex friend	.43**	.31*	.33*	.52**	.62**	1		
7. Average family conflict	.83**	.86**	.74**	.12	.42**	.45**	1	
8. Average peer conflict	.40**	.36**	.34**	.73**	.91**	.81**	.45**	1

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

Family support was also significantly negatively correlated with the conflict with family measure ($r(79) = -.29, p = .008$); however, peer support and peer conflict were not significantly correlated ($r(79) = .21, p = .06$).

Expected developmental differences in perceived support and conflict were also observed in this sample, specifically that adolescents reported significantly more conflict with their family than children did ($t(79) = 2.28, p = .025$; M teenagers = 2.56, $SD = .83$, M children = 2.14, $SD = .82$), with no differences in the amount of family support perceived by children versus adolescents ($t(79) = .04, p = .97$). When examining specific relationships, there were no developmental differences in support received from the mother, father, or sibling, but adolescents reported significantly more conflict with their mother than children ($t(73.64) = 3.57, p = .002$), with no differences in conflict with

fathers or siblings (see Figure 4 for all comparisons involving support and conflict).

There were no developmental differences in average perceived peer support ($t(79) = 1.24$, $p = .22$) or average conflict with peers ($t(79) = .94$, $p = .35$). Adolescents did, however, report significantly more support from their best same-sex friend compared to children, $t(77) = 3.28$, $p = .002$, with no other differences in support or conflict within specific relationships (see Figure 4).

Figure 4. Perceived support from and conflict with close family members and peers using 5-point ratings. 9-10-year-olds' report of support from boyfriend/girlfriend (N=4) were excluded given insufficient sample size and possibly poor validity. Asterisks mark statistically significant differences. Error bars are SEMs.

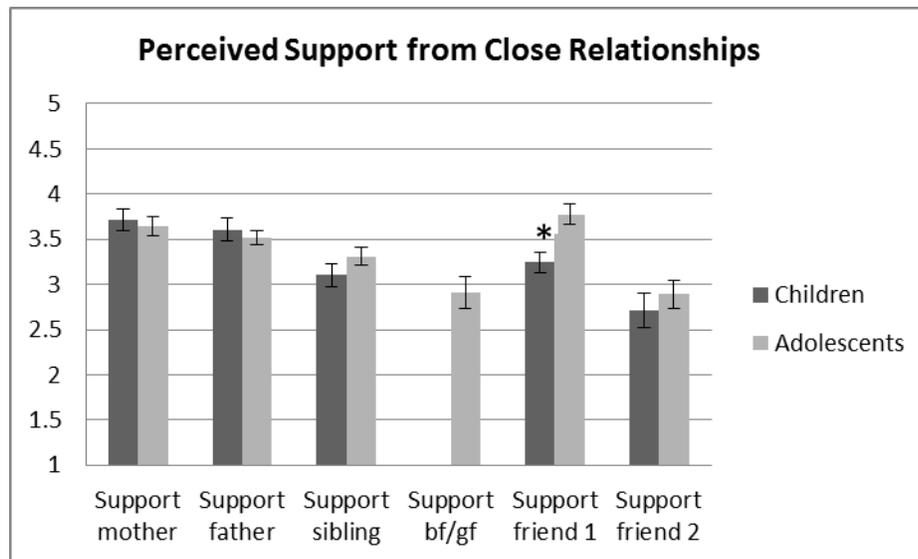
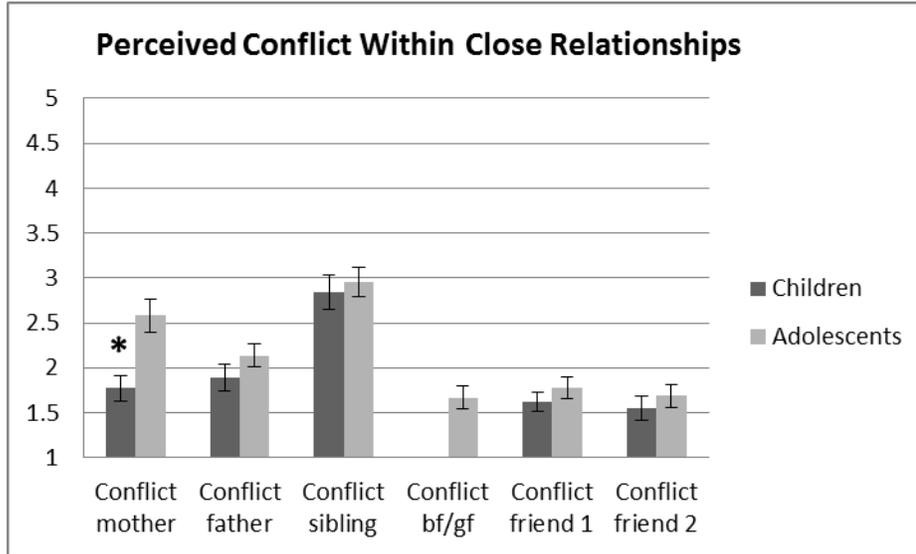


Figure 4 (cont.).



Next we examined the extent to which social support and conflict measures predicted stress reactivity or the buffering of stress by social support in the entire sample. All 2-way and 3-way interactions of age, condition, and sex found to be non-significant in previously reported analyses were removed from the present model to permit sufficient degrees of freedom for testing these hypotheses. Average support from family, conflict with family, support from peers and conflict with peers was each introduced in a separate model with its respective interactions with age and condition.

Perceived family support had no significant effects on cortisol curve parameters and no significant interactions with age or condition (p values from .11 to .99), and neither did self-reported support from peers (p values from .16 to .86). Conflict with family had significant interactions with age in predicting linear ($F(1, 197) = 4.22, p = .04$) and quadratic slopes ($F(1, 146) = 4.07, p = .045$) and marginally interacted with condition in predicting the two slopes ($F(1, 198) = 3.77, p = .054; F(1, 146) = 3.42, p = .067$). Follow-up analyses within each age group revealed the following effects. Among

9-10-year-olds, there were significant interactions between conflict with family and condition on linear ($F(1, 98.7) = 4.10, p = .045$) and quadratic slopes ($F(1, 70.9) = 3.59, p = .06$; see Table 4 for parameter estimates) such that children in the Parent condition had much lower increases in cortisol with the parent (i.e., a buffered stress response) if they had low levels of conflict with their family (see Figure 5 below for illustration) compared to high-levels of conflict.

Table 4. Parameter estimates for HLM examining conflict with family among 9-10-year-olds. Cortisol covariates were included in the model but not shown here for simplicity. $\Delta p < .10$; * $p < .05$; ** $p < .01$.

Effect	β	SE	Df	t	P
Intercept	1.20	0.57	31.6	2.12	0.04*
Linear	-0.07	0.17	102	-0.43	0.66
Quadratic	-0.02	0.05	72.7	-0.46	0.65
Conflict with family	0.05	0.10	34	0.44	0.66
Conflict with family*Linear	0.04	0.06	98.9	0.63	0.53
Conflict with family*Quadratic	-0.01	0.02	70.8	-0.68	0.50
Conflict with family*Condition	-0.06	0.14	34.4	-0.45	0.66
Conflict with family*Condition*Linear	-0.17	0.08	98.7	-2.03	0.045*
Conflict with family*Condition*Quadratic	0.05	0.02	70.9	1.89	0.06 ^Δ
Conflict with family* Sex	-0.02	0.14	32.4	-0.14	0.89
Conflict with family*Sex*Linear	0.03	0.04	29.9	0.82	0.42
Conflict with family*Sex*Quadratic	0.01	0.08	29	0.13	0.89
Condition (reference Parent)	0.14	0.31	33.3	0.47	0.64
Condition*Linear	0.53	0.19	98.3	2.76	0.01**

Condition*Quadratic	-0.15	0.06	71	-2.63	0.01*
Sex (reference Female)	-0.02	0.30	31	-0.07	0.94
Sex*Linear	-0.17	0.11	59.5	-1.55	0.13
Sex*Quadratic	0.04	0.02	71.3	1.90	0.06 ^Δ

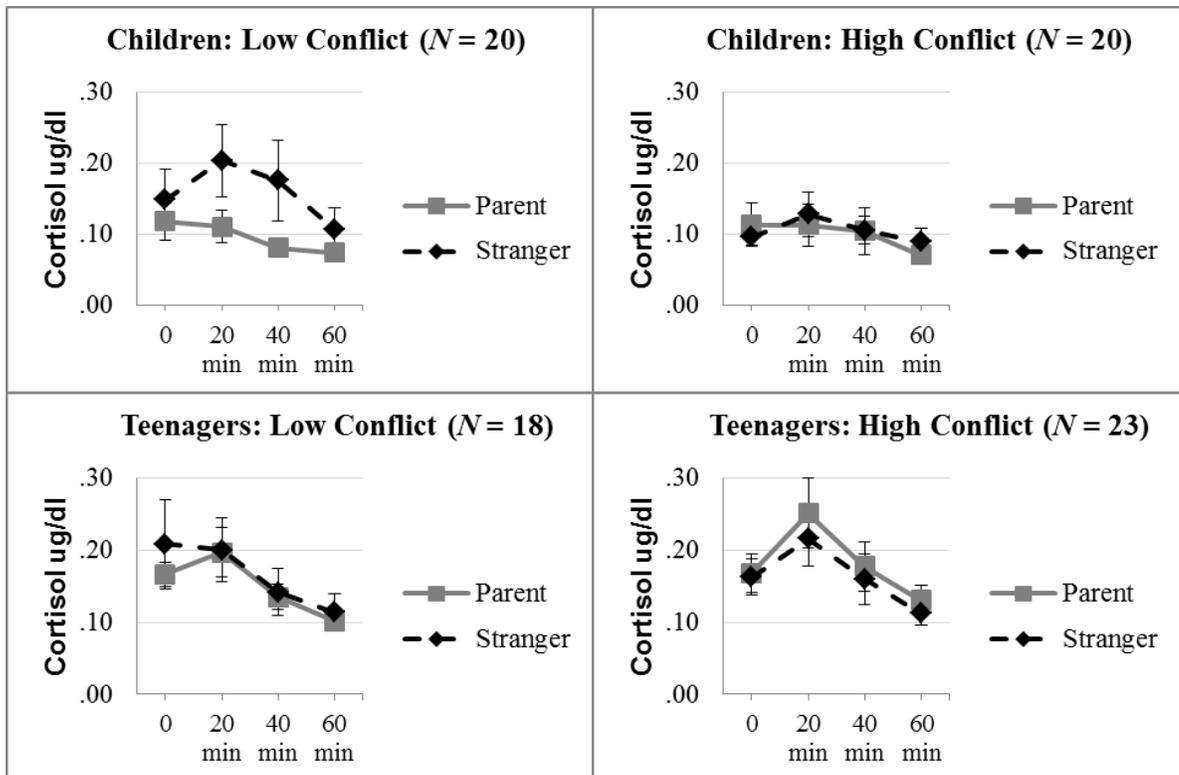
Among adolescents, there was a marginally significant effect of conflict on the linear slope ($F(1, 99.6) = 3.84, p = .053$) and the quadratic slope ($F(1, 73) = 3.08, p = .08$), but no interactions of conflict with condition (p 's of .58, .55 and .56 for the intercept, linear and quadratic slope) and still no main effect of condition –i.e., teenagers displayed an increase in cortisol regardless of condition, conflict with their family, or the interaction between them (see Table 5 for β estimates and Figure 5 for observed cortisol values by age group, condition and a high/low median split on conflict with family created for graphing purposes only).

Table 5. Parameter estimates for HLM examining conflict with family among 15-16-year-olds. Cortisol covariates were included in the model but not shown here for simplicity.

Effect	B	SE	df	T	p
Intercept	0.22	0.63	32.1	0.36	0.72
Linear	-0.23	0.16	106	-1.42	0.16
Quadratic	0.05	0.05	74	0.99	0.33
Conflict with family	0.06	0.09	33.8	0.68	0.50
Conflict with family*Linear	0.08	0.05	103	1.49	0.14
Conflict with family*Quadratic	-0.02	0.01	73.2	-1.63	0.11
Conflict with family*Condition	-0.08	0.10	34.1	-0.78	0.44

Conflict with family*Condition*Linear	-0.04	0.07	99.2	-0.6	0.55
Conflict with family*Condition*Quadratic	0.01	0.02	73.2	0.59	0.56
Conflict with family* Sex	-0.02	0.12	33.2	-0.14	0.89
Conflict with family*Sex*Linear	0.02	0.03	32.4	0.79	0.44
Conflict with family*Sex*Quadratic	0.04	0.06	30.9	0.57	0.57
Condition (reference Parent)	0.13	0.29	34.7	0.44	0.66
Condition*Linear	0.06	0.18	99.3	0.35	0.73
Condition*Quadratic	-0.02	0.06	73.2	-0.34	0.74
Sex (reference Female)	0.10	0.30	32.4	0.35	0.73
Sex*Linear	0.00	0.10	61.3	0	1.00
Sex*Quadratic	-0.02	0.02	73.8	-1.06	0.29

Figure 5. Observed cortisol responses by age group, condition, and the NRI Conflict with Family dimension. A median split within each age group was used.



