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Associations between Stress Biology Indicators and Overweight across Toddlerhood

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Abstract

Biological stress responses are proposed as a pathway through which stress exposure can “get under the skin” and lead to health problems, specifically obesity. Yet, it is not clear when such associations may emerge or whether they are bidirectional. Cortisol and salivary alpha amylase (sAA) were considered indicators of the biological stress response. We tested the longitudinal association between cortisol and sAA and weight in 215 low-income children at ages 21, 27, and 33 months (52% male; 46% non-Hispanic white). sAA and cortisol intercept and slope (representing morning level and rate of change across the day) were calculated for each age point using random effect models. Children were weighed and length measured and categorized as overweight versus normal weight (overweight defined as weight-for-length z-score $\geq 85^{\text{th}}$ percentile for age and sex). Cross-lagged models stratified by sex and controlling for birthweight z-score tested the concurrent and cross-lagged associations between each of 4 indices of stress biology individually (cortisol and sAA intercept and slope) and overweight. Overweight status was correlated across time. Cortisol and sAA were correlated across occasions of measurement, though somewhat less strongly in boys. There were no concurrent associations between stress indicators and overweight. sAA at 27 months predicted greater risk of overweight at 33 months in girls, such that both lower sAA intercept and more rapidly increasing sAA at 27 months predicted greater risk of overweight at 33 months ($\beta = -0.64$, $p < .05$ and $\beta = 1.09$, $p < .05$, respectively). For boys only, overweight at 21 months predicted lower sAA intercept at 27 months ($\beta = -0.35$, $p < .05$). Findings

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suggest that longitudinal associations of stress biology and weight status may be present only on a limited basis very early in the lifespan.

Keywords

child; obesity; stress; cortisol; salivary alpha-amylase (sAA); low-income

1. Introduction

1.1 Overweight and Psychosocial Stress among Young Children

More than one in four US preschool-aged children is overweight (Ogden et al., 2012) with children living in poverty at even greater risk (Pan et al., 2013). The chronic stress resulting from living in poverty is a hypothesized contributor to the socioeconomic disparities in overweight prevalence. One of the pathways via which chronic stress may contribute to obesity is via disrupted biological stress responses; yet, this has rarely been examined in young children.

1.2. Chronic Stress Exposure and Stress Biology

Under optimal conditions, multiple biological stress regulatory systems become activated during stress and then return to a baseline after the immediate stress has passed. Under conditions of chronic stress exposure however, such systems may fail. Systems that are routinely called upon to respond to ongoing stress can become overloaded and over time become ineffective regulators of stress (Miller et al., 2011).

A primary biological stress system, the hypothalamic-pituitary-adrenal (HPA) axis, stimulates the production of cortisol in response to stress (Gunnar and Vazquez, 2001), while also generating a strong diurnal rhythm such that cortisol levels typically peak in the early waking hours and decline into the evening. Among adults with a history of chronic stress exposure in childhood, this diurnal pattern has been observed to instead show low morning levels and a flatter rhythm across the day, which is referred to as hypocortisolism (Gunnar and Vazquez, 2001). In the current paper, we use salivary cortisol as an indicator of HPA basal activity, reflecting the adrenal (or HPA) component of the stress response.

The sympathetic nervous system (SNS) is another biological stress system that promotes the secretion of norepinephrine, which increases salivary alpha amylase (sAA) levels (Kuebler et al., 2014). sAA also demonstrates a diurnal pattern, showing a steady rise across the day into the evening (Nater et al., 2007). Chronic stress is also hypothesized to down-regulate this system, and chronic stress exposure in children has been linked with lower basal sAA patterns (Hill-Soderlund et al.). Thus, similar to cortisol, we consider sAA as a global indicator of SNS stress biology, specifically baseline activation.

1.3. Conceptual Model Linking Stress Biology and Weight Status in Children

Across systems, disrupted biological stress responses have been hypothesized to increase obesity risk via multiple complex metabolic, hormonal and/or behavioral pathways (Kyrou and Tsigos, 2009; Maniam et al., 2014), all of which converge to promote obesity. For

example, evidence supports that chronic high cortisol levels have congruent actions. First, cortisol increases the salience of pleasurable activities (e.g. ingesting sucrose, ingesting fat), that motivates eating of “comfort food” (Dallman et al., 2003; Pasquali et al., 2010). Second, cortisol also stimulates insulin secretion (Strack et al., 1995) that leads to redistribution of energy stores resulting in large abdominal depots that in turn generate an increased signal of abdominal energy stores to inhibit catecholamines in brain centers that suppress cortisol activation (Rebuffe-Scrive et al., 1992). In this way, obesity can, in turn, also alter stress physiology (Davy and Orr, 2009; Rossi et al., 2015; Soares-Miranda et al., 2011).

