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Sociodemographic Risk, Parenting, and Effortful Control: Relations to Salivary Alpha-amylase and Cortisol in Early Childhood

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Abstract

Early sociodemographic risk, parenting, and temperament were examined as predictors of the activity of children's (N = 148; 81 boys, 67 girls) hypothalamic-pituitary-adrenal axis and autonomic nervous system. Demographic risk was assessed at 18 months (T1), intrusive-overcontrolling parenting and effortful control were assessed at 30 months (T2), and salivary cortisol and alpha-amylase were collected at 72 (T3) months of age. Demographic risk at T1 predicted lower levels of children's effortful control and higher levels of mothers' intrusive-overcontrolling parenting at T2. Intrusive-overcontrolling parenting at T2 predicted higher levels of children's cortisol and alpha-amylase at T3, but effortful control did not uniquely predict children's cortisol or alpha-amylase. Findings support the open nature of stress responsive physiological systems to influence by features of the early caregiving environment and underscore the utility of including measures of these systems in prevention trials designed to influence child outcomes by modifying parenting behavior.

Keywords

cortisol; salivary alpha-amylase; intrusive-overcontrolling parenting; demographic risk; effortful control; early childhood

Developmental scientists are increasingly incorporating individual differences in the psychobiology of the stress response into conceptual models in an attempt to advance our understanding of how stress-related vulnerability moderates the effects of emotion regulation on children's social functioning (Bauer, Quas, & Boyce, 2002; Eisenberg et al., 2004; Granger, Kivlighan, el-Sheikh, Gordis, & Stroud, 2007; Kirschbaum & Hellhammer, 1992; Spinrad et al., 2007). The psychobiology of stress involves two main physiological components: the hypothalamic-pituitary-adrenocortical (HPA) axis and the autonomic nervous system (ANS) (e.g., Chrousos & Gold, 1992). The timing, duration, and intensity of ANS and HPA axis responses to stress are distinct (Engert, et al., 2011; Granger et al., 2007). Cortisol, the primary product of HPA axis activation, and alpha-amylase (sAA) a

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surrogate marker of ANS activation, can be non-invasively measured in saliva. Levels of salivary cortisol and sAA show a characteristic increase and decrease in response to physical and social psychological challenges (see Nater et al., 2009 for review). Numerous studies have employed salivary cortisol and sAA to test hypotheses regarding how stress-related reactivity of the HPA axis and ANS interact to influence individual differences in behavior (Gordis et al., 2006; Keller, El-Sheikh, Granger, & Buckhalt, 2012; El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008; Kilvighan & Granger, 2006).

In studies to date, researchers often have measured physiological responses using state-variance—momentary fluctuations in levels of activity across a controlled environmental event. Recently, however, several researchers have employed models that examine the correlates and concomitants of *stability* in the activity of physiological systems within individuals across multiple measures or time points (for review, see Granger et al., 2012). Such an approach assumes that a component part of the variability in physiological measures at any moment in time is due to stable "trait-like" intrinsic individual characteristics. Trait-like levels of physiological parameters, such as cortisol and salivary alpha-amylase (sAA), can be modeled at a latent level when multiple measurements are obtained at the manifest level. Support for modeling physiological variables at a trait-like level has been demonstrated using cortisol (Alink, Cicchetti, Kim, & Rogosch, 2012; Blair et al., 2011; Booth, Granger, & Shirtcliff, 2008; El-Sheikh, et al., 2008), as well as sAA (El-Sheikh, et al., 2008; Granger et al., 2006; Wolf et al., 2008, Out, Bakermans-Kranenburg, Granger, Cobbaert, & van IJzendoorn, 2011). Overall, the above studies have demonstrated that both cortisol and sAA are fairly stable across time and that part of the variance in these physiological markers is due to stable, heritable individual differences. However, there remain significant gaps in our understanding of the predictors of individual differences in trait-like variability in sAA and cortisol in early childhood.

In the present study, we address this knowledge gap by examining possible precursors to children's physiological systems using trait-like measures of both cortisol and sAA (henceforth called cortisol or sAA level). There are clear individual differences in cortisol levels, and these differences have important developmental concomitants (Granger, Weisz, & Kauneckis, 1994; Gunnar, Sebanc, Tout, Donzella, & van Dulmen, 2003). For example, temperament, psychopathology, previous experiences, and perceived control have been found to predict HPA responses (for a review, see Granger et al., 2007). Data from both human and animal studies have demonstrated that characteristics of the social environment (e.g., quality and stability of social relationships, and one's relative status within the environment) are associated with neuroendocrine activity and responses to stimuli (Czoty, Gould, & Nader, 2009; McCormack, Newman, Higley, Maestripieri, & Sanchez, 2009; Seeman & McEwen, 1996). Similarly, individual differences in sAA levels have been linked to problem behavior, intense affective states, executive function, academic performance, and social perception and relationships (Adam, Till Hoyt, & Granger, 2011; Berry, Blair, Willoughby, & Granger, 2012; Keller, El-Sheikh, Granger, & Buckhalt, 2012; Kidd et al., in press; Kreher, Powers, & Granger, 2012; Laurent, Ablow, & Measelle, 2011). Therefore, examining factors that contribute to children's physiological activity is important given their relations to children's development and adjustment. Given that children who experience high levels of cortisol and sAA have been found to be at risk for adjustment problems, it is critical to understand factors that are associated with children's physiological activity.

