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Abstract

This study explored the associations between the frequency and effectiveness of habitual stress coping strategies on physiological and psychological stress responses to an acute laboratory stressor in adolescents with overweight/obesity (51 adolescents; 47% female; ages 14-19 years). Coping strategies were assessed using the Schoolager's Coping Strategies Inventory. Acute physiological stress responses were measured as salivary cortisol and α-amylase output during the Trier Social Stress Test (TSST) and during a control condition. Acute psychological stress was measured using a Likert-type scale, and systolic blood pressure (SBP) and heart rate (HR) were measured at baseline. Results revealed that higher coping effectiveness was associated with lower log-based α -amylase during the stress (β = -0.025, p=0.018) and control (β = -0.030, p=0.005) conditions, but not with cortisol across either condition (all ps>0.05). SBP moderated the association between coping effectiveness and α -amylase during the stress condition, with higher coping effectiveness associated with lower α -amylase only among individuals with lower SBP (β =0.002, p=0.027). Coping frequency was not associated with cortisol responses, neither was habitual stress coping strategies associated with psychological stress (all ps>0.05). These findings provide preliminary evidence that effective use of stress coping strategies may provide a dampening effect on sympathetic activity in an at-risk adolescent population. Key words: Stress reactivity, overweight/obesity, coping, alpha-amylase, cortisol

INTRODUCTION

Adolescence is a critical developmental period often marked by heightened stress exposure, appraisal, and reactivity (Bos et al., 2014; Pervanidou & Chrousos, 2012). This period of heightened stress is due, in part, to the numerous biological and social-environmental changes experienced by adolescents during these formative years (Rith-Najarian et al., 2014). For example, the transition to high school is highlighted by increased academic and achievementrelated expectations from parents and teachers. Furthermore, adolescents face increased challenges and conflicts related to peers, friends, parents, and romantic relationships (Markova & Nikitskaya, 2017; Rith-Najarian et al., 2014; Roemmich et al., 2014). These demands often serve as novel stressors for many, leading them to perceive these transitional events as more stressful (Rith-Najarian et al., 2014).

Evidence also suggests that adolescents with overweight/obesity experience even greater stress exposure because of their weight. In comparison to their healthy weight counterparts, adolescents with overweight/obesity experience greater exposure to psychological stressors in the form of weight teasing, social devaluation, and bullying (Brixval et al., 2012; Pont et al., 2017). Additionally, adolescents with overweight/obesity are also more likely to encounter interpersonal stress and criticism from close family members because of their weight (Puhl & Luedicke, 2012). These stressors can be both acute and chronic in nature, in that a brief, timelimited stress exposure can become a chronic stressor over time when the stressor is experienced repeatedly. For example, if an adolescent experiences weight-based teasing or bullying during the lunch hour at school, this one-time isolated encounter can be conceptualized as an acute social stressor. However, if the adolescent has repeated exposure to bullying (e.g. daily occurrence during the lunch hour) or perceives the continual threat of such events with no clear ending, then this acute stressor can manifest into a chronic stressor. Given the increased stress exposure experienced by adolescents with overweight/obesity, there is a critical need to understand the relationships between psychological stress and physiological stress responses in this at-risk group.

Psychological stress is a negative affective state that can trigger a cascade of stress hormones that produce physiological changes throughout the body (Baum, 1990; Pervanidou & Chrousos, 2012). Activation of this internal stress system works primarily through the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) (Murison, 2016; Pervanidou & Chrousos, 2012). Activation of the HPA axis triggers the release of cortisol (Schumacher et al., 2013) whereas activation of the ANS, which includes both parasympathetic and sympathetic effects, can result in heightened vigilance, heart rate (HR), blood pressure, and α -amylase (Gordis et al., 2006; Jayasinghe et al., 2014). Salivary α -amylase is believed to be an indirect marker of sympathetic nervous system (SNS) activity in that the release of epinephrine at sympathetic nerve terminals on adrenergic receptors in salivary glands leads to the increased production of the salivary enzyme α -amylase (Granger et al., 2007). In healthy individuals, both salivary cortisol and a-amylase concentrations are elevated during exposures to acute psychological stressors and quickly return to baseline levels shortly after the stressor is no longer experienced. In the case of repeated exposures to a stressful event, or chronic stress, a disruption in the physiological stress response can occur. This, in turn, can lead to elevated and diminished cortisol and α -amylase concentrations both at rest and in response to an acute stressor, both of which are associated with pathological conditions ranging from psychiatric disorders to cardiometabolic diseases in children and adults (Bauer et al., 2002; Cohen et al., 2007; Roemmich et al., 2014).