The interplay of the autonomic system and obesity is also complex. Low SNS activity has been suggested as a risk factor for obesity in susceptible populations since low SNS activity is associated with low resting metabolic rate (Davy and Orr, 2009; Ravussin and Tataranni, 1996; Tataranni et al., 1997) and medications that increase SNS activity reduce food intake (Tentolouris et al., 2006). However, beyond this ‘global’ SNS action on metabolism, SNS outflow is also regulated in a highly tissue-specific manner (Morrison, 2001). As such, SNS outflow to one region may not reflect the magnitude or even the direction of SNS outflow to another. What this means is that although low sympathetic activity may be present in obesity, high SNS activation at or targeting specific organs such as the heart, blood vessels and kidneys may promote the development of the well-documented associations between obesity and obesity-related pathology (e.g. hypertension)(Morrison, 2001). Few studies have used longitudinal designs to examine the potential bidirectional associations between stress biology, onset and persistence of excessive weight gain, and none have done so in young children.

1.4. Prior Studies of Stress Biology Indicators and Weight in Children

The few studies of the association between diurnal cortisol and overweight in children have had mixed results, finding positive (Papafotiou et al.; Reinehr et al., 2014; Veldhorst et al., 2013), negative (Hillman et al., 2012; Kjölhede et al., 2014; Lumeng et al., 2014; Törnhage and Alfvén, 2006), and no association (Knutsson et al., 1997; Netherton et al., 2004; Rosmalen et al., 2005). These studies all used cross-sectional designs and all but one (Lumeng et al., 2014) focused on school-aged children or adolescents. The one longitudinal study (Ruttle et al., 2013) suggested that among adolescents, blunted cortisol was associated with higher BMI concurrently and predicted increasing BMI across time.

Studies have also investigated resting SNS activity and weight in children, and have yielded similarly mixed results, finding positive (Latchman et al., 2011; Rodríguez-Colón et al., 2011; Soares-Miranda et al., 2011) and negative (Baum et al., 2013; Vanderlei et al., 2010) associations. Only two identified studies have examined sAA as the marker of SNS activity in relation to weight in children. sAA across the day was lower among obese compared to normal weight school-aged girls (Papafotiou et al.), and lower morning sAA levels were associated with higher body mass index z-scores (BMIz) among preschool-aged children (Miller et al., 2015). The one longitudinal study that assessed SNS activity in relation to weight in young children examined cardiac reactivity to stress (not resting SNS activity) and found no cross-sectional associations, but higher BMIz at age 2.5 years predicted reduced SNS response to stress at age 5 years (Alkon et al., 2014).

1.5. Purpose of the Current Study

Young children are in a unique developmental period both for the establishment of effective responses for coping with stress (Cole et al., 2009), as well as obesity, which, once established during childhood, tends to persist into adulthood (Freedman et al., 2001). Stress exposure during childhood may convey more risk than exposure later in the lifespan, as early alterations in biological stress systems can become embedded and shape health and development over time (Karatsoreos and McEwen, 2013). Documenting the very early emergence of stress-obesity associations is therefore important in order to understand the development of such associations over time. Finally, we could find only one study in children that examined indicators of both SNS and HPA axis functioning in relation to child weight (Papafotiou et al.). Therefore, in the context of conflicting findings, the lack of data in young children, and the lack of longitudinal data to inform understanding of potential causality, we sought to test two hypotheses regarding directionality of associations. First, we tested the hypothesis that cortisol and sAA patterns, specifically blunted patterns, would predict future overweight, and second we tested the hypothesis that overweight would predict future cortisol and sAA patterns, specifically blunted patterns, among low-income toddler-aged children. As there are sex differences in weight and growth across this age period (Ogden et al., 2012), and as studies have identified sex differences in cortisol (Rosmalen et al., 2005) and sAA (Vigil et al., 2010) patterns in children, as well as in associations between cortisol and weight (Lumeng et al., 2014; Rosmalen et al., 2005), we examined associations in the full sample and in boys and girls separately.

2. Methods

2.1. Study Design and Participants

Participants were recruited via flyers posted in community agencies serving low-income families between 2011 and 2014. The study was described as examining whether children with different levels of stress eat differently. Inclusion criteria were that the biological mother was the legal guardian, had an education level less than a 4-year college degree, and was at least 18 years old; the family was eligible for Head Start, the Women Infants and Children (WIC) Program, and/or Medicaid and was English-speaking; and the child was between 21 and 27 months old, was born at a gestational age ≥ 36 weeks, and had no food allergies or significant health problems, perinatal or neonatal complications, or developmental delays. The research was approved by the [blinded] Institutional Review Board. Written informed consent was provided by the legal guardian (100% mothers; henceforth we refer to “mother”) and age-appropriate assent from children; families were compensated for their time.

Mother-child dyads were invited to participate in three data collections at ages 21, 27, and 33 months; the data collection procedures at each age spanned across 5 days and included measures regarding eating behavior and biobehavioral self-regulation. A total of 244 dyads participated. Most ($n = 186$) dyads entered the study when the child was age 21 months, but 58 entered the study when the child was age 27 months to maximize recruitment; measures obtained at study entry are henceforth referred to as “baseline” measures. This report is limited to children who provided at least one saliva sample and one anthropometric

measurement. A total of 215 of the 244 participants had mother-reported due date and birth weight for index child and at least one age point at which they provided both a saliva sample and anthropometry. Characteristics of participating children are shown in Table 1.