Conflict with peers also showed significant interactions with age and condition in predicting cortisol trajectories (linear slopes: $F(1, 197) = 6.03, p = .01$; quadratic slopes: $F(1, 144) = 3.32, p = .07$). Follow-up analyses within condition revealed an interaction of conflict and age (linear: $F(1, 100) = 4.34, p = .04$; quadratic: $F(1, 70.5) = 3.03, p = .086$) in the Stranger condition such that children in this condition who reported high levels of conflict with peers had lower linear slopes ($\beta = -.25, SE = .12, t(100) = -2.08, p = .04$) – i.e., more flat cortisol curves- compared to teenagers. In the Parent Support condition, there was no effect of peer conflict or interaction of peer conflict x age on cortisol responses (p values ranging from .34 to .65).

Support and conflict from each specific relationship was then entered in a separate model to test if each of them was predictive of cortisol reactivity by itself or in interactions with age, condition, or age x condition. Given the large number of statistical tests using correlated independent variables measuring support or conflict, a family-wise Bonferroni correction was applied and only significant effects with $p < .008$ were considered (given 6 measures of family support or conflict and 6 measures of peer conflict or support). The only support measure predicting cortisol variance was the interaction of condition with support from one's best same-sex friend (on linear slope: $\beta = -.20, SE = .07, t(198) = -2.79, p = .006$; on quadratic slope: $\beta = .06, SE = .02, t(146) = 2.88, p = .005$) such that participants with more support from their best friend had a much smaller increase in cortisol in the stranger condition. Support from the other providers had no significant effects (all p 's $> .05$). The only measure of conflict that was predictive was conflict with the mother (significant interaction of support with condition on linear slope: $F(1, 198) = 8.06, p = .005$ and on quadratic slopes: $F(1, 146) = 7.70, p = .006$)

with follow-up analyses within condition indicating that this effect was only true in the parent support condition, such that higher levels of negativity with the mother increased cortisol reactivity in this condition (higher linear slope: $\beta = .09$, $SE = .04$, $t(89.5) = 2.49$, $p = .01$, stronger quadratic deceleration: $\beta = -.02$, $SE = .01$, $t(70.3) = -2.18$, $p = .03$). It must be noted that 91.4% of parents attending the sessions were mothers, thus results were similar when using participant-reported conflict with the session parent as a measure (interaction with condition in predicting linear slopes: $F(1, 198) = 7.16$, $p = .008$ and quadratic slopes: $F(1, 146) = 6.71$, $p = .01$). Thus conflict with the session parent increased reactivity in the parent condition for both age groups.

Testing Shy/Behaviorally Inhibited Temperament as a Moderator

To examine the role of shy/behaviorally-inhibited (BI) temperament, the BI composite was introduced in an HLM including the main effects of BI on intercepts and slopes, as well as its interactions with age and condition, in addition to factors previously found to be important (age, age x condition, sex, age x sex, and cortisol covariates). In this sample, parent-reported BI temperament drawn from the EATQ-R questionnaire did not have any main effects or interaction effects (i.e., moderating roles) on stress reactivity or stress-buffering (p values for F tests ranging between .43 and .98).

Discussion

To our knowledge, this is the first experimental study showing that parent support does not reduce cortisol reactivity to a laboratory stressor in adolescence, whereas in 9-10-year-olds this source of support is still a potent stress buffer. There are several possible explanations for an absent stress-buffering by parent support during adolescence. Firstly, it is well-recognized that adolescents from many species and human cultures

leave their native family group (Steinberg, 1988; Schlegel & Barry, 1991), a process that has likely been evolutionarily favored given its advantages in terms of reproductive fitness, since this adaptation reduces inbreeding and promotes genetic diversity (Steinberg, 1988). It may be that there are evolved puberty-related biological mechanisms that switch off the anti-stress protective features of the close family group to promote distancing from relatives. Indeed, there is some evidence that psychological distancing from parents and conflict increases with pubertal maturation specifically and not just with chronological age more generally (Hill et al., 1985a, b; Steinberg, 1987, 1988). Secondly, adolescents also show increased social motivation and affiliative tendencies towards peers and potential romantic partners (Collins, Welsh, & Furman, 2009; Forbes & Dahl, 2010), thus it is possible that the rewarding nature of these social interactions and the sheer increase in the amount of time spent with peers (Larson & Richards, 1991) builds up to an automatic shift in the relative rankings of primary support figures that places peers before parents as resources in times of stress. This gradual decline in intimacy with parents and increase in the importance of peers has been observed in questionnaire data collected from teenagers between childhood and early adulthood (Buhrmester & Furman, 1987; Furman & Buhrmester, 1992; Hunter & Youniss, 1982) and the same patterns were observed in this study. Thirdly, the fact that adolescence is a period of increased self-conscious emotions and neural activation in reaction to social evaluation (Somerville et al., 2013), a time of identity development (Allen et al., 1994; Steinberg & Morris, 2001) and dramatic functional and structural changes in brain circuits implicated in social information processing (Blakemore, 2008; Choudhury et al., 2006; Nelson et al., 2005) may render social-evaluative stressors particularly difficult if not impossible to buffer by

any support figure during this period. Fourth, it is possible that our findings are not specific to social evaluation and self-presentation, but rather that stress reactivity in adolescence is more generally up-regulated and perhaps more difficult to suppress by any means. Lastly, it could be argued that the increased conflict or antagonism in the parent-child relationship that occurs as teenagers negotiate their increasing needs for autonomy sours relations and diminishes the buffering role of parents. However, this is unlikely based on the present findings, which showed that adolescents did not differ from children in their perceived support from family and that conflict with family and that teenagers did not show the buffered stress response exhibited by children regardless of conflict levels with the session parent or with their family. To test the other possible explanations described, future studies should explore the buffering role of peers, test different types of social as well as non-social laboratory stressors of different intensities, and follow participants longitudinally to investigate the exact inflection point for the decline in the role of parental buffering. This would allow an examination of whether this phenomenon is triggered by puberty and by spikes in levels of gonadal hormones or more generally by social changes occurring gradually over several years.

It must be noted that the developmental differences in social buffering were only observed with respect to HPA reactivity, and were evident when using participants' subjective ratings of experienced stress. This is consistent with prior adult studies of social buffering, where self-report does not always correlate with hormonal output –e.g., in one study, women reported feeling more supported than men but men were the only ones exhibiting the diminished cortisol responses with support from romantic partners (Kirschbaum et al., 1995). The dissociation between behavior, emotion, and HPA

reactivity has also sometimes been observed in infants, who can show socially-buffered responses while still displaying behavioral signs of distress or fear (Nachmias et al., 1996) and vice-versa, can show reduced distress with pacifiers while still exhibiting spikes in salivary cortisol (Gunnar, Fisch, Korsvik, & Donhowe, 1981). For these reasons, HPA measures continue to be critical in future investigations of the beneficial roles of social support for coping with stress.

With respect to developmental differences in the activity of the HPA axis, adolescents tended to exhibit higher basal levels during the rest day, a pattern that is consistent with some prior reports of increasing average levels of cortisol across the day with puberty (see Gunnar & Vazquez, 2006 for a review). We did not find significant age-group differences in the slopes defining reactivity once any differences in intercepts were accounted for. When examining the combined effects of sex and age, 9-10-year-old girls tended to have higher cortisol slopes than males, possibly due to females being closer to puberty than males at this age. Among teenagers there were no sex differences in cortisol reactivity. Prior findings on sex differences in TSST responses in similarly-aged samples are mixed, with some reporting no sex differences with puberty (Gunnar et al., 2009; Kudielka et al., 2004; Stroud et al., 2009; Sumter et al., 2010), whereas others indicate that males tend to have higher HPA responses to performance stressors than females, especially in late puberty (Klimes-Dougan et al., 2001; Zijlmans et al., 2013). It is possible that studies finding sex by age interactions with puberty may differ in the demands placed by the stressor tasks on the participants. For instance, studies which enhance the performance or competitive aspects of the task (e.g., children have to prove to the judges that they were the best when compared with four other children in the study

by Zijlmans et al., 2013) tend to find higher levels of male reactivity. Additionally, a study that included a social manipulation where participants had to initiate a conversation with a female experimenter who was described as “shy” before a public speaking task also revealed enhanced responses in older adolescent males but not females or younger adolescents of both genders (Klimes-Dougan et al., 2001), but this study likely added a different demand on late-pubertal male participants by asking them to interact with someone of the opposite gender. The paradigm used in our study (hypothetically introducing oneself to a new classroom of students and being made to believe that the audience is made up of one male and one female teacher judge) may have equalized the demands of the task, leading to a lack of sex differences among pubertal participants.

There was no evidence that observational ratings of parent support or negativity in the laboratory moderated the effectiveness of social buffering within the parent condition, however participant-rated conflict with the mother, who tended to be the parent attending the session in the vast majority of cases, increased cortisol reactivity in the parent support condition for both age groups, lending support to the prediction that the buffering effect would be moderated by the quality of the relationship. These findings are consistent with theory and prior work suggesting that it is the history of the relationship (captured by participant ratings) that plays an important role (Broadwell & Light, 1999) rather than the individual display of encouraging or supportive behaviors observed in the laboratory, since these behaviors do not confer the same anti-stress protection when delivered by strangers (Kirschbaum et al., 1995; Smith et al., 2009). Thus, variability in laboratory behavior may not be a particularly powerful predictor if it doesn't capture typical patterns of behavior. Future studies should ask children and adolescents to rate how typical they

believe parent's attitudes and help during speech preparation were compared to those encountered at home.

The only other measure of a specific relationship quality predicting cortisol trajectories was that of support from one's best friend, which tended to reduce reactivity in the unbuffered stranger condition for both age groups. These children and adolescents have perhaps acquired a superior ability to contain their stress responses when their parents are not present.

When examining aggregate measures of support and conflict with family or peers, it became apparent that social-buffering by parent support tended to only be effective among 9-10-year-olds who experienced low levels of conflict with their family. Among adolescents, conflict did not moderate the likelihood of experiencing a stress-buffering effect since teenagers with both high- and low-conflict with family evinced a marked increase in cortisol production regardless of Parent or Stranger Support condition.

We also found that 9-10-year-olds experiencing high conflict with their family had a flat or absent pattern of reactivity in both experimental conditions (Figure 4). There are two possible interpretations to this finding. The first is that children from high-conflict families experience buffering effects from both the parent and the stranger, which would be suggestive of disruptions in forming a selective attachment to one's parents. The second interpretation stems from reports of blunted reactivity to laboratory challenges in younger children who experience interparental conflict or high maternal emotional unavailability (Sturge-Apple et al., 2012), and findings that kindergartners who experience conflict with their family at home (coded from electronically activated recorders) showed lower levels of morning cortisol and flattened diurnal cortisol slopes

(Slatcher & Robles, 2012). Chronic stressors such as conflict between family members likely elicits these patterns by initially elevating cortisol levels acutely, followed by down-regulation of the HPA axis over time such that basal or reactivity levels become lowered (Miller et al., 2007). Conflict may have been a stronger predictor of reactivity and buffering than self-reported perceived support due to the low-risk nature of the sample, where ratings of perceived family support were fairly high (a mean of 3.5 on a scale from 1-5) and conflict was perhaps the only relationship feature where more differentiation among families occurred.

The measure of temperament used was not a significant predictor or moderator, possibly due to exclusively using a measure of parent-report of child temperament. Future studies should combine this measure with participants' self-ratings and observational ratings, to possibly remove as much of the measurement error and reporter bias from this variable as possible.

The present study had a number of strengths, including the experimental control of the stressor and of support provision, the balanced 2 x 2 x 2 design (age group x condition x sex) and the careful matching of the two developmental groups on age and sex. Additionally, the results provided novel and important results regarding the social buffering of stress by parent support in two previously neglected developmental periods, late childhood and adolescence. The study also had a number of limitations. Its cross-sectional design makes it impossible to draw conclusions about developmental change, urging future replication of the same process within the same individuals observed over time. However, there is some evidence that repeating the Trier Social Stress Test causes some habituation of HPA axis reactions (Schommer, Hellhammer, & Kirschbaum, 2003),

making the current design preferable in this regard. Information from both types of designs should be combined to inform and strengthen conclusions. The second limitation concerns omitting adolescents' ratings of how helpful they thought the parent or the stranger were during the speech preparation period. However, given the mismatch between self-report and hormonal response profiles with respect to stress responses, it is unclear that self-report would have provided more insight into this process than observational ratings of support. Lastly, another previously discussed limitation of this study was the unintentional recruitment of a largely low-risk, highly-educated and high-income sample of families. This might have inadvertently restricted the range of parenting behavior observed. Given the known detrimental effects of poverty and family stress on parent-child relationships (Blair & Raver, 2012; Evans, 2004; Evans & English, 2002), it would be ideal to attract a larger percentage of these high-risk families, which may provide increased power to detect the moderating effects of parenting quality. The nature of the sample may also somewhat restrict the generalizability of these findings to other socioeconomic strata, thus future studies should replicate these results with families where conflict is much more intense and chronic.

While the present study was underway, Chen et al. (2011) reported that an oxytocin receptor gene (OXTR) polymorphism moderated the magnitude of the social buffering effect in adult human males, such that only individuals with one or two G alleles on the rs53576 polymorphism of the OXTR gene showed lower salivary cortisol levels in response to a social evaluative stressor task when receiving support, compared to individuals receiving no support. Individuals with the AA genotype did not show the same stress-buffering effect. It would be an important future direction to assess OXTR

genotype in developmental studies of social stress-buffering to examine its potentially moderating role in children and adolescents and also in females.