Early Experiences and Children's Stress Markers

Early sociodemographic adversity and family experiences are two major environmental factors associated with individual differences in reactivity and regulation of children's responses to stress, threat, and challenge (Cicchetti & Rogosch, 2001; Gunnar & Cheatham,

2003; Gunnar & Donzella, 2002). It is evident that the family acts as a conduit for socioeconomic influences on the development of children (Conger, Conger, & Martin, 2010) and demographic risk factors such as families' low income, low education, large family size, and young age of parents are consistently associated with detrimental parenting behaviors and maladjustment of children (Kochanska, Aksan, Penney, & Boldt, 2007; Popp, Spinrad, & Smith, 2008). Children from low SES families are more likely to be in family environments that lack warmth and support and have a higher risk of experiencing mistreatment or abuse (Cicchetti & Rogosch, 2001; McLoyd, 1998). Moreover, economic stressors are associated with higher levels of hostile and coercive parenting (Conger, Ge, Elder, Lorenz, & Simons, 1994). In turn, children living in families with parents who are hostile, unsupportive, and neglectful have a higher risk of poor adjustment across a wide variety of developmental domains (for a review, see Repetti, et al., 2002).

Developmental scientists are increasingly assessing the relations between the family environment and children's physiological functioning. To our knowledge, there are no studies that have examined the relations of parenting behaviors to levels of children's sAA. However, early adversity and negative family experiences have been related to children's andrenocortical responding (e.g. Alink, et al., 2012; Cicchetti & Rogosch, 2001; Gunnar & Cheatham, 2003; Gunnar & Donzella, 2002; Repetti et al., 2002), particularly in high-risk samples, such as children who have been maltreated or abandoned (e.g. Alink et al., 2012; Gunnar, Morison, Chisholm, & Schuder, 2001; Fisher, Gunnar, Chamberlain, & Reid, 2000; Flinn & England, 1995). Other researchers have examined the relations of parenting to cortisol functioning in low-risk samples (Blair et al., 2008; Blair et al., 2011; Mills-Koonce, Garrett-Peters, Barnett, Granger, Blair, & Cox, 2011). Investigators have found that parents who use punishment, such as spanking or slapping, have children with elevated cortisol levels after stressful situations (Bugental, Martorell, & Barraza, 2003; Hastings, Ruttle, Serbin, Mills, Rosemary, Stack, & Schwartzman, 2011). In contrast, researchers have found that warm, sensitive, and responsive parenting is associated with relatively low levels of children's stress reactivity (for a review, see Loman & Gunnar, 2010). For example, Blair and colleagues (2008) reported a negative association between maternal engagement and the toddlers' cortisol levels, although they did not find a significant relation between maternal intrusiveness and children's cortisol levels with both types of parenting behaviors included in the analysis.

Researchers have found that children who live in poorer socioeconomic environments have higher cortisol levels (Chen, Cohen, & Miller, 2010). However, it is important to note that children exposed to high levels of stressors or prolonged stress also have been found to have hypocortisolism, which is characterized by low cortisol, flat daytime production patterns, and blunted responses to stressors (Gunnar & Vazquez, 2001). Investigators have hypothesized that frequent early adversity could lead to down-regulation of the HPA system that results in low rather than high levels of cortisol later in life (Gunnar & Vazquez, 2001). Supporting this theory, Alink et al. (2012) found that maltreated children were more likely to have low trait levels of morning cortisol. Thus, relations between environmental conditions and cortisol responding are still not fully understood and likely take time to emerge.

Effortful Control and Children's Physiological Reactions

Temperamental characteristics have also been increasingly linked to physiological functioning. An aspect of temperament that has been associated with both parenting quality and biological reactions is effortful control, which is believed to reflect individual differences in self-regulation (Rothbart & Bates, 2006). Effortful control is defined as "the efficiency of executive attention, including the ability to inhibit a dominant response and/or

to activate a subdominant response, to plan, and to detect errors" (Rothbart & Bates, 2006, p. 129). It includes the abilities to voluntarily or willfully focus and shift attention and inhibit or initiate behaviors, processes that likely contribute to the modulation of emotional experience and emotion-associated behavior (Rothbart, Ziaie, & O'Boyle, 1992).

In general, low-quality parenting has been negatively associated with children's effortful control during early childhood. For example, Graziano, Keane, and Calkins (2010) found that maternal overcontrol and intrusiveness at age two negatively predicted children's effortful control over three years later. In studies with preschoolers, investigators have additionally found that mothers' unsupportive (i.e., minimizing or punitive) responses to negative emotions were associated with low levels of effortful control (Eisenberg & Fabes, 1994; Eisenberg, Spinrad, & Eggum 2010; Spinrad et al., 2007). When mothers are unresponsive, punitive, or insensitive to their child, children may experience heightened arousal that can disrupt their ability to self-regulate (Eisenberg, et al., 1998). Furthermore, mothers who are disapproving or hostile may model dysregulation, whereas more positive, supportive, and sensitive mothers likely model constructive ways to manage stress and relationships (Eisenberg, Cumberland, & Spinrad, 2010).

Moreover, individual differences in effortful control appear to be negatively associated with children's physiological traits (Davis, Bruce, & Gunnar, 2002; Fortunato et al., 2008; Watamura, Donzella, Kertes, & Gunnar, 2004). Laurent and colleagues (2011) found that effortful control was concurrently related to lower levels of sAA levels, although was not significantly associated with cortisol levels, in 18-month-old infants. However, Gunnar and colleagues (2003) found that poorly controlled preschoolers had lower levels of baseline cortisol, whereas over-controlled, shy, and less impulsive children tended to have higher cortisol levels. Studies are also mixed in regard to relations between effortful control and physiological reactivity. For example, Blair and colleagues (2008) found that toddlers with higher levels of attention and cognitive development were less physiologically reactive during a stressful task. However, Spinrad and colleagues (2009) found positive relations between effortful control and cortisol and sAA reactivity in preschoolers when they were treated unfairly during a game.

The Present Study

We investigated the longitudinal relations of demographic risk measured at 18 months to intrusive-overcontrolling parenting and effortful control measured at 30 months and relations of parenting and effortful control to trait-like levels of sAA and cortisol levels at 72 months. We expected that demographic risk would negatively predict children's effortful control and positively predict intrusive-overcontrolling parenting; in turn, we expected EC and intrusive-overcontrolling parenting to negatively predict levels of sAA and cortisol.