The interrelationships between the HPA-axis and the ANS have been well documented (Allwood et al., 2011; Flaa Arnljot et al., 2008; Kapuku et al., 2002). Allwood and colleagues conducted a study in children and adolescents and observed a significant positive association between salivary α -amylase with systolic blood pressure (SBP) and HR responses during laboratory stress tasks. In the same study, investigators also observed a significant positive association between cortisol and blood pressure responses (although not with HR response). In a longitudinal study conducted in older adolescents, researchers found that SNS activity (catecholamine response) during the mental arithmetic task predicted future SBP (Flaa Arnljot et al., 2008). When examining the relationship between cortisol secretion and cardiovascular responses during two laboratory stressors (video game stressor and a cold pressor test) Kapuku and colleagues observed a significant association between cortisol and SBP responses in a sample of African-American adolescent males (Kapuku et al., 2002). Given the interrelation of the parameters (cortisol, α -amylase, blood pressure, and HR), repeated exposure to acute stressors may lead to the independent or collective dysregulation of each of these parameters. Identifying factors that may buffer the effects of stress on HPA and ANS activity is critical to decelerating the progression towards cardiovascular disease in adolescents with overweight/obesity.

Habitual stress coping behaviors may help to alleviate stress or produce adaptive physiological responses to stress as previous studies have shown that coping influences both blood pressure and HR responses to stress (Florence et al., 2018; Kato, 2017). Coping is defined as dynamic cognitive and behavioral efforts used by an individual to minimize stress (Lazarus & Folkman, 1984). The frequency and effectiveness of coping strategies employed by adolescents provide important information about ways in which they attempt to manage their stress. Coping

frequency provides the relative use of coping strategies that adolescents engage in during and/or following a stressor, whereas coping effectiveness describes the degree to which coping strategies alleviate negative emotions caused by stress (Nicholls et al., 2009). Effective coping promotes growth and generally leads to positive outcomes, whereas ineffective coping may increase adolescents' risk for emotional and behavioral issues (Connor-Smith & Compas, 2004; Kobus & Reyes, 2000). Examining both aspects of coping is essential, as increased coping frequency may not necessarily be indicative of effective coping behavior (Nicholls et al., 2009). If an adolescent frequently and effectively copes with a stressor, then resilience to stress may occur. On the other hand, if an adolescent frequently employs ineffective coping strategies, heightened stress responses and greater stress vulnerability will likely ensue.

Previous studies conducted in adolescents have demonstrated the stress-reducing effects of frequent and effective coping strategies (e.g. (Drake et al., 2015; Sladek et al., 2017; Slatcher et al., 2015; Wadsworth et al., 2018). Sladek and colleagues examined whether individual and day-to-day differences in adolescent girls' coping responses were associated with cortisol activity. They found that girls who engaged in more frequent active coping strategies when faced with an interpersonal stressor, displayed lower total diurnal cortisol output, a sharper decline in cortisol slopes, and lower awakening cortisol responses compared with girls who engaged in less frequent active stress coping strategies (Sladek et al., 2017). Similarly, Drake and colleagues observed that older adolescents with lower coping efficacy (defined as their belief in being able to cope with future stressors) coupled with higher loneliness displayed a dampened stress response throughout the day. Those with high coping efficacy and high loneliness had the steepest cortisol slopes—suggesting a protective effect of coping effectiveness on cortisol recovery and overall HPA axis functioning (Drake et al., 2015). Slatcher and colleagues in a