The present findings are consistent with prior work on normative developmental changes in social networks from childhood to adolescence, but reveal the novel and important results that adolescents may not be able to benefit from parent support during this challenging transitional period. These results have useful clinical implications for mental health treatment and prevention strategies, since adolescents who shift from using parental support to seeking peer support but who fail to concomitantly establish close friendships with peers may be essentially left with few or no stress buffers. This may, in turn, increase their risk for exacerbated stress reactivity and psychopathology. Secondly, recent findings show that maternal nurturance can also buffer against metabolic risk factors (Miller et al., 2011) and inflammatory states (Chen, Miller, Kobor, & Cole, 2011) associated with low childhood socioeconomic status and with heightened risk of affliction with chronic diseases of aging. Thus, an absent or diminished stress-buffering role of parent support during adolescence may pose health risks for adolescents that have much broader ramifications for long-term health in adulthood. For these reasons, it will be important to continue research in this arena in order to identify support figures (e.g., peers) or other coping mechanisms that are efficacious in protecting teenagers from stress, psychopathology, and other deleterious health outcomes.

Chapter 2

Early Life Social Deprivation and the Social Buffering of Stress during Childhood and Adolescence

Early life adversity predicts approximately 45% of childhood-onset and 30% of adult-onset psychopathology (Green et al., 2010), yet the psychobiological mechanisms that form the basis for this vulnerability have not been fully elucidated. There is accumulating evidence that alterations in the functioning of stress systems at least partially mediates some of the associations between early adversity and the enduring harm inflicted on psychological and physical health (Luecken & Lemery, 2004; McEwen, 2008; Shonkoff, Boyce, & McEwen, 2009). Animal studies have shown that the hypothalamic-pituitary-adrenocortical (HPA) axis, one of the primary effectors of the mammalian stress response, is developmentally programmed by early life stress (ELS) and shaped by the quality of early caregiving experiences (Claessens et al., 2011; Levine, 2005; Meaney, 2010). Much less is known about protective factors or interventions that could diminish or reverse these effects of ELS, if any have such capability. For instance, social support is a widely-recognized protective factor for physical and psychological well-being in human epidemiology (Cohen, 2004; Holt-Lunstad, Smith, & Layton, 2010; Taylor, 2011; Thoits, 2011; Uchino, 2006), however individuals who experience early life adversity often also have a history of continuously disrupted relationships, making it impossible to disentangle the effects of ELS from those of absent social support resources later in development. The study of children internationally adopted from orphanages overseas provides the unique opportunity to study a natural experiment in which ELS is circumscribed to the first few years of life and children subsequently experience a major

environmental switch into welcoming families, thus providing them with access to parent support moving forward. One of the questions that remain mostly unanswered in this natural experiment is whether children retain the ability to use their caregivers as a buffer against stress and a coping resource, despite their history of adversity in the form of neglect and social deprivation in the first few years. The present study aimed to answer this precise question, which would have the potential to inform interventions with pediatric populations experiencing a range of early life experiences. Before charting the hypotheses posed by the present study and describing the results, a brief review of the background literature on some of the most salient findings regarding early life stress, caregiving, and the activity of the HPA axis is necessary.

Animal models have shown that early life experiences shape the later activity of the HPA axis (Levine, 2005), whether to create vulnerability (Sanchez, Ladd, & Plotsky, 2001) or resilience (Lyons & Parker, 2007). Experimental studies in both rodents and primates have shown that maternal care is a powerful regulator of HPA stress responses early in development (Coe, Franklin, Smith, & Levine, 1982; Coe, Mendoza, Smotherman, & Levine, 1978; Levine, 2001; Stanton & Levine, 1985). Both high-quality maternal care (e.g., high levels of licking and grooming in rodents) and maternal separation, neglect or abuse have long-lasting effects on the developing brain and the functioning of stress systems (Meaney, 2010; Plotsky et al., 2005). For instance, rat pups reared by dams that are high in licking and grooming behavior exhibit lower endocrine and cardiovascular stress responses later in life and exhibit less fearful behavioral phenotypes compared to animals reared by dams that exhibit lower levels of these caregiving behaviors (Caldji et al., 1998; Francis, Diorio, Liu, & Meaney, 1999).

Importantly, cross-fostering of rat pups and random assignment to maternal caregiving phenotypes have demonstrated the experiential basis for these alterations in brain and behavior (Francis et al., 1999). The animal literature has recognized a rich set of inputs that are embedded in maternal care that serve to regulate offspring's physiology and behavior, inputs that have been termed "hidden regulators" (Hofer, 1984) and include tactile stimulation, feeding, temperature regulation, etc. Research on nonhuman primates additionally suggests that adverse rearing conditions in the form of early-life maternal deprivation and peer rearing greatly attenuate the ability of social stimuli to socially buffer stress responses in adulthood (Winslow, Noble, Lyons, Sterk, & Insel, 2003), which have otherwise been extensively noted in primates and other mammalian species (Hennessy, Kaiser, & Sachser, 2009; Kikusui, Winslow, & Mori, 2006).

In humans, there is a wealth of studies showing associations between adverse or disrupted childhood relationships and alterations in the functioning of the HPA axis in adolescence or adulthood, whether predicting cortisol reactivity (Bouma et al., 2011; Carpenter et al., 2007; Engert et al., 2009; Fisher, Kim, Bruce, & Pears, 2012; Goldman-Mellor, Hamer, & Steptoe, 2012; Gump et al., 2009; Hackman et al., 2013; Heim et al., 2000) or basal circadian secretion of cortisol (Engert et al., 2011; Murray, Halligan, Goodyer, & Herbert, 2009; Nicolson, 2004; Trickett et al., 2010; van der Vegt, van der Ende, Kirschbaum, Verhulst, & Tiemeier, 2009). Many previous studies have used retrospective measures of ELS or have objectively documented ELS in high-risk populations, such as children experiencing maltreatment, where chronic neglect and/or stress throughout development is superimposed on possible developmental programming effects occurring in the first few years of life. These studies provide important

information about the deleterious effects of these experiences on child development, but may need to be supplemented with other types of designs (e.g., intervention studies) to answer questions regarding plasticity and the possibility of recovery.

Studying children internationally adopted from orphanages after they experienced a period of socio-emotional and physical deprivation has provided the opportunity to investigate such hypotheses regarding plasticity and recovery. These children experience neglect and minimal cognitive or social stimulation in such group care situations, often starting at birth and continuing for the entire duration of their pre-adoptive lives (Zeanah, Smyke, & Dumitrescu, 2002). They have also been reported to exhibit abnormal patterns of HPA axis function. For instance, Carlson and Earls (1997) were the first to report that toddlers living in an orphanage in Romania had extremely low early morning cortisol levels and a flatter cortisol slope across the day, suggestive of down-regulation of the axis that is typical under chronic stress conditions (Miller et al., 2007). The same findings have also been replicated with children living in a Russian orphanage (Gunnar, 2001). However, by 6 or more years after being adopted into welcoming families, children from this population tend to exhibit elevated basal cortisol levels (Gunnar, Morison, Chisholm, & Schuder, 2001), especially if they are growth-stunted at adoption (Kertes, Gunnar, Madsen, & Long, 2008). Growth stunting is associated with more severe or prolonged levels of pre-adoptive adversity (Kertes et al., 2008) and with higher levels of dysregulated behavior years post-adoption (Johnson, Bruce, Tarullo, & Gunnar, 2011).

There are very few studies examining associations between early life stress and HPA axis function in later childhood and early adolescence. One study showed that post-institutionalized children aged 10-12 did not differ from a non-adopted comparison group

in their pattern of cortisol responses to a TSST-C task (Gunnar, Frenn, Wewerka, & Van Ryzin, 2009). When examining the cortisol awakening response (CAR) in 12-13-year-old post-institutionalized youth, Quevedo et al. (2011) showed that the effect of early life stress depended on pubertal stage such that pre/early pubertal internationally adopted teenagers showed a blunted morning CAR whereas mid/late pubertal teenagers did not significantly differ from their non-adopted counterparts. Another study based on a longitudinally-followed cohort that was internationally-adopted into the Netherlands showed that stress during the pre- and immediately post-natal period or during middle childhood (ages 6-11) or adolescence (ages 12-15) were associated with altered cortisol responses in adolescents exposed to a social stress test similar to the TSST, whereas adversity during the ages of 0-5 was not associated with altered HPA function (Bosch et al., 2012).

These results are beginning to provide support for the hypothesis that the HPA axis may recalibrate to the new life circumstances experienced during adolescence. Some proponents of these ideas point to evidence from experiments with rodents showing interactions between chronic early life stress and pubertal development such that the HPA axis undergoes a period of plasticity during the process of sexual maturation (Romeo et al., 2006, 2010; Romeo, Karatsoreos, & McEwen, 2006). These experiments not only reveal differences in patterns of HPA reactivity between the peripubertal period and adulthood (e.g., protracted corticosterone responses with slower recoveries to baseline during puberty), but brain plasticity in response to stress seems to also differ between these two periods, with adolescent rats displaying increased neurogenesis in the dentate gyrus after chronic stress and adult rats showing decreased neurogenesis in the same

region (Romeo et al., 2010). It has also been proposed that stressors experienced during adolescence may also exert enduring, programming effects on adult stress reactivity (Romeo, 2010). Given these emerging hypotheses, it would be extremely important to investigate possible interactions between early adversity and current social experiences as predictors of current stress system functioning. If such recalibration does occur, it is possible for instance that internationally-adopted youth, who experience a multiplicity of socio-emotional difficulties in childhood (reviewed below), may begin to benefit from their more supportive family context around the time of adolescence, suggesting that it may be particularly profitable to implement clinical interventions during this period.

Prior research has indeed shown that early social deprivation influences childhood trajectories of social development post-adoption. For instance, internationally adopted children exhibit less frequent secure attachments and more frequent disorganized attachment patterns with their adoptive parents 13 months after adoption (van den Dries, Juffer, van IJzendoorn, Bakermans-Kranenburg, & Alink, 2012). Furthermore, they exhibit other socio-emotional impairments such as difficulties with understanding social boundaries in interactions with strangers (Chisholm, Carter, Ames, & Morison, 1995; Rutter et al., 2007; Zeanah et al., 2002), with emotion understanding (Wisner Fries & Pollak, 2004), challenges in establishing close interactions and relationships with peers (Vorria, Rutter, Pickles, Wolkind, & Hobsbaum, 1998), and under- or over-reactions to environmental stimuli (e.g., Ames, 1997; Beckett et al., 2002; Zeanah et al., 2002). Furthermore, a study of 4-5-year-olds adopted internationally after social deprivation in orphanage care revealed higher levels of urinary cortisol after a laboratory interpersonal interaction with their mother and also a failure to elevate urinary oxytocin production

during the same parent-child interaction (Wismer Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005). In non-adopted children, an expected lowering of cortisol responses and increases in oxytocin output with the mother compared to that with a stranger was observed (Wismer Fries, Shirtcliff, & Pollak, 2008). Even though this study had a small sample size (18 adopted, 21 non-adopted children), the findings are noteworthy as they revealed not only a stress-buffering effect of the parent for non-adopted children, but also the failure of this protective mechanism to occur in children who did not experience a normative attachment relationship early in life.

Despite the richness of information on the socio-emotional development of post-institutionalized children in early and middle childhood, much less is known about the quality of interpersonal relationships with family or peers in late childhood or adolescence. Some studies suggest that their emotional difficulties persist into early adolescence (e.g., age 11, Colvert et al., 2008) and that socio-emotional problems persist for many adopted youth even later (e.g., a study on a sample aged 4-18 by Gunnar, van Dulmen, and the International Adoption Project Team, 2007), but to our knowledge there is a major gap in the literature on the quality of social networks during late childhood and adolescence for internationally-adopted youth. Specifically, little is known about the ability of these teenagers to use parent support as a coping resource in times of stress. Furthermore, it is not known if they would report comparable levels of support and conflict with their families during adolescence, a period that tends to bring about elevated levels of conflict in some typically-developing families, as reviewed more extensively in Chapter 1.

For these reasons, the present study aimed to examine the extent to which early life social deprivation may impact the reactivity of the HPA axis and/or the effectiveness of parent support in lowering or regulating HPA stress responses during late childhood. Furthermore, the current study had the ability to test the hypothesis that the HPA axis recalibrates during adolescence by using a cross-sectional design that recruited equal numbers of participants before (ages 9-10) and after (ages 15-16) the pubertal transition. We present the predictions and hypotheses derived from the background literature next.

Study Hypotheses

Based on the prior evidence described above, we aimed to test the following hypotheses/predictions: 1) parent support would fail to dampen stress reactivity in 9-10-year-old children in comparison to the stranger condition, as a reflection of the enduring effects of severe early social deprivation; there is insufficient evidence to make a strong prediction for adolescents, however the following two competing hypotheses will be tested: 1a) either adolescents show the same pattern as typically-developing children, with normal cortisol responses in both the parent support and stranger support conditions –i.e., no buffering effect given the diminishing role of parents during adolescence; or 1b) adopted teenagers exhibit a buffering effect of parent support due to a recalibration of the HPA axis to recent and current circumstances, when the effect of supportive parents in recent years is finally beginning to be noted; 2) observational ratings of parent support do not moderate the magnitude of the social buffering effect, consistent with the developmental findings in Chapter 1; 3) post-institutionalized (PI) children report comparable levels of perceived support and conflict with family members as non-adopted children, but report greater conflict and lower support with peers given their peer

difficulties reviewed above; 4) similar to the study described in Chapter 1, perceived conflict with family and specifically with mothers was hypothesized to impact cortisol reactivity; 5) given the correlational nature of this study, measures of pre-adoptive adversity including linear growth stunting were used to ascertain that current outcomes follow a gradient of early life adversity and explain previously noted individual differences in these samples across the range of adversity; and lastly, 6) by comparing the PI sample to the non-adopted group in Chapter 1, we also tested the effects of early life stress, age, and their interaction on cortisol reactivity and the buffering of stress by social-support. Sex and sex by age interactions were explored in all models, but firm hypotheses could not be formulated given insufficient prior work. Summary information on the quality of support and level of conflict with family members and peers were also explored given the scarcity of pre-existing information on the social development of post-institutionalized youth in our age ranges.