The 215 participants providing birth weight, at least one saliva sample and one anthropometry measure included in this analysis did not differ from the 29 excluded participants with regard to child sex, child race/ethnicity, child age, maternal education, or food security. Excluded participants were more likely to be from households headed by a single parent ($p=.03$) and have lower maternal BMI ($p=.02$). A total of 53 children (24.7%) participated at only one age point, 82 (38.1%) participated at only two age points, and 80 (37.2%) participated at all three age points. Mother-child dyads who participated at two or three age points did not differ at baseline from those who participated at only one with regard to child sex, child race/ethnicity, child age, maternal BMI, maternal education, food security, or family structure.

2.2. Procedure and Measures

2.2.1. Collection of Cohort Characteristics by Parent Report—Mothers reported children's sex, race/ethnicity (categorized for this report as non-Hispanic white vs. not), age, maternal education (< high school, high school diploma or generalized equivalency diploma (GED), or some college), family structure (single parent vs. not) and children's birth weight. To assess food security, mothers completed the US Department of Agriculture 18-item Household Food Security Survey that categorizes households as food secure vs. not (Bickel et al., 2000). Mothers also reported annual household income; this value was divided by the federal poverty line for a family of a specific size to generate the income-to-needs ratio. An income-to-needs ratio <1.00 indicates that the family was living below the poverty line.

2.2.2. Saliva Sampling—Saliva samples were collected by trained research assistants who were familiar to the child. Children of this age range were unable to reliably provide the saliva by passive drool sampling. Using a consistent method for all children in the study is important for saliva sampling (Rohleder et al., 2006), therefore all saliva samples were gathered using an absorbent swab placed in the child's mouth. Research assistants maintained contact with the mothers throughout each sampling day to determine the child's wake time and location and waited until at least 45 minutes after the child had napped or eaten prior to taking each sample. Children with food or beverage debris in their mouth provided samples after their mouth was rinsed with water, and waited 10 minutes after rinsing to minimize contamination or dilution of saliva samples. The primary caregiver reported for the child on the day of each sample any medication use, whether the child had recently been playing very actively (outside play; running, jumping, etc.), illness, unusually good or bad events, exact time of morning awakening (and if it was the usual time), the number of times the child woke up during the night prior, and the last time the child slept or ate prior to saliva collection. The location of sample collection (e.g., home, daycare) was recorded. Children provided saliva samples 3 times per day on 3 consecutive days (weekdays only). The first sample each day was collected approximately 45 minutes after the child woke for the day, with the following samples occurring at 3 to 4-hour intervals afterwards.

2.2.3. Cortisol and sAA Assays—Following collection, saliva samples were stored in Thermo Scientific Matrix Racks at -80° C until assayed. Saliva samples were submitted to the Center of Chemical Genomics (CCG) at the University of Michigan to perform assays. Assays were conducted by the same technician using the same equipment. On the day of the assay, the sample was thawed completely, vortexed, centrifuged at 3000 rpm for 15 minutes, separated from debris and submitted to the steps for cortisol or alpha amylase activity detection following manufacturer's instructions.

Cortisol was assayed using an Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Catalog No. 1-3002, 96-Well Kit, Salimetrics LLC, PA, USA) with a detection limit of 0.007µg/dL. Inter-assay coefficient of variation of was 14% and intra-assay coefficient was 7.5%. We report cortisol in µg/dL.

sAA was assayed using an alpha amylase kinetic reaction assay kit (Catalog No. 1-1902, 96-Well Kit, Salimetrics LLC, PA, USA), which uses a chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose. The enzymatic action of alpha amylase on this substrate is spectrophotometrically measured 2 minutes after the start of the reaction using a calibrated plate reader. The amount of alpha amylase activity present in the sample is directly proportional to the increase in absorbance detected at 405 nm. High, medium and low salivary alpha amylase controls were included in each assay. Samples below the controls were re-assayed using a dilution to achieve a higher concentration of the sample and thus, a greater absorbance reading. The inter-assay coefficient of variance was 10.8%, 6.5%, and 9.3% across 3 different lots of assay kits. The intra-assay coefficient of variation was 8.9% when using standards of the kit and 15% when pooled saliva samples were used. Results are reported in enzyme units per milliliter (U/ml) of alpha amylase.

2.2.4. Anthropometry—Trained research staff conducted all anthropometric assessments for child and mother and were re-certified based on video review by a pediatrician on a yearly basis. Children were weighed using a Seca Baby Scale Model 334. Child length was measured recumbent using a Seca Infantometer Model 417. Mothers' weight was measured using a Detecto DR-550C scale and height was measured using a Seca 213/217 stadiometer. All measurements were taken twice and the average was calculated for use in analyses. For both children and mothers, if measurements were off by 0.5 centimeter or more for length/height, or by 0.1 kilogram or more for weight, participants were measured two additional times and the new average was calculated.

2.3. Statistical Analysis

Data analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC) and MPLUS version 4.1 (Muthen & Muthen, Los Angeles, CA)). Outliers as described below were excluded from the analysis.