A primary focus of the study was to examine whether these relations were significant across time and whether our predictions applied to both the hypothalamic-pituitary-adrenal and the autonomic nervous system. We tested these predictions using structural equation modeling in order to test the unique relations of both child and parent characteristics to levels of sAA and cortisol. Few researchers have examined children's physiological characteristics using measures of multiple physiological systems (for exceptions, see Laurent, et al., 2011; Spinrad et al., 2009), although examining both cortisol and sAA would allow a better understanding of multiple facets of children's physiological responses (Bauer et al., 2002). Finally, the longitudinal nature of the study allows researchers to understand how both social and temperamental factors predict components of the stress response across development and to determine whether regulation-related abilities that are maturing in

young children, such as effortful control, might be a focus for intervention (Granger et al., 2012).

Method

Participants

Participants were preschool-aged children residing in a large city who were part of a longitudinal study of children's social and emotional development (blinded for review). Initially, families ($N = 256$) were recruited through three local hospitals following the birth of the target child. The current study consisted of a subsample of 148 children who completed saliva collection at the 72-month assessment (81 boys, 67 girls) and whose physiological values did not include outliers ($n = 11$). Outlying values (above three standard deviations) for cortisol and sAA were removed from the data set. Four children had extreme values for cortisol, and seven children had outlying values for sAA. Data on non-physiological variables from the 18- and 30-month assessments (T1 and T2, respectively) were also used.

The majority of the children in the present sample were White, non-Hispanic (70.1%), with others identified as Hispanic (15.7%), African American (5.8%), Native American (4.7%), Asian (2.3%), or other (1.7%). Family income ranged from less than $15,000 to over $100,000 (median income ranged from $60,000 to $75,000). At T1, the majority of parents were married (86.5%), and those parents were married an average of 4.21 years ($SD = 3.96$).

Individuals with physiological data at 72 months ($n = 148$) were compared with the individuals from the full sample at 18 months who did not participate at 72 months ($n = 97$) using *t*-tests. We examined differences on demographic variables (household income, mother and father education level, mother and fathers age at child's birth, and marital status), as well as our study variables. Families that were lost because of attrition were younger in age at child's birth ($t = -2.70$, $df = 347$, $p > .01$, $M = -1.96$, $SE = .73$ for mothers; $t = -3.24$, $df = 327$, $p > .01$, $M = -2.45$, $SE = .76$ for fathers) and were married for fewer years ($t = -3.31$, $df = 350$, $p > .01$, $M = -1.74$, $SE = .53$). There were no group differences between on any of the study variables (demographic risk, intrusive-overcontrolling parenting, and effortful control).

Procedures

At the 18-month (T1) and 30-month (T2) assessments, mothers accompanied their child to laboratory visits. For the 72-month (T3) assessment, research assistants usually conducted the assessment in the participants' homes. Laboratory sessions were similarly structured at all assessments and lasted approximately 1.5 to 2 hr. At these visits mothers and children participated in a series of tasks, during which various measures of socioemotional development and mother-child interactions were observed. Questionnaires were also sent to the mothers when children were 18, 30, and 72 months old and were returned by mail, brought back to the laboratory, or collected at the home visit. Parents reported on multiple characteristics of their children's behavior, as well as on some of their own characteristics and demographic information (e.g., racial identity, ethnicity, and family income level). Primary caregivers (nearly always mothers) also provided the contact information for an additional non-parental caregiver who knew the child well (e.g., babysitter, preschool teacher) and their consent to contact the non-parental caregiver. The non-parental caregivers were contacted by telephone. If the non-parental caregivers agreed to participate, questionnaires and an informed consent form were mailed to them. Caregiver questionnaires were available for 112 children at T2.

Saliva samples were taken at 72 months of age during the T3 visit. Children participated in a task designed to elicit frustration (a modified not-sharing task; Goldsmith, Reilly, Lemery, Longley, & Prescott, 1999). In this task, children played a card game with an experimenter that involved candy being unfairly divided based on what cards they received. Saliva samples were collected prior to the not-sharing task, and at 10 and 20 minutes post-task; these samples were later assayed for sAA and cortisol.

Measures

Family demographic risk index—Family demographic risk (Kochanska, et al., 2007) was calculated from demographic information provided by mothers at 18 months. The demographic risk index by assigning graded 'risk points' for parents' education level and age, family income, and number of children. The risk points were calculated in the following manner: *parental education* (for each parent) 3 = did not complete high school, 2 = completed high school, 1 = some college or associates degree, and 0 = completed college or beyond; *parental age* (for each parent) 3 = younger than 20, 2 = 19 or 20, 1 = 22 or 23, and 0 = 24 or older; family annual income 3 = less than $30,000, 2 = $30–45, 000, 1 = $45–60,000, and 0 = greater than $60,000; *number of children* 3 = five children, 2 = four children, 1 = three children, 0 = one or two children. For each family these scores were then summed into the score of demographic risk, and the mean score was calculated resulting in a range from 0 to 3 (mean = .62, *SD* = .57).

Intrusive-overcontrolling parenting—The latent variable for *intrusive-overcontrolling* parenting consisted of observed behaviors during three mother-toddler interaction tasks at 30 months. The first indicator consisted of mothers' intrusiveness during a free-play interaction. Mothers were presented with a basket of toys and asked to play as they normally would at home for 3 min. Mothers were rated for intrusiveness on a 4-point scale every 15 s: 1 = no intrusive behavior, 2 = minimal intrusiveness, 3 = moderate intrusiveness, 4 = high intrusiveness. Examples of intrusive behavior included offering a continuous barrage of stimulation or toys, not allowing the infant to influence the pace or focus of play, or pulling the child's hands off objects he or she is holding (Fish, Stifter, & Belsky, 1993). Interrater reliability (intraclass correlation coefficients [ICCs]), assessed for approximately 30% percent of the sample, was .86.