Methods

Participants

A sample of 81 internationally-adopted participants were recruited, half of whom were children ($N = 41$, M age = 9.7 years, $SD = .56$, range 8.87 – 10.99 years old; 51.2% females) and half adolescents ($N = 40$, M age = 15.6 years, $SD = .67$, range 14.2 -17.2; 55% females). Participants and their parents were recruited from the International Adoption Project registry at the University of Minnesota. The registry has been recruiting adopting families across the state for many years, drawing from a diverse set of adoption agencies. During recruitment, children and adolescents were excluded if they met any of the following criteria: being diagnosed with an Autism Spectrum Disorder, Fetal Alcohol

Syndrome (FAS), or any developmental disorder including but not limited to Pervasive Developmental Disorders, taking steroid medications (due to their interference with cortisol assay results), or having any medical or psychological condition that parents deemed would make participation challenging or too stressful for their child (e.g., reading delays such that completing questionnaires would be impossible). One participant (9-year-old male) reported a diagnosis of FAS at the time of the session, however the results did not differ with or without this participant thus he was included in the results presented below. Two participants (a male and a female from the 9-10-year-old group) reported taking steroid medications at the time of testing despite not using them at the time of recruitment, thus they were excluded from all cortisol analyses (final sample size $N = 79$), however their complete questionnaire data were available and used in analyses. PI participants had been adopted as children between the ages of 11 mos. – 5.08 years (with one outlier age of adoption at 7.08 years), M age = 26.4 mos., $SD = 15.5$ mos. The vast majority of children and adolescents (91.2% of them) had spent at least 50% of their pre-adoptive life in an institution –e.g., orphanage or baby home. They were adopted from 20 countries from diverse regions of the world: Russia and Eastern Europe (50%), East and South-East Asia (33.7), India/Nepal (11.3%), Latin America (3.8%), and Africa (1.2%).

Family income and education levels were high, as is typical of internationally-adopting families, with a median yearly family income of \$100,000-\$125,000 (range from 2.5% of families being in the \$15,001-\$25,000 bracket to 15% of the sample in the \$200,000 or more income bracket. Parental education had a median of 16 years of education for both the parents attending the session and their spouses or partners. Four

parents declined to provide their income information and one did not provide educational information.

Procedure

The present research protocol was approved by the Institutional Review Board at the University of Minnesota. Using a cross-sectional developmental design to compare individuals before and after the pubertal transition, 9-10 and 15-16 year-olds were recruited. The study had a balanced design, with three factors: Age Group (2) x Sex (2) x Experimental Condition (2). Participants completed the modified Trier Social Stress Test for Children (TSST-M, Yim, Quas, Cahill, & Hayakawa, 2010; an adaptation of the TSST-C by Buske-Kirschbaum et al., 1997), which included a public speaking task and a mental arithmetic task performed in front of a two-way mirror behind which participants were told there were judges and while being videotaped. All sessions had start times between 3:30 - 4:30 pm in order to control for diurnal variation in cortisol. Participants were randomly assigned to prepare their speech with a Stranger (a female experimenter) or their parent. For a more detailed description of the stress task and the timing of saliva sampling, please see Chapter 1 (pp. 23-25).

Measures

Salivary cortisol. Participants expelled saliva through a straw into pre-labeled vials. They were instructed to refrain from eating large, protein-filled meals and consuming caffeine or energy drinks 2 hours prior to the appointment. After each session, the samples were stored in a laboratory freezer at -20°C until being shipped to the University of Trier, Germany for being assayed using a time-resolved fluorescence immunoassay (dissociation-enhanced lanthanide fluorescent immunoassay [DELFI]);

intra-assay CV < 7%, interassay CV < 10%). All of the samples from a child were included in the same assay batch, and the assay batches were balanced by adoption group, age, and condition. Samples were assayed in duplicate and averaged.

Observational ratings of parental support. Speech preparation with the parent (for participants randomized to this condition) was rated in real time by the experimenter (E) using 5-point Likert items yielding two subscales: E-rated Parent Support (4 items measuring parental sensitivity, overall positive affect towards the child, effective and clear communication, and validation of the child's perspective; Cronbach's alpha = .88) and E-rated Parent Negativity (3 items measuring intrusiveness, criticism/hostility, and authoritarianism; scale Cronbach's alpha = .68). Additionally, parent behavior during the 5-minute speech preparation was double-coded from videotapes by two other independent coders (C; inter-rater reliability: $r(82) = .88$). This coding also used 5-point Likert scales and yielded a measure of C-rated Parent Support (6 items: encouragement, validation, assistance coping with stress/nervousness, positivity, sensitivity, and helpfulness; Cronbach's alpha = .84) and a measure of C-rated Parent Negativity (2 items: criticism and intrusiveness; Cronbach's alpha = .67). E-ratings and C-ratings were standardized and averaged to create two observational measures of Parent Support and Parent Negativity.

Daily diaries. The parent and the child each completed a daily diary on session days, containing information relevant to cortisol collection (all the information concerned the child participant on the day of the diary): time of wake-up, time of breakfast and lunch, medication usage, caffeine consumption, distressing events experienced on testing day from a list of 5 common events for children and adolescents, and number of hours of

sleep during the previous night. Child report was used as a primary source of information, and parent data was used for imputation when the child’s information was missing, contradictory, or incomplete (e.g. vague or misspelled medication names).

Demographics and pre-adoption history. The participating parent completed a demographics questionnaire, including information on family education, income, child current and pre-adoption history when applicable and known (including age at adoption, percent of pre-adoptive life spent in an institution, parent knowledge of any physical or sexual abuse prior to adoption, and parent impression of the quality of overall pre-adoptive care in institution if they had the chance to observe it or had any information about it, using a 5-point Likert scale ranging from 1 = “good” to 5 = “extremely neglectful care”). We created a cumulative Adversity Index by adding a score of 1 for each risk factor gleaned from these variables (see Table 6 for definitions of risk factors). The Adversity score had a range of 0-4 in this sample (Median = 1; 6.3% with a score of 0, 50.6% with a score of 1, 25.3% with a score of 2, 7.6% with a score of 3, and 2.5% with score of 4).

Table 6. Pre-adoptive adversity factors summed to create the Adversity Index and frequency of each risk factor in the PI sample.

Adversity Factor	Median	Range	Risk Factor Coded “1” if:	% Participants with Risk Factor
Age at adoption	19 mos.	11-85 mos.	Child older than (or equal to) median of 19 mos.	51.3% (N=39)
% time spent in institutional care	100%	18-100%	Child spent 100% of pre- adoptive life in institution	60.3% (N=47)

Prior physical abuse	N/A	N/A	Parent has knowledge of it	8% (N=6)
Prior sexual abuse	N/A	N/A	Parent has knowledge of it	0%
Overall quality of care in institution	2	1-5	Ratings of 2 or higher (i.e., more neglectful)	67.1% (N=51)

Internationally adopting parents also completed a HIPAA medical release form to authorize our laboratory to obtain the height, weight, and head circumference from the child’s first post-adoption medical visit in the United States. A measure of linear growth stunting was obtained by converting the height at adoption to standardized scores based on World Health Organization (WHO) norms by gender for the child’s age at the time (this information was available on N = 53 of participants; missing data was due to one or more of the following reasons: parent’s lack of records, information missing from clinic records, the clinic provided on the release form was not the one used for first post-adoption visit, or parents declined to sign the HIPAA form). This factor was examined given prior work on the potentially lingering influence of growth stunting at adoption on later HPA axis function (Kertes et al., 2008). Of the 53 participants with available data, we found that 32% of children were growth-stunted at adoption using a definition of being at least 2 standard deviations below the mean for their age and gender according to WHO norms. This variable was analyzed separately given the extent of the missing data.

Child Life Events Scale (Boyce et al., 1995). The parent selected any major life events that have happened in their child’s life in the 3 months prior to the scheduled laboratory session (from a list of 40 possible events –e.g., serious illness of parent, death

of a grandparent, change in school, problems with teachers, moving to a new home, etc.) and also rated the impact they thought the event had on the child. A total Life Events score was created by adding 1 for each event experienced by children/adolescents during this period ($M = 1.37$, $SD = 1.37$, range = 0-5). Parental ratings of each stressor's impact on the child were not used in the analysis to eliminate subjectivity and obtain a more standardized count of stressors across all participants.

Self-Assessment Manikin (Lang, 1980). Participants used 5-point Likert scales to rate how stressed they felt at five time points: upon arrival to the laboratory, during speech preparation, while giving the speech, during the subtraction task, and at the time of filling out the questionnaire (at least 30 minutes after the end of the stressor).

Male and Female Puberty Scales (Petersen, Crockett, Richards, & Boxer, 1988). This questionnaire assessed participants' physical changes indicative of puberty, including skin and body hair changes, age of first menstruation for women, etc. Both the parent and the child completed these questionnaires for increased accuracy. Puberty measures were used to examine whether all 9-10-year-olds were indeed pre-pubertal and 15-16-year-olds had begun puberty. The scale yields a score from between 1-4, with 1 signifying a lack of any signs of pubertal changes in a domain (e.g., body hair) and 4 representing completed changes. Participants were deemed to have reached puberty if they had average scores of at least 2.5 on the Petersen scale. In the PI group, all 9-10-year-olds were pre-pubertal and 7 teenagers self-reported not being fully pubertal (mean score 2.11; however, 6 out of these 7 participants were deemed to be pubertal according to parent report, $M = 2.67$, thus participants likely underestimated or were too

embarrassed to admit some of the changes that their parents had noticed; only one seemed to be truly delayed according to both parent and self-report).

Network of Relationships Inventory: Social Provisions Version (NRI-SPV) (Furman & Burhmester, 1985). The NRI-SPV is a well-validated 39-item questionnaire that was designed to be used with children and adolescents. It assesses ten relationship qualities including seven aspects of support (Companionship, Instrumental Aid, Intimate Disclosure, Nurturance, Affection, Reassurance of Worth, Reliable Alliance) and three other relationship features (Relative Power, Conflict, and Antagonism). Subjects rated these qualities using three Likert-type questions for each of eight specific relationships (whenever applicable): mother, father, sibling, a relative, the closest same-sex friend, the closest opposite-sex friend, a romantic partner, and any one extra individual. For instance, participants had to use 1-5 scales to rate “how much does this person like or love you” or “how often do you and this person get mad at or get in fights with each other.” Principal Components Analysis with a Varimax rotation conducted in the PI group indicated that the seven aspects of support all loaded highly on a single factor (loadings between .64 and .89), whereas Conflict and Antagonism loaded highly on a second factor (loadings of .90 and .92). Similar to results in the Non-adopted group, the Relative Power scale was not examined further as it only contained 3 questions and it loaded on a third factor by itself. Given these two Support and Conflict factors derived, four composite measures were created by averaging across the 7 positive dimensions or the 2 negative relationship dimensions and then averaging across relationships (mother, father, and sibling were averaged for family composites; opposite-sex or same-sex friends and romantic partners were averaged for peer composites; ratings about the distant

relative were not included due to hypotheses pertaining to close family support, whereas the eighth extra person could not be included since some participants listed a family member and others listed extra peers). The four composite measures derived and used in analyses were Support from family ($M = 3.59$, $SD = .67$, range = 1.57 - 4.81; Cronbach's alpha = .94), Support from peers ($M = 3.29$, $SD = .72$, range = 2.07 - 4.57; Cronbach's alpha = .89), Conflict with family ($M = 2.32$, $SD = .84$, range = 1- 4.67; Cronbach's alpha = .88) and Conflict with peers ($M = 1.52$, $SD = .50$; range = 1-3.83; Cronbach's alpha = .74). In addition, measures of support and conflict for each of six close relationships (mother, father, sibling, best same-sex friend, best opposite-sex friend, and boyfriend/girlfriend) were also explored throughout.

Data Analysis Plan

Data preparation. Preliminary analyses were conducted to identify outliers in cortisol concentrations and values more than 3 SD from the mean were Winsorized and replaced with the value at the 99.7th percentile. Since cortisol measures displayed high skewness and kurtosis, a log₁₀ transformation was applied to these concentrations to normalize their distributions and meet assumptions for statistical analyses.

Experimental Manipulation Check. A preliminary analysis using a Sample (2) x Day (2) repeated-measures ANOVA with age and sex as fixed factors was conducted to analyze differences between four cortisol values: baseline and peak from Day 1 and baseline and peak from Day 2. This initial analysis explored whether the TSST-C served its purpose of eliciting a cortisol response above baseline. There was indeed a significant effect of Day ($F(1, 74) = 11.05$, $p < .001$) such that cortisol values were significantly higher on Day 1 compared to Day 2. However, there was also a significant day x age x

sex interaction ($F(1,74) = 5.33, p = .02$). These subgroup differences in reactivity will be explored below, in the Results section. As an additional check that the stressor elicited a response in the sample as a whole, the peak measure from Day 1 was significantly higher than the peak measure on Day 2 ($F(1, 74) = 13.1, p < .001$).