sample comprised of children and adolescents observed that greater use of positive coping strategies (e.g. problem solving and cognitive reframing) was associated with greater awakening cortisol (a beneficial response), while greater use of negative (maladaptive) strategies was associated with lower awakening cortisol and a flatter diurnal cortisol slope (Slatcher et al., 2015). Steeper cortisol slopes and better cortisol recovery following a laboratory stressor have also been observed in children who employed less disengagement coping compared with those who employed high disengagement coping (Wadsworth et al., 2018). Together, these studies provide preliminary evidence of the benefits associated with frequent and effective coping strategies to minimize physiological stress responses. Additional studies are needed however, to determine whether habitual coping strategies are effective in minimizing psychological and physiological stress response to an acute stressor in adolescents with obesity-related HPA and ANS dysregulation.

The purpose of this study was to explore the associations between the frequency and effectiveness of habitual stress coping strategies on psychological, cortisol, and α -amylase stress responses during an acute laboratory stressor—the Trier Social Stress Test (TSST). It was hypothesized that greater habitual coping frequency and effectiveness would be associated with lower psychological, cortisol, and α -amylase stress responses during the laboratory stressor. Given the interrelationships between cortisol, α -amylase, and other cardiovascular parameters, a secondary aim of this study was to examine the moderating role of SBP and HR on the relationship between habitual coping and acute stress responses. It was hypothesized that the significant associations between habitual coping measures and stress reactivity would be moderated by SBP and HR; with greater coping frequency and effectiveness associated with

lowered stress responses among those with low to normotensive blood pressure and HR, compared with those with higher blood pressure and HR.

METHODS

Study Participants

Adolescents with overweight/obesity (ages 14-19 years) were recruited from the greater Ann Arbor and Ypsilanti, Michigan areas to participate in the laboratory-based experimental study, Stress Reactivity in Adolescents Study (SRAS). Participants were excluded from SRAS if: (i) they were taking medications known to influence body composition or metabolism; (ii) had a previous history of metabolic diseases; (iii) had a clinical diagnosis of depression or other mental health disorders; (iv) were current smokers; (v) had any food allergies, intolerances, and other dietary restrictions; (vi) had enrolled in a weight loss program in the last 6 months; (vii) or if a female was pregnant or currently taking birth control. Data collection for SRAS occurred between the fall of 2014 to the summer of 2017. Due to the possible influence of pubertal maturation on coping and stress among adolescents (e.g. Nicholls et al., 2009), and the desire to have greater internal validity, all enrolled adolescents were post-pubertal. Prior to any data collection procedures, written informed consent and assent were obtained from parents and adolescents. Assent was obtained if the participant was younger than 18 years of age. Participants were compensated fifty dollars for completed participation in the study. The University of Michigan institutional review board approved this study.

Stress Reactivity Study in Adolescents (SRAS) Study

The primary aim of SRAS was to examine the physiological (cortisol and α -amylase reactivity) and behavioral (caloric intake) responses to an acute laboratory stressor in adolescents with overweight/obesity. The present study is a secondary analysis of SRAS examining habitual

coping strategies and their relation to acute psychological and physiological stress responses during an acute laboratory stressor. Experimental visits were randomized and completed on the same day of the week for two consecutive weeks, between 1500-1630 hours to control for the diurnal rhythmicity of cortisol. All participants took part in both the stress and control experimental conditions and were instructed to refrain from food or drink at least 1 hour before each visit.