2.3.1. Cortisol and sAA Outliers—Individual cortisol values were excluded if the value was > 3 SD's from the mean for the sample for that timepoint, as recommended by others (Gunnar and White, 2001). Of the 4440 samples assayed across all age points, 289 (6.5%) were excluded as such values could reflect taking medications or be otherwise biologically implausible (Crowley et al., 1993; Luthold et al., 1985). To be included in the cortisol

analysis, a child needed to have at least 5 valid cortisol data points over 2 days in order to have a value for at least half of the measurement points and values on more than one day to account for possible day to day variation due to a range of daily factors. The mean number of valid cortisol data points per child per age point was 8.4 (*SD* 1.5).

sAA values were similarly excluded if the value was > 3 SD's from the sample mean for that timepoint, resulting in the exclusion of 323 (7.2%) of 4489 samples. To be included in the analysis, a child needed to have at least 5 valid sAA values over 2 days; the mean number of valid diurnal sAA values per child per phase was 8.4 (*SD* 1.3).

2.3.2. Cortisol and sAA Variable Creation—Using methods described previously (Lumeng et al., 2014) we used hierarchical linear modeling (HLM) to generate random intercept and slope parameters for cortisol and sAA to capture individual diurnal curves for each participant using the restricted maximum likelihood method (REML). The HLM approach is a powerful modeling technique for estimating unbiased individual trajectories, provided that trajectories have a known parametric form (e.g. linear, log-linear, quadratic) (Hruschka et al., 2005). This approach is also powerful because it accounts for the time differential in saliva sampling in a direct way using the parametric function of the diurnal pattern. For both cortisol and sAA, each sample for each day is included in the model with the corresponding time since waking based on wake up time that particular day, and time that sample was taken. Having data over a range of post-wake time points within and between subjects allows us to estimate the slope based on the log-linear parametric form. Random effect parameters thus estimated the child's expected cortisol and sAA pattern over the three sample days. The random intercept and slope estimates from the HLM analysis were then used as individual-level predictor variables for all subsequent analyses.

The diurnal cortisol curve follows a pattern such that cortisol increases initially after morning awakening, reaches a peak usually within 30 minutes, and afterward decays exponentially over the course of the day (Clow et al., 2004). For sAA, the known diurnal pattern differs from that of cortisol such that sAA decreases initially after morning awakening, reaching a nadir within about 30 minutes and after that rises gradually over the course of the day (Nater et al., 2007). However, both cortisol and sAA follow a log-linear pattern (for time ≥ 60 minutes), albeit cortisol has a negative slope whereas sAA has a positive slope both in log-scale. A log transformation was implemented to capture the log-linear pattern (for time ≥ 60 minutes) of the diurnal cortisol and sAA, and to ensure normality for the residuals. Thus using the log transformed cortisol and sAA as the outcome and the time (since awakening) at which the sampling occurred as the independent variable, the diurnal cortisol and sAA patterns are therefore linear on time in a log-scale with the linear trajectory captured by two parameters, intercept and slope. Preliminary analysis showed sAA values to be very sensitive to whether or not the child was reported to have eaten before the sample, thus for sAA the HLM model was controlled for whether the child ate before each sample. The random intercept is an estimate of the expected cortisol (or sAA) level at 60 minutes after awakening for a given individual, and the random slope is the expected rate of change on cortisol (or sAA) after 60 minutes post-awakening. Thus, both the random intercept and the random slope capture the diurnal cortisol (or sAA) patterns of an individual.

2.3.3. Cortisol and sAA Covariates—None of the variables recorded in the daily logs obtained at the time of saliva collection were associated with cortisol or sAA intercept or slope and thus were therefore not included as covariates in analyses.

2.3.4. Overweight—Child weight-for-length (WFL) was calculated as weight divided by length. Because the normal distribution of children's weights-for-lengths differs based on age and sex, children's weights-for-lengths were percentiled based on the US Centers for Disease Control (CDC) reference growth curves. Per CDC guidelines children were categorized as overweight/obese (WFL $\geq$ 85th percentile for age and sex), or non-overweight (WFL < 85th percentile for age and sex). Mother's BMI's were calculated as weight (in kilograms) divided by height (in centimeters) squared, and then categorized as overweight (BMI $\geq$ 25) versus not.

2.3.3. Analysis Plan—We conducted descriptive statistics to assess central tendency and examined correlations. We used an alpha level of 0.05 (two-tailed) to determine statistical significance. Path models were conducted (using MPLUS version 4.1 (Muthen & Muthen, Los Angeles, CA)) to test our a priori hypotheses regarding the concurrent and cross-lagged associations between weight status and cortisol or sAA intercept and slope (see Figure 1). The cross-lagged models were conducted separately in boys and girls. Bayesian estimation technique in MPLUS was used to fit models which contained both continuous and binary variables. Bayesian posterior predictive checks (PPC) using Chi-square statistics and the corresponding posterior predictive p-values were used to assess the goodness of fit in each model (Gelman, 2004).