A RM ANOVA was then used to examine participants' 5 consecutive ratings of subjective stress on the Self-Assessment Manikin. This analysis also showed increased levels of stress during the speech and arithmetic tasks, with a significant within-subjects effect of Sample ($F(4, 308) = 86.8, p < .001$) such that subjective stress levels increased leading up to and during the stress task and then decreased (see Figure 6). Pair-wise comparisons revealed that the stress experienced during speech preparation, speech, and the arithmetic task was each higher than either stress upon arrival or stress after recovery (at least 30 minutes more post-stressor), with all p values for these pair-wise comparisons $< .001$.

Statistical Analyses. Hierarchical Linear Modeling (Raudenbush, 2001; Raudenbush & Bryk, 2002) was used to analyze the cortisol data, since it is ideal for modeling change over time, it accounts for the auto-correlated nature of multiple samples collected from the same individual and it allows for greater statistical power than testing the same effects using traditional Repeated Measures ANOVA models (Raudenbush & Bryk, 2002) or using averages across the session. The analyses were implemented using the PROC MIXED procedure for linear mixed modeling in the SAS 9.2 Software (SAS Institute, Inc., 2009). Linear and quadratic time terms were used to model the increase in cortisol levels in response to the TSST-C. The Level 1 model represented individual change in levels of cortisol as a function of time, whereas the Level 2 model explained

between-subjects differences based on multiple independent variables, including three dummy variables coding for Age group (child versus adolescent, with adolescent being the reference), Sex (female was the reference), and Condition (Stranger versus Parent support, with Parent being the reference). For additional details on the HLM set-up, please see Chapter 1 (pp. 31-32).

As described in Chapter 1, the linear and quadratic slopes estimated through growth-curve modeling captured the dynamics of cortisol reactivity, whereas anticipatory stress responses were measured through a difference score between pre-speech levels (sample 1) and recovery levels (sample 4), in the same manner as Sumter et al. (2010). These difference scores were also log₁₀-transformed after an initial linear transformation (adding 10 to all values to ensure they were all positive and could be log-transformed). Univariate analyses of variance (ANOVA) were then used to examine age, condition, and age x condition differences in anticipatory stress responses subsequent to analyzing the dynamics of reactivity.

Cortisol covariates. Variables with potential impacts on the HPA axis (Kudielka, Hellhammer, & Wüst, 2009) were each examined in separate models: number of hours slept the previous night, time of breakfast, time of lunch, caffeine consumption, medication usage (see Chapter 1, pp. 33-34 for details on the coding chosen for this variable), number of distressing events on testing day, number of major life events in the previous 3 months, and family income. Time since wake-up was a significant predictor of intercepts ($F(1, 76) = 7.56, p = .008$) and was included in all models to control for the fact that individuals were at different points in their diurnal rhythm despite their comparable afternoon session times. Analyses showed that hours slept had a significant

effect on the intercept ($F(1, 84.1) = 7.01, p = .01$), whereas the day's distressing events had significant effects on the linear ($F(1, 200) = 4.13, p = .04$) and quadratic slope ($F(1, 154) = 6.10, p = .01$), with no other significant covariates. Given hypotheses about group differences in cortisol responses between adopted and non-adopted groups, we also examined whether they differed on any of these covariates, since they could serve as confounding variables for group effects. The PI group slept significantly more hours the previous night ($t(157) = 2.04, p = .04$; M PI = 9.44 hours, $SD = 2.02$, M non-adopted = 8.83 hours, $SD = 1.8$), which was another reason to control for this variable, and the PIs also used significantly more medications (difference in medication code: $t(158) = 3.98, p < .001$; overall, 30.4% of PI subjects were using medications versus 3.7% of the non-adopted group, a significant difference: $\chi^2(1) = 20.3, p < .001$). Most of the medications (75%) used by PI participants were psychotropic. To account for this potential confound, we also controlled for the effect of medications on cortisol curve parameters in all analyses involving PI children, in addition to the effects of hours slept on the intercept and of day's distress on the linear and quadratic slopes.

Results

Age, Sex, and Condition Differences in Stress Reactivity

The fixed effects of age, sex, condition and all their 2-way and 3-way interactions were tested as predictors of cortisol trajectories (including relevant cortisol covariates – see Data Analysis plan). There was a significant interaction of age x sex on the intercept ($F(1,75) = 5.95, p = .02$), with follow-up analyses within age group revealing a significant effect of sex on intercepts among teenagers ($F(1, 34.9) = 4.62, p = .04$) such that males tended to have higher cortisol intercepts than females, and no sex differences among 9-

10-year-olds. There was also a significant interaction of age group and condition on the linear slope ($F(1,191) = 3.99, p = .047$) and on the quadratic slope ($F(1,146) = 4.15, p = .043$). The 3-way interactions of age, sex, and condition on the intercept, linear and quadratic slope did not reach statistical significance ($F(1,74.9) = 3.01, p = .087$; $F(1,192) = 2.79, p = .097$; and $F(1,146) = 3.16, p = .077$), thus they were not followed up and there were no other significant effects (see Table 7 for parameter estimates in the model).

The age x condition interaction was followed-up within each age group and within each condition, revealing some differences in reactivity among these subgroups (see Figure 6). Specifically, children and teenagers differed significantly in their linear and quadratic slopes in the Stranger condition ($F(1,91.5) = 4.49, p = .04$ and $F(1,70.1) = 4.13, p = .046$), but not in the Parent condition ($F(1, 96) = 0.62, p = .43$; $F(1, 74.4) = 1.07, p = .30$). Within-age analyses revealed no differences in the intercepts and slopes of 9-10-year-olds by condition (p values of .46, .36, and .49) and no statistically significant differences by condition in 15-16-year-olds (p values of .53, .28, and .11).

Figure 6. Observed cortisol responses by age group and condition in the PI group. Data shown in two right-side panels are redundant with the left panels in order to show all four pair-wise comparisons by condition and age. All trajectories in the Parent condition are represented in black and all those in the Stranger condition are shown in gray. Error bars are SEMs.

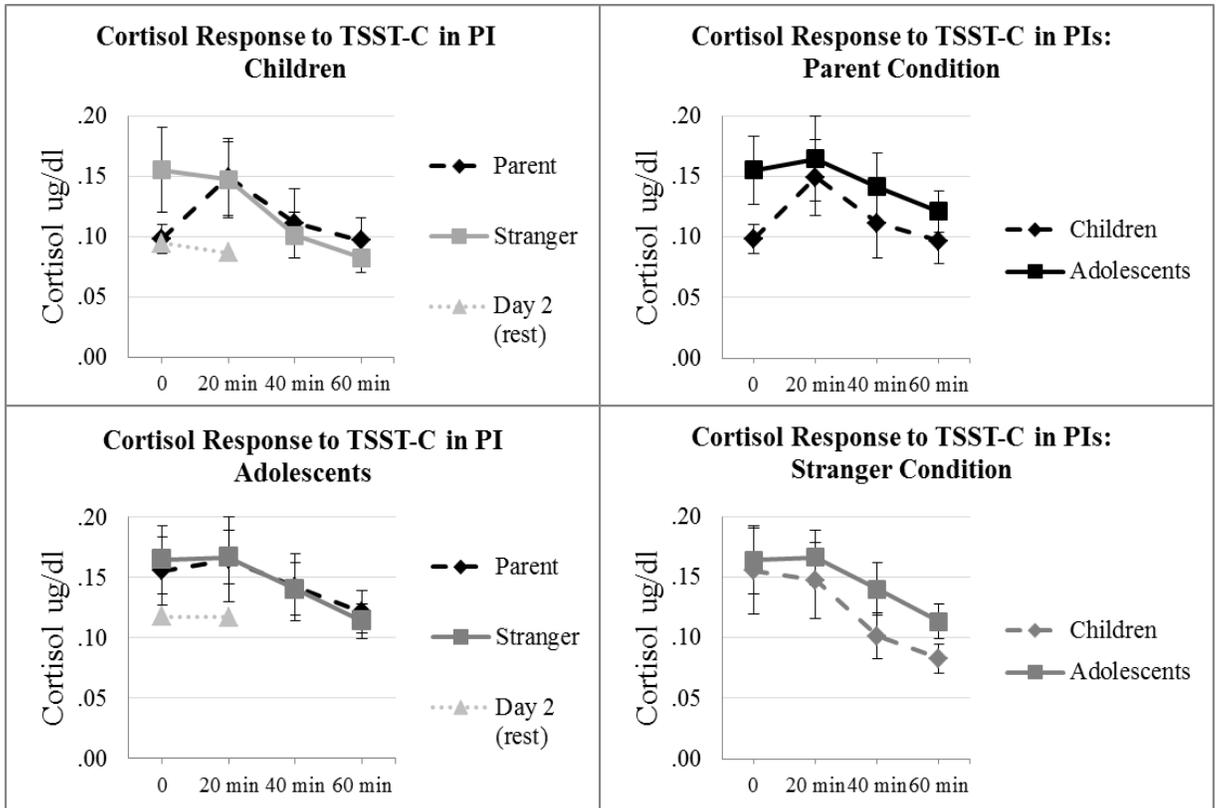


Table 7. Parameter estimates for model including age, condition, sex and their interactions as fixed effects. The following covariates were included in the model but not shown here for simplicity: hours of sleep the previous night, effects of day’s distress on linear and quadratic slopes, and of medication code on all curve parameters. $\Delta p < .10$; * $p < .05$; ** $p < .01$.

Effect	β	SE	df	t	P
Intercept	1.42	0.28	68.1	5.09	<.001*
Linear	0.05	0.06	192	0.79	0.43
Quadratic	-0.02	0.02	146	-1.34	0.18
Age group (ref Teenager)	0.04	0.12	75.2	0.31	0.76
Age group*Linear	0.15	0.09	191	1.66	0.10
Age group*Quadratic	-0.05	0.03	145	-2.01	0.046*

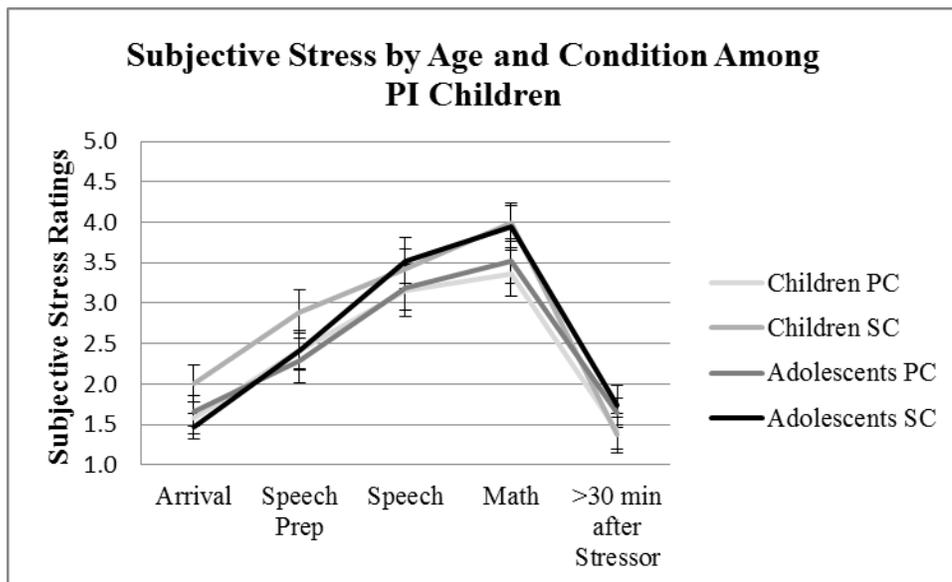
Sex (ref Females)	0.04	0.12	75	0.33	0.74
Sex*Linear	-0.01	0.09	191	-0.13	0.89
Sex*Quadratic	-0.01	0.03	146	-0.23	0.82
Condition (ref Parent)	-0.06	0.11	74.9	-0.51	0.61
Condition*Linear	0.09	0.09	192	1.04	0.30
Condition*Quadratic	-0.04	0.03	146	-1.41	0.16
Age Group*Sex	-0.09	0.17	75	-0.51	0.61
Age Group*Sex*Linear	-0.19	0.13	192	-1.47	0.14
Age Group*Sex*Quadratic	0.07	0.04	147	1.75	0.08 ^Δ
Condition*Age Group	0.20	0.17	74.9	1.23	0.22
Condition*Age Group*Linear	-0.34	0.13	192	-2.67	0.01*
Condition*Age Group*Quadratic	0.11	0.04	146	2.78	0.01*
Condition*Sex	0.27	0.17	74.9	1.63	0.11
Condition*Sex*Linear	-0.01	0.13	192	-0.1	0.92
Condition*Sex*Quadratic	0.01	0.04	146	0.17	0.87
Condition*Age Group*Sex	-0.41	0.24	74.9	-1.73	0.09 ^Δ
Condition*Age Group*Sex*Linear	0.31	0.18	192	1.67	0.097 ^Δ
Condition*Age Group*Sex*Quadratic	-0.10	0.06	146	-1.78	0.08 ^Δ

Given the goal of analyzing possible age or age by condition differences in anticipatory stress responses (see Data analysis plan), ANOVAs were used to test the main effects of age, condition, and age x condition on this measure, while controlling for the effects of sex and cortisol covariates (time since wake-up, hours of sleep, day's distress, and medications). There was a significant main effect of condition ($F(1, 68) = 4.15, p = .045$) such that PI participants overall had higher anticipatory stress responses to

the Stranger condition than the Parent condition, but no effect of age group ($F(1, 68) = .27, p = .60$) or age x condition interactions ($F(1, 68) = 1.26, p = .27$). Figure 6 above also makes it evident that PI participants from both age groups exhibited anticipatory stress responses –i.e., early elevations in cortisol that were already evident in the first sample, which was collected approximately 15 minutes after leaving the parent with the stranger to walk to a different room and prepare their speech.