Stress condition

The stress condition consisted of participants completing an acute laboratory stressor, the TSST, resembling real-world cognitive and psychosocial stressors experienced by adolescents in school environments (e.g. oral presentations, timed math examinations, social evaluation). Moreover, the social evaluation and mental arithmetic segments of the TSST also functioned to reinforce the harmful, pervasive, and negative stereotypes that are often attributed to individuals with overweight/obesity [i.e. weak-willed, unintelligent, and unsuccessful (Puhl & Heuer, 2010)]. The TSST has been shown to be a validated means to induce glucocorticoid responses in children and adults (Kirschbaum & Hellhammer, 1994). The TSST lasted a total of 20 minutes and included an instructional and preparation period before the test. As a means of controlling for the potential influence of body position on the physiological stress responses, participants were instructed to maintain an upright posture during the TSST (Nair et al., 2015). Following the stress condition, participants were taken to a separate room and were instructed to rest with the option to eat at their leisure for one hour.

Control (rest) condition

The control condition followed the same timeline as the stress condition, with the TSST substituted with a segment from a low-affect educational film. All participants watched the same

film. Similar to the stress condition, participants were instructed to stand during the film to control for any potential positional effects on physiological responses. Following the low-affect educational film, participants were taken to a separate room and were instructed to rest with the option to eat at their leisure for one hour. A detailed description of the study protocol has been previously published (Nagy et al., 2019).

Dependent Variables

Physiological responses during the stress and control conditions

Physiological responses were measured as cortisol and α -amylase responses during the stress and rest conditions. Salivary cortisol and α -amylase samples were collected at five time points throughout both experimental conditions: minutes 10, 25 (pre-TSST), 58 (post-TSST), 88 and 118. Saliva was collected using 2 mL SalivaBio Cryovials (Passive Drool; Salimetrics LLC, State College, PA) and stored at -80°C upon the completion of the experimental visits. Samples were shipped on dry ice to Salimetrics' SalivaLab (Carlsbad, CA), where cortisol levels were determined using the Salimetrics high-sensitive cortisol assay kit (Salimetrics, Carlsbad, CA), without modifications to the manufacturers' protocol. Cortisol samples were assayed in duplicate. The average coefficient of variation for all samples tested were less than 3%. Sample test volume was 25 µL of saliva per determination. The assay has a lower limit of sensitivity 0.007 µg/dL, standard curve range between 0.012 µg/dL and 3.0 µg/dL.

Alpha-amylase samples were assayed in duplicate using the Salimetrics Kinetic Reaction Assay Kit without modifications to the manufacturers' protocol. The average coefficient of variation for all samples tested was less than 5.1%. 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. Results were computed in U/mL of α -amylase using the following formula: [Absorbance difference per minute * total assay volume (328 ml) * dilution factor (200)]/[millimolar absorptivity of 2-chloro-p-nitrophenol (12.9) * sample volume (.008 ml) * light path (.97)]. *Psychological stress responses*

Acute psychological stress responses were assessed prior to being given the instructions for the TSST and upon completion of the TSST. Before being given instructions, participants were asked how much they agreed or disagreed with the following statement: "*The upcoming task is very stressful.*" Participants were asked to respond to the question by marking their answers on a Likert-type scale anchored by strongly disagree (-4) to strongly agree (4). Following the completion of the TSST, participants were asked how much they agreed or disagreed with the statement: "*The prior task was very stressful*," with responses again anchored by strongly disagree (-4) to strongly agree (4). Raw scores for acute psychological stress pre- and post-TSST were standardized and then subtracted to create a standardized change score (Δ acute stress) between the acute anticipatory and retrospective stress scores of each participant. These change scores were calculated as the standardized difference between the retrospective and anticipatory scores. Likert-type scales are commonly used to measure momentary changes in psychological stress, and have been previously used among children and adolescents (Feda et al., 2016; Roemmich et al., 2007).

Independent Variable

Habitual stress coping strategies

Before completing the experimental protocol, participants completed psychological and health behavior questionnaires including the Schoolager's Coping Strategies Inventory (SCSI). The SCSI is a 26-item questionnaire that assesses the frequency and effectiveness of coping strategies used by individuals when stressed, nervous, or worried (Ryan-Wenger, 1990). An

example item from the SCSI includes: "*Think about when you feel stressed, nervous, or worried. How often do you eat or drink? (Part A) How much does it help?*" (*Part B*). Both frequency (Part A) and effectiveness (Part B) of each question were measured on a 4-point Likert-type scale. Frequency was measured on a scale anchored by "never" (0) to "most of the time" (3), while effectiveness was measured on a scale anchored by "never do it" (0) to "helps a lot" (3). The Cronbach's alpha for the SCSI was: coping effectiveness (α =0.86), coping frequency (α =0.89), and for the full survey (α =0.93). The SCSI has been previously validated in children (Ryan-Wenger, 1990) and has been used in both children and adolescents (Mohammad et al., 2015).