The second indicator consisted of mothers' *overcontrolling/intrusive* behavior during a three-minute teaching paradigm task (adapted from Calkins & Johnson, 1998) in which mothers and toddlers were presented with a difficult puzzle (pegs/geometric shapes). Mothers were instructed to teach their child to complete the puzzle with whatever strategies they would use at home. Mothers were rated for intrusiveness on a 4-point scale every 30 s (1 = no intrusive behavior, 2 = minimal intrusiveness, 3 = moderate intrusiveness, 4 = high intrusiveness). Mothers' overcontrolling, intrusive behaviors included overstimulating the child with toys, employing intrusive physical interactions, or intervening to help the child when not required (ICC = .71 for 23% percent of the sample).

The third indicator consisted of mothers' *controlling* behavior during a clean-up task (Kochanska, Coy, & Murray 2001). Mothers were told through headphones to have their children pick up the toys as if they were at home. The interaction was videotaped until the clean-up was finished or 3 min had passed (whichever came first). Mothers' controlling behavior (such as firmly holding the child, moving the child decisively, removing the toys from the child's hand) was observed. Maternal control was rated every 15 s as either present or absent (1 = yes; 0 = no; ICC = .82 using approximately 26% percent of the sample).

Children's effortful control—The latent variable for effortful control consisted of three indicators obtained at 30 months. The first indicator was a composite of mother and non-parental caregiver ratings of toddlers' EC using three subscales from the Early Childhood Behavior Questionnaire (Putnam, Gartstein, & Rothbart 2006): (a) attention-focusing—the ability to concentrate on a task (alpha = .82 for mothers, .84 for caregivers, and .81 combined); (b) attention shifting—the ability to move attention from one activity to another (alpha = .88 for mothers, .89 for caregivers, and .79 combined); and (c) inhibitory control—the ability to voluntarily control behavior (alpha = .71 for mothers, .68 for caregivers; and .69 combined). Each 12-item scale ranged from 1 = never to 7 = always. Reports were averaged across scales and across reporters to form the first indicator.

The second indicator consisted of an observed measure of a snack delay task coded from videotapes (Kochanska, Murray, & Harlan, 2000). In this task, children were asked to wait until a bell was rung to get a piece of candy from under a clear cup during four trials (with delays of 10, 20, 30, and then 15 seconds). The time that children waited to eat the candy on each trial was calculated. Scores were then averaged across trials.

The final indicator consisted of an adapted version of the Infant Behavior Record (IBR; Popp, et al., 2008) that captures children's behaviors across the entire lab visit. At the end of each lab visit, four researchers rated the child's attention and persistence during the laboratory tasks that included several episodes designed to elicit negative emotion and regulation. Attention and persistence items (1 item each) were rated on a five-point scale (1 = consistently off task or lacks persistence to 5 = continued absorption in toy/activity/person or consistently persistent; attentive) and scores were averaged across items and then reporters (average agreement intraclass correlations [ICCs] = .85).

Cortisol and sAA—Saliva samples were collected at 72 months prior to the not-sharing task, 10 minutes after the task, and 20 minutes after the task. However, although the task was designed to elicit frustration, it did not produce the predicted pattern of reactivity (an increase in either sAA or cortisol and then a decline) across the three samples (see means, Table 1). Moreover, although behavioral indices of negative emotion were coded, there were no significant changes in emotion across the task, providing further evidence that the task did not elicit emotional reactivity. Given the lack of physiological reactivity to the task, and because cortisol and sAA were not significantly correlated, measuring cortisol and sAA separately at a trait-like level was most appropriate. Thus, modeling was employed with the three sample collections as indicators (see Booth, et al., 2008; Out et al., 2011) and a trait-like measure of these variables was computed at a latent level.

Following Granger and colleagues (2007), samples were assayed for alpha-amylase using a commercially available kinetic reaction assay (Salimetrics, State College, PA). The assay employed a chromagenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose. The enzymatic action of alpha-amylase on this substrate yielded 2-chloro-p-nitrophenol, which was spectrophotometrically measured at 405 nm using a standard laboratory plate reader. The amount of α-amylase activity present in the sample is directly proportional to the increase (over a 2 minute period) in absorbance at 405 nm. All samples were assayed for salivary cortisol by enzyme immunoassay (Salimetrics, State College, PA). This test required 25 μl of saliva that has a range of sensitivity from .007 to 3.0 μg/dL, and average intra- and inter-assay coefficients of variation 5% and 10% respectively. Detailed saliva collection procedures are described in prior work (deleted for blind review).

Control variables—We included two control variables in the analyses: child sex (0 = boys, 1 = girls), and time of day that salivary samples were taken. Time of day was scored on a range of 1 to 5 with 1 = 8 am to 10 am, 2 = 10 am to 12 pm, 3 = 12 pm to 2 pm, 4 = 2

pm to 4 pm, and 5 = 4 pm to 6 pm (M = 3.01, SD = 1.43) (Kirschbaum & Hellhammer, 1989).

Analysis Strategy

Statistical models were fit to data using the Mplus program (Version 6; Muthén & Muthén, 2007) with full information maximum likelihood (FIML). FIML estimation has been found to be efficient and unbiased when data are missing at random and appears to be less biased than standard approaches (Arbuckle, 1996). To evaluate fit of a structural model to data, we used the standard chi-square index of statistical fit that is routinely provided under maximum likelihood estimation of parameters as well as other indices of practical fit, including the Root Mean Square Error of Approximation (RMSEA), the Tucker-Lewis index (TLI), and the comparative fit index (CFI). The RMSEA is an absolute index of fit, with values below .05 indicating close fit to the data. For both the TLI and CFI, fit index values should be greater than .90, and preferably greater than .95, to consider the fit of a model to data to be acceptable (Browne & Cudeck, 1993). Given our small sample size and fairly complex model, we ran two separate analyses—the first predicting cortisol level and the second predicting sAA level.[i]

Results

Descriptive Analyses

In a preliminary step, we examined zero-order correlations among variable indicators as well as among latent variables. Correlations among indicators from the variables were largely as expected (Table 1). For example, all indicators of intrusive-overcontrolling parenting were positively correlated with demographic risk. Correlations among latent variables also were as expected (Table 2).