When using growth-curve modeling to capture participants’ changes in self-reported stress they experienced across the session (Figure 7) using the paper-and-pencil Self-Assessment Manikin, there were no effects of age, condition, sex or any of their 2-way or 3-way interactions on intercepts, linear or quadratic terms for this curve (p values between .10 and .95).

Figure 7. Self-reported stress (5-point Likert scale) across the session. PC = Parent Condition; SC = Stranger Condition. Error bars are SEMs.



Observed Support and Stress Buffering

The composite observational measure of parent support received during speech preparation (for the 38 participants in that experimental condition) was not significantly correlated with the participant's own ratings of support generally received from that same parent ($r(37) = .16, p = .32$), whereas the measures of daily negative interactions reported by the participant and those captured during the laboratory observation were also not significantly related ($r(37) = -.17, p = .31$). Given the fact that these measures captured different aspects of the relationships, they were analyzed separately.

To examine whether the quality of the support provided in the laboratory moderated the magnitude of the stress-buffering effect, the composite measures of our two observational ratings of Parent Support and Parent Negativity were analyzed next. These observational measures were available for participants randomized to the Parent Support condition with cortisol data and not taking steroids ($N = 38$). In addition to age group, sex, age x sex and the cortisol covariates previously included, observed parent support and its interaction with age was introduced as a main effect on the intercept, linear and quadratic slopes. This observational measure did not have significant effects on the intercepts, linear or quadratic slopes and did not interact with age group on any of these parameters (p values ranging between .38 to .79). Parent negativity observed in the laboratory also did not have significant effects on the three curve parameters and also did not interact with age in predicting them (p values ranging between .12 and .92). Lastly, ANOVAs also revealed no significant differences in age, sex, or age x sex in terms of either observed support ($F(1, 35) = 2.23, p = .14$; $F(1, 35) = .22, p = .64$; and $F(1, 35) = .41, p = .53$) or observed negativity ($F(1, 35) = .007, p = .93$; $F(1, 35) = .001, p = .97$; and $F(1, 35) = 2.31, p = .14$).

Perceived Support, Conflict, and Stress Responses

Similar to findings in the non-adopted group, family measures of support were highly correlated with each other, whereas peer measures also had some significant associations among them (see Table 8). Participants who on average perceived more support from family members also reported more support from peers.

Table 8. Bivariate correlations between measures of perceived support from close family members and peers in the PI group. *N* ranges from 62 to 77 for peers and family members; *N* = 23 for boyfriend/girlfriend.

	1	2	3	4	5	6	7	8
1. Support mother	1							
2. Support father	.74**	1						
3. Support sibling	.71**	.60**	1					
4. Support boyfriend/girlfriend	-.27	-.20	.01	1				
5. Support same-sex friend	.36**	.28*	.45**	-.08	1			
6. Support opposite-sex friend	.19	.37*	.32*	.01	.63**	1		
7. Average family support	.87**	.87**	.86**	-.12	.41**	.40**	1	
8. Average peer support	.34**	.34**	.45**	.49*	.85**	.87**	.46**	1

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

Turning to conflict measures, similar patterns were observed such that measures of conflict with family members were correlated with each other and they were also correlated with at least some measures of conflict with peers (Table 9).

Table 9. Bivariate correlations between measures of self-reported conflict with close family members and peers among PI participants. *N* ranges from 62-77 for peers and family; *N* = 23 for boyfriend/girlfriend.

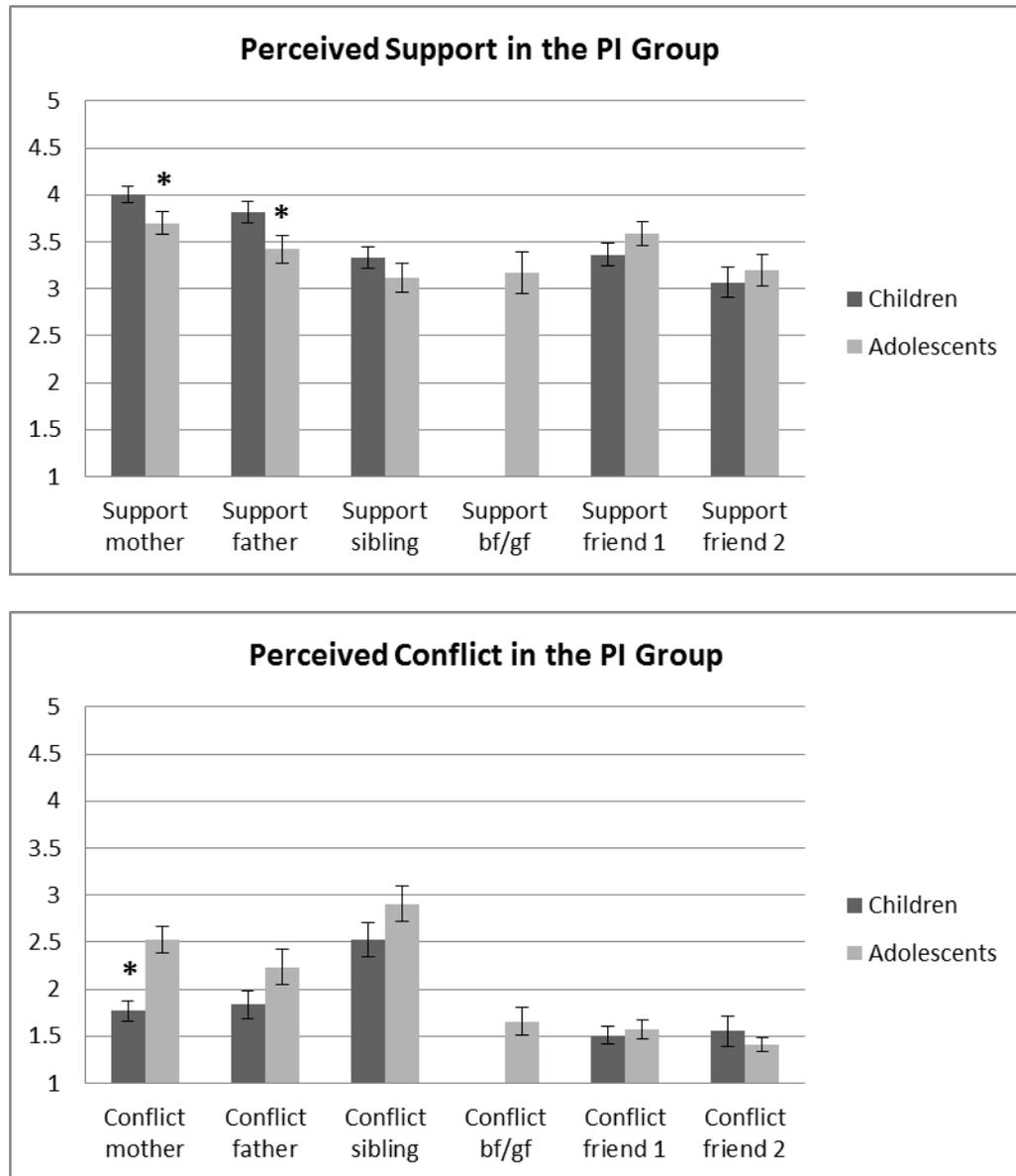
	1	2	3	4	5	6	7	8
1. Conflict mother	1							
2. Conflict father	.70**	1						
3. Conflict sibling	.46**	.45**	1					
4. Conflict boyfriend/girlfriend	-.17	-.05	.09	1				
5. Conflict same-sex friend	.29*	.20	.08	.11	1			
6. Conflict opposite-sex friend	.24	.23	.21	.23	.64**	1		
7. Average family conflict	.79**	.87**	.76**	.06	.22	.32*	1	
8. Average peer conflict	.26*	.19	.12	.70**	.80**	.75**	.24*	1

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

It must also be noted that family support was not significantly related to family conflict in PI children ($r(75) = -.20, p = .09$) and peer support was also not related to peer conflict ($r(74) = -.14, p = .22$).

With respect to developmental differences, PI adolescents reported significantly more conflict with family ($t(77) = 2.92, p = .005$; M teenagers = 2.58, $SD = .87$, M children = 2.05, $SD = .72$) and less support from with their family than children did ($t(77) = -2.13, p = .04$; M teenagers = 3.43, $SD = .75$, M children = 3.75, $SD = .59$). When examining specific relationships (see Figure 8) teenagers reported significantly less support from their mother ($t(75) = -2.03, p = .046$), father ($t(63) = -2.01, p = .049$), and more conflict with their mother ($t(71.9) = 4.26, p < .001$) than children. There were no developmental differences in average perceived peer support ($t(77) = .26, p = .80$), conflict with peers ($t(77) = .28, p = .78$), or measures of support/conflict with specific peers (Figure 8).

Figure 8. Perceived support from and conflict with close family members and peers in the PI group (5-point scales). 9-10-year-olds' ratings of support from boyfriend/girlfriend (N=5) were excluded given insufficient sample size and possibly poor validity. Asterisks mark statistically significant differences. Error bars are SEMs.



We then proceeded to examine hypotheses concerning the extent to which social support and conflict measures predicted stress reactivity or the buffering of stress by social support in the entire sample. All 2-way and 3-way interactions of age, condition,

and sex found to be non-significant in previously reported analyses were removed from the present model to permit sufficient degrees of freedom for testing these hypotheses. Average support from family or from peers and conflict with family or with peers was each introduced in a separate model with its respective interactions with age and condition. There was a significant main effect of family support on the linear and quadratic slopes ($F(1, 187) = 8.25, p = .005$; $F(1, 141) = 8.56, p = .004$) and a significant interaction of family support x age group in predicting the two slopes ($F(1, 189) = 5.18, p = .02$; $F(1, 143) = 7.25, p = .008$). Follow-up analyses within each age group revealed a significant effect of parent support on the cortisol trajectories among children (fixed effect of support on linear slope: $F(1, 90.2) = 4.93, p = .03$; on quadratic slope: $F(1, 68.2) = 5.67, p = .02$; intercepts did not differ by support: $F(1, 34.2) = .71, p = .41$), with parameter estimates shown in Table 10. Among adolescents there was no effect of support (effect of parent support on intercept: $F(1, 34) = .15, p = .70$; on linear slope: $F(1, 95.5) = 0.08, p = .78$; on quadratic slope: $F(1, 73.2) = 0.02, p = .88$; for full list of parameter estimates, see Table 11).

Table 10. Parameter estimates for HLM examining family support and cortisol trajectories among 9-10-year-olds. Cortisol covariates (hours slept, day's distress, and medication code) were included in the model but not shown here for simplicity. $\Delta p < .10$; $*p < .05$; $**p < .01$.

Effect	β	SE	df	T	p
Intercept	1.83	1.01	31.6	1.81	0.08 ^{Δ}
Linear	1.08	0.42	87.9	2.55	0.01*
Quadratic	-0.30	0.13	66.7	-2.3	0.02*

Family Support	0.00	0.13	34.3	0.02	0.98
Linear*Family Support	-0.25	0.11	88.1	-2.26	0.03*
Quadratic*Family Support	0.06	0.03	66.9	1.86	0.07 ^A
Family Support*Condition	-0.16	0.19	34.2	-0.86	0.40
Linear*Family Support*Condition	0.11	0.17	88.3	0.67	0.50
Quadratic*Family Support*Condition	0.00	0.05	67	0.05	0.96
Sex	-0.12	0.09	34.2	-1.31	0.20
Linear*Sex	-0.09	0.08	87.7	-1.1	0.28
Quadratic*Sex	0.03	0.03	67.2	1.01	0.32
Condition	0.65	0.70	34.2	0.93	0.36
Linear*Condition	-0.53	0.63	88.1	-0.84	0.40
Quadratic*Condition	0.02	0.19	67	0.1	0.92

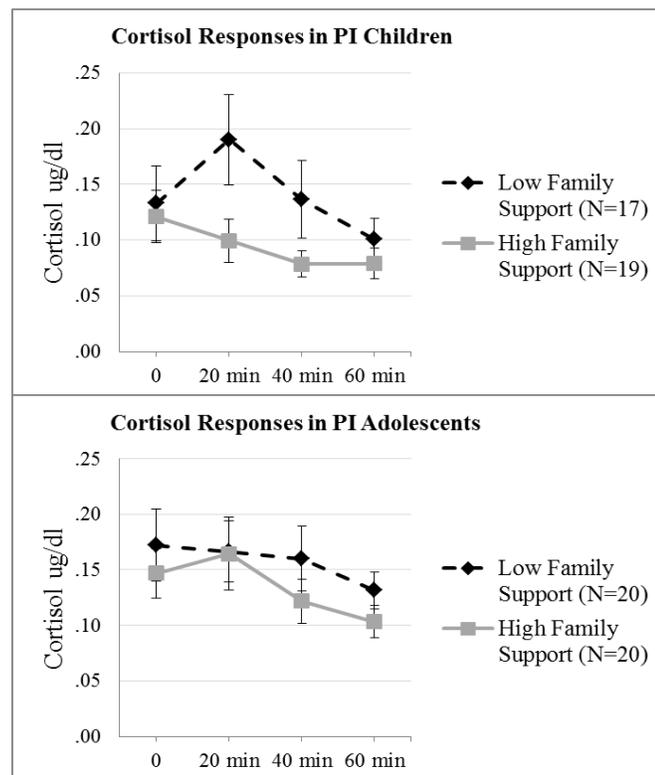
Table 11. Parameter estimates for HLM examining family support and cortisol trajectories among 15-16-year-olds. Cortisol covariates were included in the model but not shown here for simplicity. $\Delta p < .10$; * $p < .05$; ** $p < .01$.