3. Results

Mean child WLZ was 0.54 (SD = 1.06) at 21 months, 0.42 (SD = 1.08) at 27 months, and 0.41 (SD = 1.01) at 33 months. Of the children, prevalence of overweight was 30.1% at 21 months, 29.4% at 27 months, and 26.5% at 33 months. The morning saliva sample was collected at a mean of 0.9 hours (SD 0.7, range 0–5.6, interquartile range 0.4–1.1 hours) since awakening, the midday sample was collected at a mean of 4.0 hours (SD 1.1, range 0.7–12.8, interquartile range 3.3–4.5 hours) since awakening, and the afternoon sample was collected at a mean of 7.5 hours (SD 1.2, range 4.3–11.7, interquartile range 6.7–8.3 hours) since awakening. Mean cortisol morning level (i.e., “intercept”) was 0.27 $\mu\text{g/dL}$ (SD = 0.17) at 21 months, 0.26 $\mu\text{g/dL}$ (SD = 0.14) at 27 months, and 0.29 $\mu\text{g/dL}$ (SD = 0.18) at 33 months and mean cortisol slope was -0.09 (SD = 0.03) at 21 months, -0.09 (SD = 0.01) at 27 months, and -0.10 (SD = 0.03) at 33 months, indicating that the general pattern was for children to show higher morning cortisol levels that decreased over the course of the day at all three timepoints. Mean sAA morning level (i.e., “intercept”) was 24.49 U/dL (SD = 17.26) at 21 months, 30.91 U/dL (SD = 20.22) at 27 months, and 32.33 U/dL (SD = 21.20) at 33 months. Mean sAA slope was 0.04 (SD = 0.01) at 21 months, 0.05 (SD = 0.02) at 27 months, and 0.03 (SD = 0.03) at 33 months, indicating that the general pattern was for children to show lower morning sAA levels that increased over the course of the day at all three timepoints. There were no sex differences in cortisol or sAA at any age (all p 's < .05).

Cross-lagged analysis results for the conceptual model depicted in Figure 1 are presented stratified by sex in Table 2. Results are presented for effect size and statistical significance to indicate magnitude of the effect. All of the models showed good fit, with posterior predictive p-values ranging from .36 to .64, well within the 0.05–0.95 range.

Among girls, child weight status was positively associated across ages (paths b1, b2 in Figure 1), and stress biology indicators were also generally positively associated across ages (paths b3, b4). There were no concurrent associations between stress biology indicators and overweight at any age (paths b5, b6). There were two significant associations between earlier stress biology indicators and later overweight, such that a more positive sAA slope at 27 months predicted overweight at 33 months (path b9) and that lower sAA intercept at 27 months predicted overweight at 33 months (path b9). There were no associations between earlier overweight and later stress biology indicators (paths b10, b11).

Among boys, child weight status was positively associated across ages (paths b1, b2 in Figure 1), and stress biology indicators were positively associated across 21 to 27 months, with fewer significant associations from 27 to 33 months (paths b3, b4). There were no concurrent associations between stress biology indicators and overweight at any age (paths b5, b6). There were no significant associations between earlier stress biology indicators and later overweight (paths b8, b9). There was one significant association between earlier overweight and later stress biology indicators such that overweight at 21 months predicted lower sAA intercept at 27 months (path b10).

4. Discussion

There were several main findings of this study. First, overweight status was highly correlated across toddlerhood for both girls and boys. Second, stress biology, as indexed by cortisol and sAA intercept and slope, was also highly correlated across toddlerhood, though somewhat less strongly so in boys. Third, there were no concurrent associations between stress biology indicators and overweight in girls or boys. Fourth, with regard to cross-lagged associations, lower sAA intercept and more rapidly increasing sAA at 27 months predicted greater risk of overweight at 33 months in girls. For boys only, overweight at 21 months predicted lower sAA intercept at 27 months.

The observation that overweight status was stable across toddlerhood is consistent with known patterns of weight gain in childhood. For example, children who have a BMI greater than the 95th percentile for age and sex at age 2 years are 5 times as likely to be overweight as age 12 years compared to children with a BMI < 50th percentile at age 2. Of children who are obese at age 2 years, 50% will be overweight at age 12 (Nader et al., 2006). Fewer than 1% of obese adults will return to a normal weight and fewer than 15% will achieve 5% weight loss (Fildes et al., 2015). Overweight, once established, is very likely to persist.

The observation that stress biology, as indexed by diurnal cortisol and sAA intercept and slope, was stable across toddlerhood, is a new contribution to the literature. The finding is consistent with the one prior study that also examined salivary cortisol and sAA among low-income children across approximately this age range (12–36 months; (Hill-Soderlund et al.);

however the prior study gathered cortisol at only one point during the day, and in a laboratory waiting room context rather than naturalistic home settings. Prior research has also shown stability of single-timepoint assessments in infants (Berry et al., 2016) but less is known about the stability of multiple diurnal parameters in children. The fact that stress biology indicators were somewhat less strongly correlated across time in boys differed from the prior study most similar to ours, which did not report sex differences in the stability of association between cortisol or sAA over time (Hill-Soderlund et al.). We found no sex differences in cortisol or sAA. Prior longitudinal work in children under age 5 years found that girls had higher cortisol secretion than boys, but this study did not examine sAA and reported no sex differences in stability (Hatzinger et al., 2013), and no studies of children have reported sex differences in the stability of sAA responses over time. This question could be examined in future longitudinal work, as early-emerging biological sex differences in stress-response patterns could have implications across multiple areas of functioning and could be the focus of intervention efforts to reduce the negative effects of stress exposure.