Structural Equation Models

We next ran separate measurement models for both cortisol and alpha amylase (with three indicators for the three assessments) with correlations among all variables. Both models demonstrated good fit of the data to the model. For cortisol, χ^2 (46, N= 148) = 45.07, *ns*; RMSEA = .00, CFI = 1.00, and TLI = 1.00. For alpha amylase, χ^2 (46, N = 148) = 54.43, *ns*; RMSEA = .03, CFI = .97, and TLI = .96. We then tested our structural models. In these models, T1 (18-month) demographic risk was a predictor of T2 (30-month) effortful control and intrusive-overcontrolling parenting, which in turn predicted T3 (72-month) physiological responding. Factor loadings of manifest indicators on latent variables were all statistically significant and ranged from .44 to .99. The statistical model predicting cortisol (Figure 1) demonstrated good fit of the data to the model, χ^2 (44, N= 148) = 44.40, *ns*. The practical fit indices were also acceptable, with an RMSEA of .001 and CFI and TLI values of 1.00 and 1.00, respectively. The statistical model predicting sAA (Figure 2) also

[i]We first ran a model with both sAA and cortisol included. However, the model would not converge with all the paths included. Removing a variable (demographic risk) resulted in a good-fitting model: χ^2 (69, N= 148) = 75.34, $p < .05$; CFI = .99; TLI = .99; RMSEA = .03. However, cortisol and sAA were not significantly correlated (β = .06, SE = .10, p = .55). Zero-order correlations also did not show significant relations between sAA and cortisol (Table 1). Given these findings, and because other investigators also have found no relation between cortisol and sAA (Engert et al., 2011), we made the decision to run the analyses as two separate models in order to keep demographic risk in the model.

We also attempted to run a model using observations of both intrusive-overcontrolling parenting and sensitive-warm parenting in order to determine if negative parenting had unique effects above and beyond positive parenting. However, our model would not converge with both variables in the model, most likely because the observed negative and positive parenting scales were highly negatively correlated. Given this problem, we decided to run the models separately for each parenting behavior. The results for positive and negative parenting were highly similar (e.g., the path from positive parenting to sAA: β = −.46, SE = .21, $p < .05$; and from negative parenting to sAA: β = .48, SE = .16, $p < .01$). Thus, we chose to only present the analyses with intrusive-overcontrolling parenting because this type of parenting fit better with our demographic risk variable.

demonstrated good fit of the data to the model, χ^2 (46, N = 148) = 54.62, *ns*. The practical fit indices were also acceptable, with an RMSEA of .036 and CFI and TLI values of .97 and .96, respectively.

For both models, demographic risk measured when a child was 18 months old negatively predicted children's EC at 30 months (β = −.39, SE = .09, p < .01) (Figure 1 and 2). Demographic risk at 18 months positively predicted intrusive-overcontrolling parenting at 30 months for both the cortisol (β = .61, SE = .09, p < .01) and sAA models (β = .62, SE = .09, p < .01). Intrusive-overcontrolling parenting at 30 months was negatively correlated with children's EC at 30 months (cortisol: β = −.50, SE = .15, p < .01; sAA: β = −.53, SE = .14, p < .01). Mothers' intrusive-overcontrolling parenting at 30 months predicted higher levels of both cortisol (β = .45, SE = .22, p < .05) and sAA (β = .45, SE = .15, p < .01) at 72 months; note that these relations were unique from any prediction by EC of physiological responding. Unexpectedly, 30-month EC did not predict either cortisol or sAA at 72 months when prediction by parenting was taken into account.

We also tested mediating paths in the model. The indirect effect of demographic risk on children's trait cortisol through intrusive-overcontrolling parenting was small in magnitude, .28 (SE = .14) and significant, z = 1.96, p < .05. The indirect effect of demographic risk on children's sAA level through intrusive-overcontrolling parenting was small in magnitude, .27 (SE = .10), and statistically significant, z = 2.86, p < .01.

Discussion

Our study examined the unique environmental and temperamental precursors of children's physiological activity using trait-like measures of both salivary cortisol and alpha-amylase. Of most importance, we found that demographic risk measured at 18 months predicted mothers' intrusive-overcontrolling parenting as well as children's effortful control at 30 months. In turn, parenting predicted children's higher cortisol and sAA levels 3.5 years later. Parenting also mediated the relations between demographic risk and children's levels of both cortisol and sAA. These findings suggest that children's early environment is associated with their overall levels of cortisol and sAA later in childhood, although more stringent tests (i.e., controlling for stability of the outcomes) of this mediated pathway are needed.

The finding that intrusive-overcontrolling parenting predicted children's stress characteristics is consistent with other research on parenting behaviors and children's cortisol levels and reactivity. At an early age, parent-child relationships and quality of parenting are associated with children's physiological responses (Loman & Gunnar, 2010). Prior research has found associations between positive parenting behaviors such as engagement (Blair et al., 2008) and warmth and responsiveness (Loman & Gunnar, 2010) and children's cortisol levels. Other researchers have found relations between children's elevated cortisol and more extreme parental behavior such as spanking or slapping (Bugental et al., 2003; Hastings et al., 2011). The present study expands this prior literature by demonstrating longitudinal associations between controlling-intrusive parenting and children's later levels of both cortisol and sAA. It is likely that children who have controlling or intrusive mothers experience more stress, which in turn leads to a more chronically overaroused state. On the other hand, when mothers are non-intrusive and less controlling, children's overall stress response most likely remains regulated and lower.