Effect	β	SE	df	T	p
Intercept	1.17	0.39	33.8	3.04	<0.001*
Linear	0.05	0.14	95.3	0.34	0.74
Quadratic	-0.01	0.04	73.1	-0.21	0.84
Family Support	0.01	0.07	33.7	0.11	0.91
Family Support*Linear	-0.01	0.04	95.1	-0.17	0.87
Family Support*Quadratic	0.00	0.01	73	-0.2	0.84
Family Support*Condition	0.03	0.12	33.7	0.26	0.80
Family Support*Condition*Linear	0.00	0.07	95	-0.07	0.94

Family Support*Condition*Quadratic	0.00	0.02	72.9	0.11	0.91
Sex	0.17	0.08	33.7	1.99	0.05 ^Δ
Linear*Sex	-0.02	0.05	94.9	-0.4	0.69
Quadratic*Sex	0.00	0.01	72.9	-0.18	0.86
Condition	-0.06	0.43	33.7	-0.14	0.89
Condition*Linear	0.09	0.24	95	0.39	0.70
Condition*Quadratic	-0.04	0.07	72.9	-0.53	0.60

The pattern of reactivity by age and a median split on family support within each age group conducted for graphing purposes is also displayed below in Figure 9. The figure shows cortisol reactivity collapsed across conditions, given non-significant 3-way interactions of family support, age, and condition (p values of .43, .93, and .65 for the intercept and slopes, respectively).

Figure 9. Cortisol responses in PI children/adolescents by self-reported social support from family (median split used within each age group for graphing purposes).



When examining average support from peers, a similar pattern emerged, with a significant effect of support on the linear and quadratic slopes ($F(1,185) = 4.59, p = .03$; $F(1, 140) = 4.55, p = .035$) and also an interaction of age and peer support in predicting cortisol trajectories (linear slope: $F(1,187) = 3.32, p = .07$; $F(1, 140) = 7.05, p = .01$). Follow-up analyses revealed a comparable pattern to that described for parent support, with lower cortisol reactivity in children with high peer support compared to those with low support and no differences in cortisol reactivity by peer support among adolescents. Given the large number of parameters needed for estimating the effects of peer support and parent support on all elements of cortisol trajectories simultaneously in the same analysis, it was not possible to examine whether these effects of peer support are unique and independent of those due to family support.

None of the parameter estimates for the effects of average conflict with family or conflict with peers on cortisol curve parameters were significant, and these two conflict measures also did not interact with age group or condition in predicting cortisol variance.

Support and conflict from each specific relationship was then entered in a separate model to test if each of them was predictive of cortisol reactivity by itself or in interactions with age, condition, or age x condition. Given the large number of statistical tests using correlated independent variables measuring support or conflict, a family-wise Bonferroni correction was applied and only significant effects with $p < .008$ were considered (given 6 measures of family support or conflict and 6 measures of peer conflict or support). The only specific support measure with significant effects was support from the mother, which interacted with age group to predict cortisol linear ($F(1,174) = 7.93, p = .005$) and quadratic slopes ($F(1,138) = 11.16, p = .001$). Follow-up

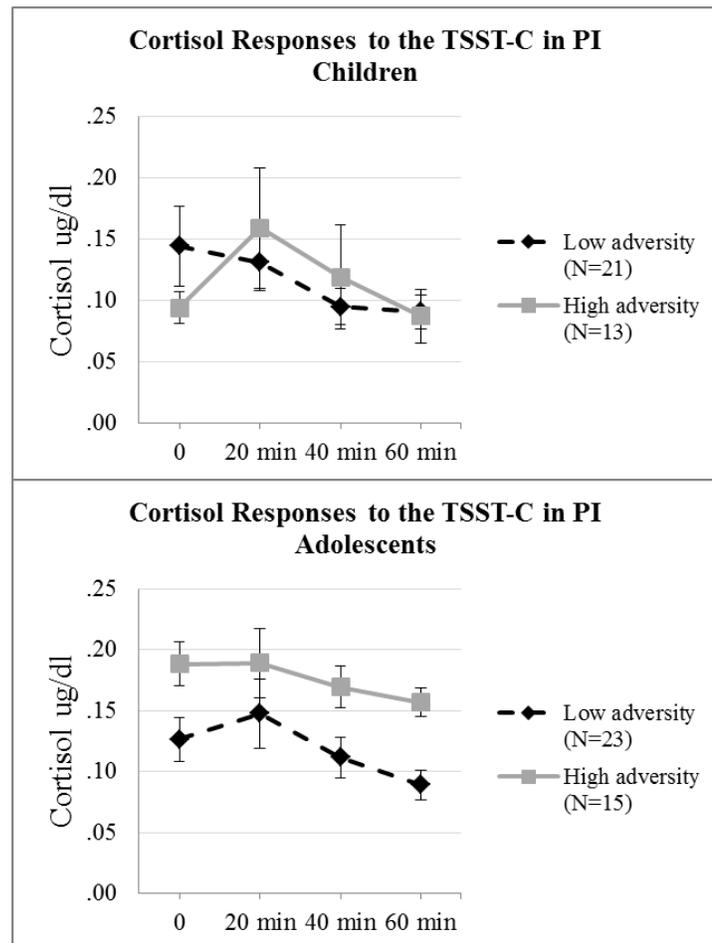
analyses within age group indicated that maternal support significantly lowered cortisol reactivity for 9-10-year-olds (effect on linear slope: $\beta = -.22$, $SE = .08$, $t(81.4) = -2.71$, $p = .008$; on quadratic slope: $\beta = .07$, $SE = .03$, $t(67.3) = 2.84$, $p = .006$), but had no effects on cortisol curve parameters for teenagers (p 's between .32 and .75). Given that mothers were the parent attending the session in the vast majority of cases (85.9%), identical results were obtained when using the support ratings for the parent-attending the session as when mother support was used. None of the conflict measures with specific individuals predicted significant variance in cortisol by itself or in interactions with age, condition, or age x condition.

Pre-adoptive Adversity and Cortisol Reactivity

We examined the role of pre-adoptive factors in explaining cortisol reactivity next. The Adversity Index was entered in models along with its interactions with age, condition, and age x condition. There was a significant interaction of Adversity and age on all curve parameters (intercept: $F(1, 68.3) = 4.21$, $p = .04$; linear: $F(1, 175) = 4.24$, $p = .04$; quadratic: $F(1, 135) = 6.08$, $p = .01$). Adversity alone did not exert main effects (intercept: $F(1, 67.7) = .20$, $p = .66$; linear: $F(1, 176) = 1.56$, $p = .21$; quadratic: $F(1, 135) = 1.43$, $p = .23$), interactions of Adversity x condition on intercepts and linear slopes were not significant ($F(1, 68.7) = .10$, $p = .75$ and $F(1, 175) = 1.59$, $p = .21$) and neither were Adversity x age x condition effects on intercepts and linear slopes ($F(1, 67.7) = .66$, $p = .42$ and $F(1, 175) = 1.41$, $p = .24$); there were, however, significant Adversity x condition and Adversity x age x condition interactions ($F(1, 135) = 3.94$, $p = .049$ and $F(1, 135) = 4.69$, $p = .03$) on the quadratic slope, suggesting different shapes of the curves. We followed up on this latter interaction and the Adversity x age interaction

with analyses within each age group. Among 9-10-year-old PI children, those who had experienced higher adversity had more abrupt positive linear slopes, $\beta = .28$, $SE = .13$, $t(82.2) = 2.25$, $p = .03$, and more negative quadratic terms, $\beta = -.11$, $SE = .04$, $t(63.4) = -2.72$, $p = .008$ (i.e., a more pronounced cortisol response) than those with low adversity, who had a more flat response (see Figure 10). Among teenagers, the effects of Adversity were not statistically significant for intercepts ($\beta = .11$, $SE = .06$, $t(31) = 1.74$, $p = .09$), linear slopes ($\beta = -.03$, $SE = .03$, $t(92.3) = -0.93$, $p = .35$), or quadratic terms ($\beta = .02$, $SE = .009$, $t(68.9) = 1.72$, $p = .09$). The differential effects of pre-adoptive Adversity before and after the pubertal transition are shown below.

Figure 10. Cortisol responses among PI children and adolescents by pre-adoptive adversity. A median split on the Adversity Index was used for graphing purposes only.



Growth stunting (defined as having a height at adoption of at least two standard deviations below the mean for age and gender according to WHO standards) did not significantly predict or interact with age, condition and their interactions in explaining any cortisol curve parameters in the subsample of 53 participants who had this growth variable available (p values between .07 and .98).

Social Network Differences between Adopted and Non-Adopted Groups

Before comparing PI and NA children with respect to their HPA axis activity in order to infer possible enduring effects of early life stress, we examined whether the two groups differed on measures of support from or conflict with either family or peers. Independent samples t -tests revealed that PIs did not differ significantly from NAs in terms of average perceived support from family ($t(158) = .95, p = .34$) or from peers ($t(156) = .96, p = .34$), and also reported no significant differences in average conflict with family ($t(158) = -.24, p = .81$) or with peers ($t(159) = -1.33, p = .19$). PI children also did not differ from NAs on any measures of conflict or support with specific family members or peers (all p values $> .06$).

Early Life Stress and HPA Axis Function

The sample of typically-developing children from Study 1 (see Chapter 1) was added to the present analyses to test differences in cortisol reactivity and social buffering of stress between the post-institutionalized children and the age- and sex-matched comparison group available. Effects of group, age, condition and all their interactions were entered simultaneously in the same hierarchical linear model. Analyses controlled for the main effect of sex on all cortisol parameters, without 2-way or higher-order interactions with sex given the lack of specific hypotheses regarding these interactions

and the need to preserve degrees of freedom for the number of parameters in this analysis. There was a significant group x age x condition interaction on the linear ($F(1, 406) = 6.83, p = .009$) and quadratic slope ($F(1, 307) = 6.14, p = .01$), but not on the intercept ($F(1, 162) = .06, p = .81$). The follow-up analysis within each age group revealed significant group x condition effects on cortisol curves in children (linear slope: $F(1, 199) = 4.93, p = .03$; quadratic slope: $F(1, 150) = 3.54, p = .06$; no differences in intercepts) but there were no group or group x condition effects in adolescents (intercepts: $F(1, 78.8) = .35, p = .55$; linear slope: $F(1, 205) = 1.11, p = .29$; quadratic slope: $F(1, 155) = 1.74, p = .19$), thus we examined group differences under each condition only for children.

In the Stranger condition, there was a significant effect of group for children's linear slope ($F(1, 102) = 8.36, p = .005$) and quadratic slope ($F(1, 73) = 5.96, p = .02$), such that PI children had lower linear slopes and higher quadratic terms (i.e., a flatter trajectory; see Table 12 for estimates and Figure 11 for illustration). In the Parent condition, the effect of group on the three curve parameters was not significant ($F(1, 39) = .16, p = .69$; $F(1, 95.4) = 1.26, p = .27$; $F(1, 74.2) = 1.28, p = .26$; see Table 13 for estimates). This pattern of results adds to previous analyses within each of the groups (Chapter 1 and within-group analyses for the PIs above), which revealed significant effects of condition in the NA group (i.e., a buffering effect) and no condition differences in the PI group (see Figure 11 for a side-by-side comparison of previously shown reactions to the paradigm in NA versus PI children).

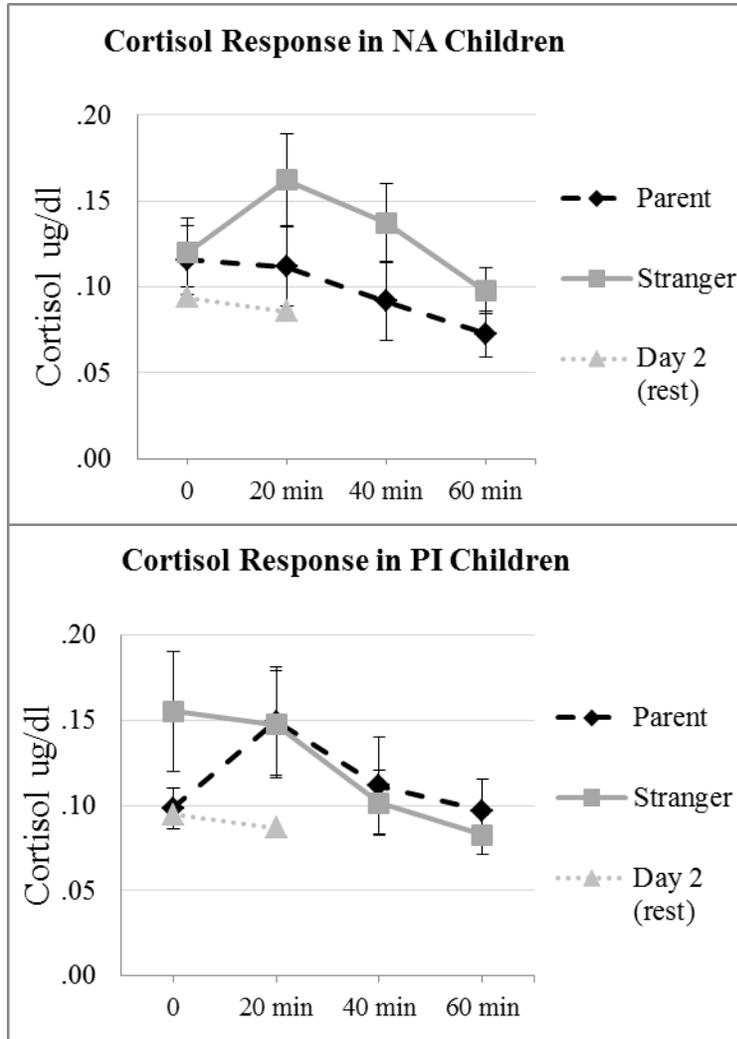
Table 12. HLM parameter estimates for group differences between PIs and NAs in the Stranger condition. Cortisol covariates were included. $\Delta p < .10$; * $p < .05$; ** $p < .01$.