Third, there were no concurrent associations between stress biology indicators and overweight in this study of toddlers, as has also been found in prior work with older children (Alkon et al., 2014; Knutsson et al., 1997; Netherton et al., 2004; Rosmalen et al., 2005). In contrast, our research team has previously reported concurrent associations between diurnal hypocortisolism (lower intercept and flatter slope) and higher risk of overweight (BMIz 85% for age and sex) among low-income preschool-aged girls (Lumeng et al., 2014), and cross-sectional studies with older children have reported either the same inverse association (Hillman et al., 2012; Kjölhede et al., 2014; Tornhage and Alfvén, 2006) or a positive association (Reinehr et al., 2014; Veldhorst et al., 2013) between cortisol and weight in school-aged children. We have also previously reported within a sample of low-income preschool-aged children that although there was no concurrent association of sAA intercept or slope with overweight, as in the current study, there was an inverse association with child BMI z-score (a continuous outcome), which differs from the finding here (Miller et al., 2015). One other cross-sectional study in school-aged children found lower sAA across the day in obese (BMIz 95% for age and sex) compared to non-obese girls (Papafotiou et al.) which is consistent with our work in preschoolers, but also differs from current findings. Most prior work, including our own, has been cross-sectional, has studied associations in older children, and has not examined boys and girls separately. Thus, differing findings could be a result of differences by sex or differences in the ages of the study samples, such that associations may emerge only later in development. Furthermore, associations may be bidirectional. Given that both stress biology and childhood weight and growth patterns involve complex and interacting systems, it will be important in future work to tease apart directional associations in more detail.

To our knowledge, ours is the first study to examine longitudinal, cross-lagged associations between stress biology indicators and overweight in children this young. We found that in girls only, lower sAA intercept and more rapidly increasing sAA at 27 months predicted greater risk of overweight at 33 months. Results are consistent with prior cross-sectional work in older children that found increased SNS activity, as assessed using cardiac measures, in association with obesity (Latchman et al., 2011; Rodríguez-Colón et al., 2011; Soares-Miranda et al., 2011). However, results are inconsistent with our own prior findings

of lower concurrent sAA intercept and higher BMIz in preschoolers (Miller et al., 2015) as noted above, and also inconsistent with other studies in older children finding reduced SNS activity in association with obesity (Baum et al., 2013; Papafotiou et al.; Vanderlei et al., 2010). The one prior longitudinal study we could find with young children was in a Latino sample, and found that higher BMI at 2.5 years predicted decreased SNS reactivity in response to stress, but early SNS reactivity did not predict BMI (Alkon et al., 2014). Prior work reported no sex differences. With the exception of our work and Papafotiou et al., these studies assessed SNS activity using resting, ambulatory, or overnight cardiac autonomic modulation. Thus, results from prior research are fairly inconsistent, perhaps as studies have included samples of different ages, different populations, and used different methods. As well, our measure of SNS activity captured a broad process, rather than tissue-specific effects, which are likely important (Morrison, 2001) and should be a focus of future work in children.

Growing up in poverty can expose children to chronic stressors that can shape both biological and behavioral coping responses to stress (Hill-Soderlund et al., 2014); early life stress may therefore increase the risk for overweight in these children over time through behavioral pathways such as eating in response to stress, as well as pathways that are mediated by stress biology. The finding that these associations are stronger in girls than boys may suggest that sex-specific pathways of influence from stress to later health are detectable early in the lifespan, and is consistent with research that cumulative social stress early in life for girls, but not boys, predicted obesity (Suglia et al., 2012), as well as work that in general, stress responses are more closely linked to weight in women (Pasquali et al., 2008). Thus results from the current study identified that a longitudinal association between sAA and later weight emerges early in development. This association may be particularly important for girls, but this finding should be replicated in future longitudinal work.

Finally, we found that on the whole, sAA was more strongly associated with weight than cortisol, an aspect of stress biology which has been examined more frequently in relation to weight although typically with older children. SNS activity may promote later obesity risk through different pathways involving alterations in energy expenditure, for example low resting metabolic rate (Ravussin and Tataranni, 1996) or increased food intake, which could contribute to weight gain over time (Tentolouris et al., 2006). Developmental considerations may explain why findings emerged for sAA but not cortisol in the current study. First, toddlerhood has been characterized as a period in development when cortisol secretion in response to stress is diminished (at least in the presence of responsive caregivers) and thus may be somewhat protected from the biological effects of stress (Gunnar & Donzella 2002). This has not been described with regard to the SNS system, which requires rapid response in “fight or flight” situations, compared to the HPA axis, which stimulates the production of cortisol in response to stress over a number of minutes. Although we did not assess response to an acute stressor in the current study, such differences in the nature of sAA compared to cortisol production in response to stress exposure over time may have different implications for how the biology of stress may affect weight gain across time. Second, as the developmental trajectory of sAA production and cortisol both change across early development, with cortisol decreasing (Watanabe et al., 2003) and sAA increasing (Hill-Soderlund et al.); the associations of weight with each of these biological stress profiles may

change over time. Thus, there may be developmental sensitivity to the effects of SNS versus HPA-axis activation with different implications for weight outcomes. Future longitudinal work would be important in order to examine these different possible pathways.