Also notable is that although our sample was predominantly low-risk, we still found significant associations between parenting and children's physiological states, suggesting that the relation of intrusive/overcontrolling parenting behaviors to physiological

functioning is not just limited to high-risk or predominantly low-income samples. Our findings suggest that intrusive/overcontrolling parenting is negatively associated with the activity of both the HPA axis and ANS. Although research has shown that sAA and cortisol represent the activity of different components of the psychobiology of the stress response, the HPA axis and ANS are functionally interrelated (Engert, 2011, Granger et al., 2007). That an overcontrolling and intrusive parenting style is associated with elevated sAA and cortisol may suggest that high levels of negative parenting would be more frequently observed among individuals in the top quartiles of the sample distributions of both sAA and cortisol.

The prediction by demographic risk of children's trait levels of cortisol and sAA via the quality of parenting is consistent with prior research (e.g. Blair et al., 2011; Blair et al., 2008). However, it should be noted that Blair and colleagues (2008) included both positive and negative aspects of parenting in their analysis, and they found that the relation between negative parenting and cortisol did not hold when positive parenting was included in the model. We were unable to include both parenting measures in a single model in the present study given our significantly smaller sample and the fact that our observed positive and negative parenting measures were highly correlated. It will be important to replicate our findings including both positive and negative parenting in order to determine whether negative parenting has unique effects beyond positive parenting behaviors.

The finding that demographic risk negatively predicted both cortisol and sAA at a trend level could be evidence of hypocortisolism, in that prolonged or extreme stress has been shown to impede regulation of cortisol (Gunnar & Vazquez, 2001). However, these paths only approached significance in our model, and the correlations (Table 1 and 2) showed mixed support for this hypothesis. Given the lack of negative correlations between risk and sAA, our results could reflect a suppression effect and should be replicated.

Unexpectedly, we did not find significant relations between effortful control and trait-like levels of either cortisol or sAA in the structural models, although sAA and effortful control were significantly negatively correlated in the zero-order correlations (Table 2). Thus, although EC was correlated with lower sAA, when parenting was included in SEM, parenting, rather than effortful control, uniquely predicted levels of sAA 3.5 years later. Because effortful control and parenting were substantially related, it was likely difficult for both constructs to uniquely predict sAA. Effortful control did not predict cortisol in the correlations, in contrast to other studies showing longitudinal associations between toddlers' effortful control and cortisol reactivity (e.g. Blair et al., 2008) and concurrent associations with sAA (Laurent et al., 2011). It could be that effortful control is more strongly linked to physiological reactivity than to trait-like physiological characteristics. Our results also suggest that effortful control may be differentially related to the HPA and ANS systems, and further examination of both systems, would be an important step for future research.

The finding that intrusive-overcontrolling parenting prospectively predicted children's physiological functioning additionally has important implications for future research. In particular, our results suggest that prevention and intervention efforts that target negative parenting behaviors could have important implications for children's physiological health and functioning. Poor family relationships in early childhood can lead to biological dysregulation that has long-term consequences for health and adjustment (Boyce & Ellis, 2005; Repetti et al., 2002). In the future, researchers could examine whether parenting programs that teach parents to use warm, supportive, and nonintrusive parenting are associated with children's physiological states and responses. Our findings suggest that the quality of the family environment is potentially important when considering children's physiological reactivity, even in relatively low-risk samples.

In future work, it would be useful to consider the mediational role of children's physiological traits in the relation between the social environment and later problem behaviors. It is logical to suggest that the association between intrusive-overcontrolling parenting and children's physiological activity is at least partly accounted for by the association between intrusive-overcontrolling parenting and children's later problem behaviors and/or psychopathology. Other family characteristics likely are precursors to children's later cortisol levels. For example, mother's depression has been associated with higher levels of young children's morning cortisol (Dougherty, Klein, Olino, Dyson, & Rose, 2009). In addition, it would be useful to examine the extent to which other intrinsic or temperamental factors relate to children's physiological functioning. For example, researchers have found that cortisol levels in preschoolers decreased across the school year for exuberant children, but remained high for highly inhibited children (Tarullo, Mliner, & Gunnar, 2011). Dougherty and colleagues (2009) found that low positive emotionality is associated with elevated morning cortisol levels in young children, even after controlling for negative emotionality. Studies that examine other temperamental characteristics that potentially could be associated with stress systems, particularly for sAA, and especially across time, could make additional, important contributions to this field.