Effect	B	SE	df	T	p
Intercept	2.10	0.63	33.7	3.33	0.00
Linear	0.04	0.08	105	0.58	0.56
Quadratic	-0.05	0.02	74.3	-2.03	0.046*
Group (reference PI)	-0.10	0.11	37.80	-0.9	0.37
Group*Linear	0.23	0.08	102.00	2.89	0.005**
Group*Quadratic	-0.06	0.02	73.00	-2.44	0.02*
Sex (reference females)	-0.10	0.10	37.10	-0.97	0.34
Sex*Linear	-0.08	0.07	101.00	-1.06	0.29
Sex*Quadratic	0.03	0.02	73.00	1.21	0.23

Table 13. HLM parameter estimates for group differences between PI and NA children in the Parent condition. Cortisol covariates were included. $\Delta p < .10$; * $p < .05$; ** $p < .01$.

Effect	B	SE	Df	T	p
Intercept	1.21	0.52	33.9	2.34	0.03*
Linear	0.14	0.07	94.7	1.94	0.06 ^Δ
Quadratic	-0.06	0.02	73.6	-2.79	0.01*
Group (reference PI)	-0.03	0.09	39.00	-0.4	0.69
Group*Linear	-0.09	0.08	95.40	-1.12	0.27
Group*Quadratic	0.03	0.02	74.20	1.13	0.26
Sex (reference females)	-0.06	0.08	39.20	-0.79	0.44
Sex*Linear	-0.14	0.07	94.40	-1.93	0.06 ^Δ
Sex*Quadratic	0.04	0.02	73.50	1.94	0.06 ^Δ

Figure 11. Group differences by experimental condition and childhood rearing experiences among 9-10-year-olds. NA = non-adopted, PI = post-institutionalized.



Discussion

The present study found atypical responses to stress and social buffering during a laboratory stressor in a sample of post-institutionalized, internationally adopted children and adolescents studied before and after the pubertal transition. As predicted, the experimental parent support condition failed to buffer stress responses among 9-10-year-old PI children. This observed pattern is consistent with prior studies showing that 4-5

year old post-institutionalized adopted children did not show the typical lowering of cortisol output or an increase in urinary oxytocin levels after an interaction with their parent in the same way noticed in non-adopted children (Wisner Fries et al., 2005, 2008). The result is also consistent with animal models showing that maternal deprivation in early life impairs the social-buffering of stress later in development (Winslow et al., 2003). Among teenagers, there was also no difference between the Parent and Stranger support conditions, however teenagers exhibited an atypical pattern of cortisol responses, with an earlier elevation in cortisol than what is typically observed in the TSST-C, likely indicative of anticipatory stress responses. The same anticipatory response was shown by 9-10-year-olds in the Stranger condition. Leaving the parent and walking with the stranger to a new room to prepare their speech may have been a powerful enough stressor for these children, a pattern consistent with the separation anxiety problems that are sometimes noted in this population. The lack of a buffering effect in the parent condition may be due to an absence of discriminate early attachments to parents and due to the experience of frequent caregiver turnover often encountered in institutions (Zeanah et al., 2002), which may have shaped neurobehavioral development in ways that prevent the manifestation of a social buffering effect by parent support. Furthermore, these experiences may reduce selective responses to parents versus strangers, given the lack of a clear discrimination between the two experimental conditions in teenagers and very minor differences in the timing but not the magnitude of peak reactivity in the two conditions among 9-10-year-olds.

However, these conclusions must be tempered by the fact that 9-10-year-olds who reported higher levels of support from family, particularly from their mothers (who were

the parent attending the session in the vast majority of cases), exhibited a lowered cortisol response to the TSST-C akin to an ongoing social buffering effect, whereas those with lower levels of support had robust cortisol responses. These differences were not observed in teenagers. The finding was independent of our experimental manipulation, suggesting that the child's overall perceptions of support from their mothers may be more important than receiving support immediately before a stressor. Given that this is a correlational finding, the result could also be due to an unobserved third variable that might both lower stress responses and allow children to elicit more supportive parenting. The same pattern was observed with respect to average perceived support from peers. It may be that a subsample of PI children that are very well-adjusted and receive high levels of both peer and maternal support mounted much lower cortisol responses to the TSST-C. However, these findings would need to be replicated before extrapolating the results. We must also remember that the same associations were not observed in PI teenagers, who did not differ in levels of peer support from NA children, but only differed in self-reported parent support.

When comparing PI and NA children on measures of perceived support and conflict, PI children did not differ significantly from NAs on average levels of support or conflict or on the ratings they provided for specific relationships, contrary to our hypotheses that they would report more difficulties with peers. Furthermore, PIs showed the expected developmental patterns of reporting higher levels of conflict and decreased support from parents in adolescence, which has been explained in terms of increased psychological distancing from parents with pubertal maturation (Hill et al., 1985a, b; Steinberg, 1987, 1988).

Similar to previously reported results in typically-developing children and adolescents, in the PI group observational measures of parental positivity and negativity during speech preparation in the laboratory did not explain any variance in our stress reactivity outcomes, which is hypothesized to occur due to the fact that relationship histories may matter more than momentary behaviors (see Chapter 1), or due to the small sample size since this question was only examined in the Parent support condition.

Another important point is that PI teenagers were more likely to show anticipatory stress responses than children, which may be explained by a study with a typically-developing sample which found that individuals who were further along in their pubertal development (late or post-pubertal) had greater anticipatory responses (defined as the difference between pre-speech level and recovery level) compared to early- and mid-pubertal participants (Sumter et al., 2010). This pattern of anticipatory responses may not be abnormal, but it may instead signify an accelerated maturation of the HPA axis with puberty signals such that even though PIs and NAs were age-matched and had comparable puberty stages, the PI HPA response looked more similar to that of post-pubertal teenagers from the study by Sumter and colleagues. Future studies should utilize clinical inventories to document anxiety levels, as it is also possible that PIs may exhibit excessive worry, rumination and anxiety that manifests as a tendency towards anticipatory responses rather than cortisol peaks that occur later and track better with the public speaking task. These problems with enhanced anxiety would be consistent with animal models showing enhanced fearfulness with the experience of chronic early life stress (Caldji et al., 1998; Francis et al., 1999; Sanchez et al., 2001).

There were also significant age-group differences in stress reactivity in the PI group that were evident in the Stranger support condition such that teenagers exhibited a slower recovery in cortisol levels compared to children, whose return to baseline had a steeper slope. Future studies should examine the cumulative implications of this slower rate of cortisol recovery on adolescent health outcomes. The only sex differences observed were that teenage males had higher cortisol intercepts than females, but there were no differences in reactivity by sex in the entire sample or within each of the age groups, which is consistent with some studies finding no sex differences in stress reactivity post-puberty (Gunnar et al., 2009; Kudielka et al., 2004; Stroud et al., 2009; Sumter et al., 2010).

It must be noted that all the age and group differences reported above were not observed when using subjective ratings of stress using a paper-and-pencil measure, highlighting the continued importance of incorporating endocrine measures in future work in this domain.

When comparing the PI group with the non-adopted group, the previous interpretations hold in that PI children failed to show a buffered effect in the Parent condition (higher reactivity than NAs), whereas in the Stranger condition NAs exhibited a robust cortisol response that is typically observed with the TSST and PIs showed a more atypical anticipatory response in the Stranger condition for both younger and older participants. Cortisol trajectories of PI adolescents did not differ, on average, from NA adolescents, despite visual indications of different trajectories (more robust and typical cortisol peaks in NAs and a tendency towards anticipatory responses in PIs), however these differences were not statistically significant when examining cortisol dynamics.

This was likely due to the great heterogeneity among PIs, which increased estimated variances and obfuscated differences in means that might otherwise be apparent.

Given this great heterogeneity among the PI children, we examined adversity indices including but not limited to growth stunting to explain some of the variability. The associations noted between HPA axis reactivity and the Adversity Index are consistent with tentative explanations provided thus far. Among 9-10-year-olds, higher adversity correlated with a more robust cortisol response to the TSST-C, whereas children who experienced lower adversity had declining concentrations of salivary cortisol indicative of a diminished or absent acute HPA stress response. Combining several of our findings, a pattern emerged such that children who experienced low adversity or had high levels of parent or peer support tended to show diminished stress responses to our laboratory stressor. Previous studies have shown negative associations between the degree of pre-adoptive adversity experienced and the parenting quality elicited from adoptive parents, with parenting quality being slightly lower in children with more severe impairments and parenting improving over time as children catch up post-adoption (Croft et al., 2001). This indicates that children with multiple symptoms and impairments are understandably more difficult to parent. Thus, our findings may suggest the possibility that children who had experienced the least amount of adversity and possibly elicit more parent support also exhibit lower stress responses, without necessarily showing an interaction with our experimental condition or a buffering effect in the laboratory. In other words, this may be a more stable trait of low reactivity that is correlated with lower adversity and higher perceptions of parent support rather than an

active process driven by parent support at the time of the experimental stressor, but obviously the two possibilities cannot be teased apart with our study.

Lastly, growth stunting did not predict cortisol reactivity or stress-buffering in our sample, as has been found in other studies of post-institutionalized children (Kertes et al., 2008; Gunnar et al., 2009). It is possible that the present sample was less stunted at adoption than participants in previous studies. Indeed, the mean standardized height-for-age score in our sample was -1.38 with a standard deviation of 1.35, whereas Gunnar et al. (2009) for instance reported a mean of -2.1 with a standard deviation of 1.9. Furthermore, the sample size for these analyses was substantially reduced due to the fact that we studied older children and adolescents, thus the ability to access older medical records was greatly diminished. Future studies should re-examine this question to strengthen confidence in previously reported associations with growth stunting.

To summarize, the present study found support for the prediction that the stress-buffering effect of parent support would be reduced among PI children given their history of early social deprivation. However, this conclusion needs to be tempered by our findings that perceived total support from family may still be correlated with lowered acute HPA responses in the laboratory. Among teenagers, perceived or laboratory-provided support did not seem to make a difference and anticipatory stress responses seemed to be the norm. The atypical patterns of stress responsivity in post-institutionalized teenagers did not support the pubertal recalibration hypothesis. It is possible that the basal activity of the HPA axis normalizes post-puberty (e.g., the findings on the cortisol awakening response by Quevedo et al., 2012), whereas reactivity to a social stress test and the social buffering of stress may still bear the sequelae of severe

early-life social deprivation. Future studies should examine behavioral and clinical correlates of these anticipatory responses to ascertain whether they are problematic or not. The correlations obtained between high levels of pre-adoptive adversity and these profiles of earlier elevations in cortisol levels suggest they may be problematic, but more research is needed to understand late/post-pubertal PI adolescents.

The present study had a number of strengths and several limitations. The study of internationally-adopted children has the advantage of shedding light on the effects of circumscribed periods of early adversity, while at the same time examining the possibility of recovery with an improved social context. The use of an experimental stressor and a laboratory paradigm for eliciting parent support allowed us to show a more complex pattern of results than if we had used a questionnaire measure of parent support alone, as was evident here. The study also had several limitations that are typical of work with this population, namely that a) it is impossible to differentiate prenatal from postnatal stress, however associations with levels of pre-adoptive adversity experienced were analyzed in an attempt to show that outcomes follow a gradient of presumed severity of post-natal stress; b) nevertheless, we must acknowledge that parent reports of the quality of institutional care are often subjective or incomplete based on brief visits or adoption agency records that are not always accurate and are rarely standardized across informants; c) the study was cross-sectional, thus it is possible that adolescents adopted 13-14 years before the study inception may have differed from children adopted 7-8 years before in ways that are not related to pubertal or chronological maturation, thus a longitudinal study is needed to tease these effects apart; and lastly, d) we noted that more than 30% of PI participants (compared to 3.7% of NA subjects) were taking medications

that have the potential to influence the HPA axis (the majority of which were psychotropic medications), thus even though the effect of these medications was controlled for statistically in all analyses it cannot be completely ruled out that some of the differences between the PI and the NA group are due to complex nonlinear effects of medications on cortisol measures that were not fully accounted for in our linear mixed models. It would be important for subsequent research to use drug washout procedures to study HPA axis reactivity when participants are not taking these medications.

Future studies should continue to explore the question of how early life stress shapes brain and behavioral development in ways that promote vulnerability or resilience (Karatoreos & McEwen, 2013). There are important societal and public policy implications to studying the ramifications of early life stress with the goal of ultimately promoting stress resilience through well-designed interventions (Gunnar, Fisher, & The Early Experience, Stress, and Prevention Network, 2006). It is only by understanding individual differences in outcomes and how they interact with normative developmental stages and possible sensitive periods that we will be able to design the most efficacious interventions and deploy them at the most beneficial developmental timing.

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