Finally, overweight predicted lower sAA but only in boys, and only from 21 to 27 months, and thus should be interpreted with some caution. This is consistent with Alkon et al. who found that obesity at 2.5 years predicted later blunted SNS reactivity in response to stress at age 5, but not vice versa, in a sample of Latino children (Alkon et al., 2014). Obesity, particularly visceral fat can cause disruptions in stress regulation systems, with some studies showing that weight gain associates with increased cortisol levels (Travison, 2007). Visceral fat may also shape SNS activation (Davy and Orr, 2009). Stress biology and obesity may also mutually reinforce each other over time through multiple pathways, including cortisol changes due to weight gain (Foss and Dyrstad, 2011).

Our study has several limitations. The sample was limited to low-income toddler-aged children, thus limiting generalizability to children of other ages and socioeconomic strata. Due to limitations of our study setting, our measurement of cortisol and sAA did not occur at a fixed time after awakening, and therefore our estimates of each child's cortisol and sAA intercept and slope include some degree of error. We did not have measures of cardiac physiology or other measures that may index SNS activity relevant to energy balance, did not examine tissue-specific SNS activity, and sAA may not be a precise measure of overall SNS activity. For example, alpha amylase secretion is determined through multiple factors including diet, and can also be influenced by the parasympathetic system (Bosch et al., 2011). Very few studies have explored relationships between sAA and cardiac physiology, as an SNS indicator, in children and this is an important area for future work in order to understand the complexity of these systems. Finally, WLZ is only one measure of weight and growth, and is thus limited. Although our field-based data collection protocols precluded us from gathering additional measures that could be used to assess adiposity indices (e.g., body fat percentage), incorporating such indicators in future work will be important in order to specify the mechanisms of association between 23 biological stress indicators and weight gain. Study strengths include the relatively large, diverse, high-risk sample with a large proportion of overweight children and a home-based biological measure of chronic stress exposure.

In summary, we found limited evidence for links between biological stress indicators and overweight in very early childhood. Excess weight develops very early in the lifespan and socioeconomic disparities in obesity prevalence are detectable in early childhood. In addition, links between psychosocial stress and biological stress indicators are detectable very early in the lifespan, and there are plausible mechanisms linking stress biology and overweight. The generally null findings from the current study may reflect that these associations do not emerge until later in the lifecourse, that their effect sizes are small, or that the physiological mechanisms linking psychosocial stress and overweight are other than the SNS and HPA axis. It is also possible that there is simply no association between stress biology and overweight in very young children. It is also quite possible that subpopulations of children may respond to stress differently (i.e., hypo- compared to hyper-responsive patterns), and/or that response patterns may change over time (i.e., first hyper- then hypo-

responsive) for reasons that are not yet well-articulated in the literature (e.g., genetic propensities, prenatal programming, chronic stress exposure), and such differences may be differentially associated with weight status over time. Finally, given that we examined these processes in a low-income sample, a population that often experiences high levels of stress, limited variability in stress exposure or stress biology patterns may have constrained our ability to detect effects. Alternatively, it is possible that we found the associations that we did because it was a low-income sample with relatively high rates of overweight and stress exposure. Future studies comparing samples who have experienced higher versus lower levels of early life stress will be important in order to inform our understanding of whether and how stress-weight associations may emerge across development. Finally, given the sex differences detected in the current study, it may be important to consider sex as a moderator of such associations, even in childhood, in future work.

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Abbreviations

BMI	body mass index
HPA	hypothalamic pituitary adrenal
sAA	salivary alpha-amylase
SDU	standard deviation unit
SNS	sympathetic nervous system

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Highlights

- Overweight, cortisol, and sAA are stable across ages 21 to 33 months.
- sAA but not cortisol at 27 months predicted overweight in girls at 33 months of age.
- Overweight at 21 months predicted sAA at 27 months in boys only.

Figure 1.

Cross-lagged model depicting hypothesized concurrent and longitudinal associations between stress biology indicator (cortisol intercept; cortisol slope; sAA intercept; or sAA slope) and child overweight. Unidirectional arrows indicate concurrent associations. Bidirectional arrows indicate temporal direction of association.

Table 1

Characteristics of the sample (n=215)