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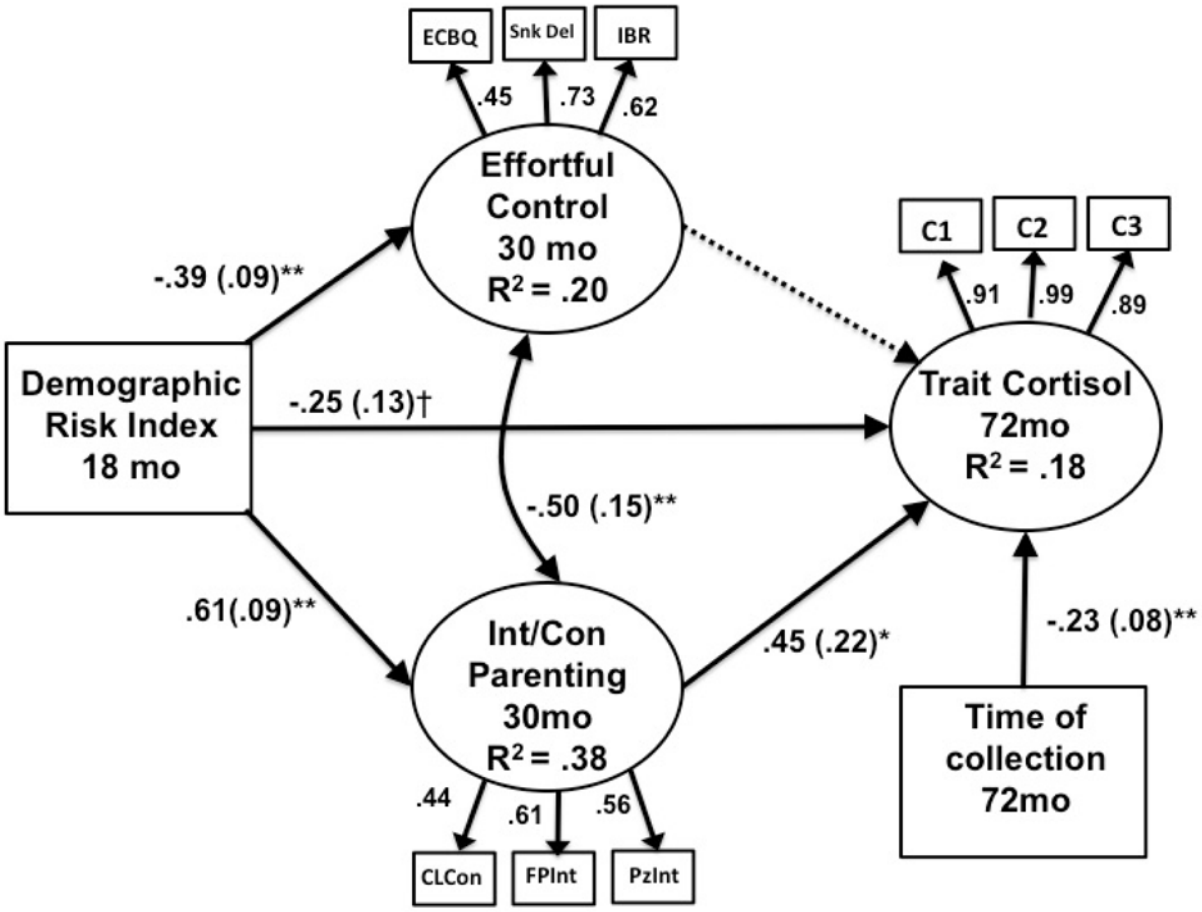

Figure 1.

Results from statistical model with salivary cortisol (standardized).

Note: χ^2 (44, N= 148) = 44.40, $p < .05$; Comparative Fit Index (CFI) = 1.00; Tucker–Lewis Index (TLI) = 1.00; Root mean square error of approximation (RMSEA) = .001.

**$p < .01$ *$p < .05$ (two-tailed test); dotted lines = non-significant. Factor loadings are all significant ($p < .01$). Standard errors are shown in parentheses.

Time of collection = Time of day salivary samples were collected; Int/Con= intrusive-controlling parenting; ECBQ 30 = attention shifting, attention focus, inhibitory control (mother and caregiver report); Snk. Del 30 = snack delay task at 30 months (observer report); IBR = observer report of child's attention and persistence; ClCon = mothers' control during cleanup task (observational report); FPint= mothers' intrusiveness during free play (observational report); Pzint = mothers' intrusiveness during puzzle task (observational report); C1 = pretest of cortisol; C2 = cortisol measured 10 minutes after task; C3 = cortisol measured 20 minutes after task.

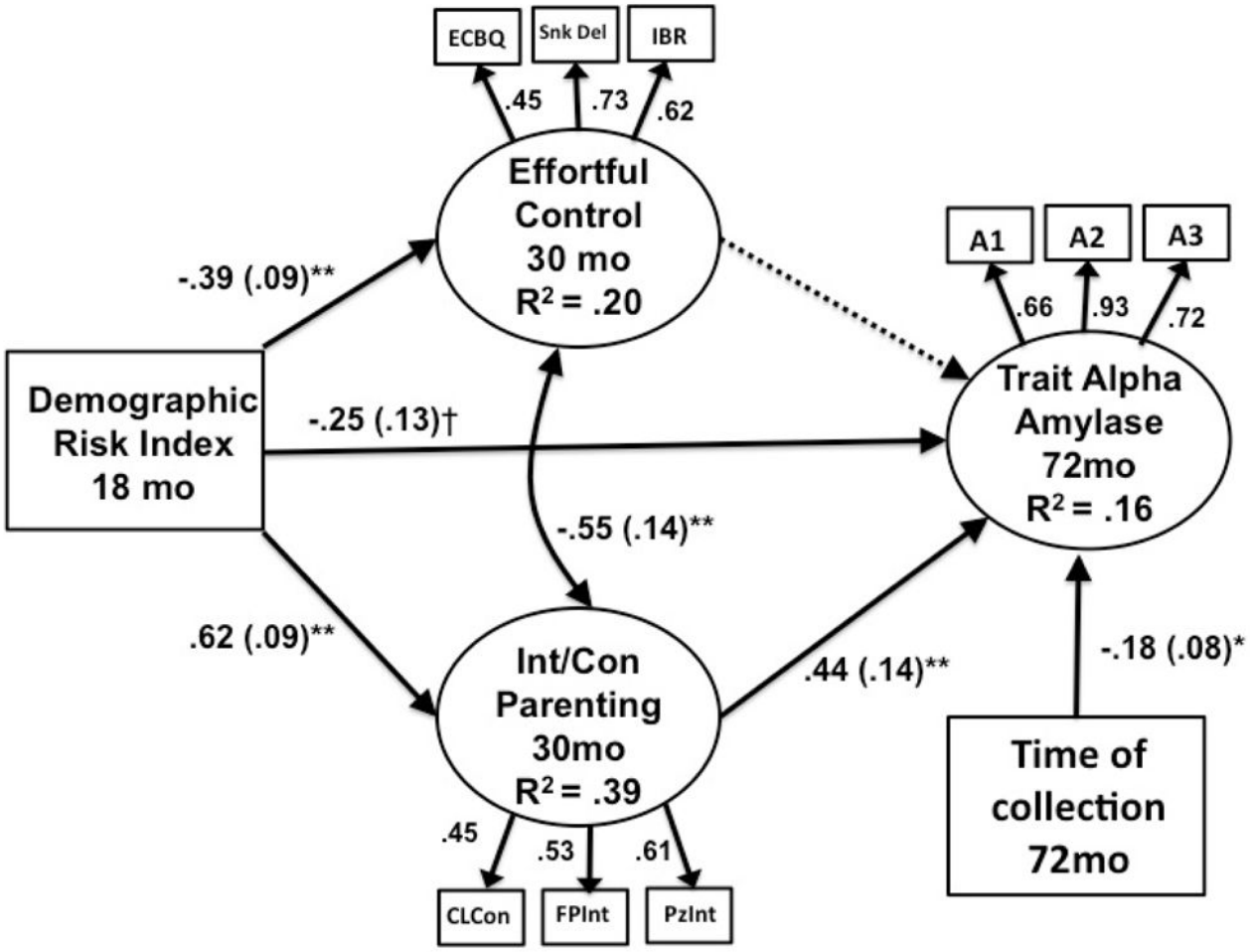

Figure 2.

Results from statistical model with salivary alpha amylase (standardized).

Note: χ^2 (46, N= 148) = 54.62, $p < .05$; Comparative Fit Index (CFI) = 0.97; Tucker–Lewis Index (TLI) = 0.96; Root mean square error of approximation (RMSEA) = .036.

**$p < .01$, *$p < .05$, † $p < .10$ (two-tailed test); dotted lines = non-significant. Factor loadings are all significant ($p < .01$). Standard errors are shown in parentheses.

Int/Con= intrusive-controlling parenting; ECBQ 30 = attention shifting, attention focus, inhibitory control (mother and caregiver report); Snk. Del 30 = snack delay task at 30 months (observer report); IBR = observer report of child's attention and persistence; ClCon = mothers' control during cleanup task (observational report); FPint = mothers' intrusiveness during free play (observational report); Pzint = mothers' intrusiveness during puzzle task (observational report); A1 = pretest of alpha amylase; A2 = alpha amylase measured 10 minutes after task; A3 = alpha amylase measured 20 minutes after task.

Table 1

Correlations among indicators (*N*=148)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Dem Risk	1.00														
2. Del. Task	−.22**	1.00													
3. ECBQ	−.18*	.38**	1.00												
4. IBR	−.26**	.46*	.22**	1.00											
5. FP Intru.	.29**	−.24**	−.22**	−.13	1.00										
6. Pz. Intru.	.37**	−.28**	−.17*	−.23**	.37**	1.00									
7. Cl. Contr.	.26**	−.24**	−.17*	−.22**	.28**	.19*	1.00								
8. Cort 1	−.03	.00	−.10	.01	.24**	.02	.02	1.00							
9. Cort 2	−.09	−.02	−.06	.04	.27**	.03	.03	.90**	1.00						
10. Cort 3	−.13	.01	−.06	.05	.21*	.01	.04	.82**	.89**	1.00					
11. sAA 1	.15†	−.05	−.03	−.08	.04	.26*	.17*	.08	.03	−.02	1.00				
12. sAA 2	−.08	−.16†	−.02	−.13	.10	.20*	.10	.11	.16†	.08	.59**	1.00			
13. sAA 3	.01	−.08	.00	−.10	−.01	.10	.19*	.06	.08	.03	.48**	.65**	1.00		
14. Sex	.17*	.13	.06	.23**	−.13	.00	−.13	−.15†	−.16†	−.20*	−.00	−.04	−.07	1.00	
15. Time	.04	−.12	.04	−.08	−.13	.00	.01	−.23**	−.26**	−.22**	−.02	−.19*	−.10	.06	1.00
Mean	.62	6.38	4.45	3.61	1.26	1.11	.24	.08	.08	.08	35.71	33.26	33.17	.45	3.01
St. Deviation	.57	2.58	.52	.82	.25	.23	.30	.05	.05	.05	26.28	25.53	26.91	.50	1.43

Note:

*$p < .05$

**$p < .01$ (two-tailed test).

Dem Risk = demographic risk index at 18 months; Del. Task = snack delay task at 30 months (observer report); ECBQ = attention shifting, attention focus, inhibitory control (mother and caregiver report); Cl. Contr. = mothers' control during cleanup task (observational report) at 30 months; Pz. Intru. = mothers' intrusiveness during puzzle task at 30 months(observational report); FP Intru. = mothers' intrusiveness during free play at 30 months (observational report); Cort 1 = pretest of cortisol at 72 months; Cort 2 = cortisol measured 10 minutes after task at 72 months; Cort 3 = cortisol measured 20 minutes after task at 72 months; sAA 1 = pretest of alpha amylase at 72 months; sAA 2 = alpha amylase measured 10 minutes after task at 72 months; sAA 3 = alpha amylase measured 20 minutes after task at 72 months; Sex = child sex (0 = boys, 1= girls); Time = time of day that saliva samples were collected.

Table 2

Zero-order correlations among variables. ($N = 148$)

	1	2	3	4	5	6	7
1. Demographic Risk T1	1.00						
2. Effortful Control T2	−.34**	1.00					
3. Int-Con Parenting T2	.59**	−.62**	1.00				
4. Cortisol T3	−.08	−.01	.23*	1.00			
5. Alpha Amylase T3	.01	−.20*	.28*	.15	1.00		
6. Child Sex	.17*	.24**	−.13	−.17*	−.09	1.00	
7. Time of collection	.04	−.12	−.05	−.25**	−.12	.06	1.00

Note:

* $p < .05$

** $p < .01$ (two-tailed test).

Sex (0 = boys, 1= girls).

T1 = 18 months, T2 = 30 months, T3 = 72 months; Time of collection = Time of day salivary samples were taken. Int-Con = intrusive-overcontrolling.