Demographic Characteristics	Total N = 215	Girls N =104	Boys N =111
Child birth weight, mean (SD), kilograms	−0.24 (0.97)	−0.30 (1.04)	−0.19 (0.91)
Male sex, No. (%)	111 (51.63)	0 (0)	111 (100)
Child race/ethnicity			
non-Hispanic white	98 (45.58)	49 (47.12)	49 (44.14)
Hispanic or not white	117 (54.42)	55 (52.88)	62 (55.86)
Maternal education at baseline, No. (%)			
< high school diploma	30 (13.95)	14 (13.46)	16 (14.41)
High school or general equivalency diploma	52 (24.19)	26 (25.00)	26 (23.43)
> high school diploma, < 4-year college degree	133 (61.86)	64 (61.53)	69 (62.16)
Household food security status at baseline, No. (%) (n=197 total, n=101 male)			
Food Secure	131 (66.50)	58 (60.42)	73 (72.28)
Food Insecure	66 (33.50)	38 (39.58)	28 (27.72)
Family structure at baseline, No. (%) (n=184 total, n=101 male)			
Single parent household	42 (22.83)	20 (24.10)	22 (21.78)
Not single parent household	142 (77.17)	63 (75.90)	79 (78.22)
Income-to-needs ratio at baseline, mean(SD) (n=172 total, n=91 male)	0.94 (0.57)	0.98 (0.59)	0.91 (0.56)
Child overweight, No. (%)			
Age 21 months (n=156 total, n=79 male)	47 (30.13)	26 (33.77)	21 (26.58)
Age 27 months (n=177 total, n=94 male)	52 (29.38)	23 (27.71)	29 (30.85)
Age 33 months (n=151 total, n=77 male)	40 (26.49)	17 (22.97)	23 (29.87)
Mother overweight at child baseline, No. (%) (n=189 total, n=98 male)	138 (73.02)	62 (68.13)	76 (77.55)
Cortisol intercept (μg/dL), mean (SD)			
Age 21 months (n=151 total, n=79 male)	0.27 (0.17)	0.29 (0.21)	0.25 (0.12)
Age 27 months (n=165 total, n=85 male)	0.26 (0.14)	0.27 (0.16)	0.24 (0.10)
Age 33 months (n=141 total, n=73 male)	0.29 (0.18)	0.29 (0.18)	0.29 (0.19)
Cortisol slope, mean (SD)			
Age 21 months (n=151 total, n=79 male)	−0.09 (0.03)	−0.08 (0.03)	−0.09 (0.02)
Age 27 months (n=165 total, n=85 male)	−0.09 (0.01)	−0.09 (0.02)	−0.09 (0.01)
Age 33 months (n=141 total, n=73 male)	−0.10 (0.03)	−0.10 (0.03)	−0.10 (0.03)
sAA intercept (U/dL), mean (SD)			
Age 21 months (n=154 total, n=79 male)	24.49 (17.26)	26.61 (18.66)	22.47 (15.66)
Age 27 months (n=164 total, n=84 male)	30.91 (20.22)	31.76 (20.99)	30.11 (19.55)
Age 33 months (n=135 total, n=71 male)	32.33 (21.20)	36.07 (22.43)	28.96 (19.59)
sAA slope, mean (SD)			
Age 21 months (n=154 total, n=79 male)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)
Age 27 months (n=164 total, n=84 male)	0.05 (0.02)	0.05 (0.02)	0.05 (0.02)
Age 33 months (n=135 total, n=71 male)	0.03 (0.03)	0.03 (0.02)	0.03 (0.03)

Table 2

Standardized path coefficients for cortisol intercepts and slopes, and sAA intercepts and slopes for model shown in Figure 1 for girls (n=104) and boys (n=111)

	Path										
	Longitudinal associations of overweight		Longitudinal associations of stress biology		Concurrent associations of overweight and stress biology			Stress biology predicting future overweight		Overweight predicting future stress biology	
	Ovwt 21m → Ovwt 27m	Ovwt 27m → Ovwt 33m	Stress biology 21m → Stress biology 27m	Stress biology 27m → Stress biology 33m	Ovwt 21m → Stress biology 21m	Ovwt 27m → Stress biology 27m	Ovwt 33m → Stress biology 33m	Stress biology 21m → Ovwt 27m	Stress biology 27m → Ovwt 33m	Ovwt 21m → Stress biology 27m	Ovwt 27m → Stress biology 33m
	b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8	b_9	b_{10}	b_{11}
Cortisol Intercept											
Girls (ppp=.49)	1.97 *	1.87 *	0.40 *	0.34 *	-0.07	-0.02	-0.22	-0.21	-0.17	0.02	-0.04
Boys (ppp=.50)	2.45 *	1.40 *	0.40 *	0.08	-0.01	-0.01	-0.01	0.03	-0.09	-0.12	-0.11
Cortisol Slope											
Girls (ppp=.40)	2.04 *	1.43 *	0.37 *	0.39 *	-0.07	0.06	-0.40	-0.29	-0.01	-0.03	-0.15
Boys (ppp=.48)	2.45 *	1.26 *	0.39 *	-0.01	-0.01	0.03	0.14	0.03	-0.10	0.07	-0.02
sAA Intercept											
Girls (ppp=.45)	2.12 *	1.88 *	0.65 *	0.63 *	-0.00	-0.07	0.02	0.22	-0.64 *	0.05	0.04
Boys (ppp=.64)	2.22 *	1.25 *	0.85 *	0.60 *	0.06	0.01	-0.06	0.14	-0.27	-0.35 *	0.05
sAA Slope											
Girls (ppp=.36)	2.31 *	2.34 *	0.37 *	-0.11	-0.01	-0.13	0.38	0.32	1.09 *	-0.02	-0.04
Boys (ppp=.61)	2.49 *	1.49 *	0.49 *	0.08	0.02	0.17	0.19	0.10	0.09	-0.27	-0.06

Note. Ovwt=overweight. 21m=21 months; 27m=27 months; 33m=33 months. ppp=posterior predictive p-value (indicates model fit). b =path indicated in Figure 1.

* $p < .05$.