Covariates

Given the possible confounding effects of body mass index (BMI), race, sex, and age (Chapman & Mullis, 2000; Hampel & Petermann, 2006; Roemmich et al., 2007; Valiente et al., 2015; Verdejo-Garcia et al., 2015) on stress coping and stress reactivity, these variables were included as covariates within the statistical models. Anthropometric measures of height and weight were measured in the laboratory by trained study staff members. Height (cm) was measured to the nearest 0.1 cm using ShorrBoard ® (Weigh and Measure, LLC. Olney, MD) with standardized procedures (Shorr, 1984). Weight (kg) was measured to the nearest 0.1 kg, using an electronic scale (Doran Scales, Inc, Batavia, IL). Height and weight were used to calculate each participant's BMI (kg/m²), which was then converted to BMI z-scores (z-BMI) based on the U.S. Centers for Disease Control and Prevention guidelines (http://www.cdc.gov/growthcharts). BMI z-scores as opposed to BMI percentile were used given the concerns about BMI percentile truncating at the 99th percentile for age and sex in youth. Race/ethnicity and biological sex were self-reported.

Moderators

Given the potential moderating effects of SBP and HR on the relationship between stress coping and stress reactivity (Allwood et al., 2011; Kato, 2017), these variables were included as moderators within the statistical models. Resting baseline SBP for each participant was taken during the baseline period, before the commencement of the TSST or the low-affect educational film. SBP was measured using a digital blood pressure monitor (Omron HEM907XL, Kyoto, Japan). All blood pressure readings were taken in a seated position using appropriate blood pressure cuff sizes and were recorded in mmHg. Resting HR was measured using HR monitors (Polar FT1 Heart Rate Monitor, Polar USA). HR was recorded during the baseline period before the commencement of the TSST or the low-affect educational film. All measurements were recorded in beats per minute (bpm).

Statistical analysis

Statistical analyses were performed using SPSS (IBM SPSS version 25.0, IBM Corp., Armonk, NY) for the confirmatory stress analyses and Stata 16.0 (StataCorp LP, College Station, TX) for multilevel modeling. To confirm that a stress response occurred as a result of the TSST, one-way repeated-measures ANOVAs were conducted to examine the main effects of time and condition on the psychological and physiological stress responses, respectively. For the physiological stress confirmatory analyses, the area under the curve with respect to ground (AUCg) was calculated for cortisol and α -amylase responses during the stress and control conditions using the trapezoidal formula (Pruessner et al., 2003).

Multilevel regression analyses were conducted using the MIXED procedure command with maximum likelihood estimation, to examine cortisol and α -amylase trajectories in response to the social-evaluative stressor and during the control condition. Multilevel modeling methodology considers the dependent nature of the physiological stress variables (i.e. α -amylase measures) nested within each participant. Multilevel modeling also allows for the examination of within-person (level 1) changes over time as well as between-person (level 2) differences in physiological stress reactivity over time. Before the analyses, cortisol (µg/dL) and α -amylase (U/mL) values were natural-log transformed to help normalize their distribution and correct for high right-skewness in the distribution of both variables. To control for any possible effect of time (i.e., diurnal cortisol rhythm) on the dependent variables, a time of day variable (i.e. the five sample collection time points) was included as a predictor in all analyses. Additionally, all continuous predictors were grand-mean centered (e.g. mean age across all participants was subtracted from each participant's age).

Preliminary analyses were conducted using unconditional (null) models with no predictors to assess the between- and within-person variance for the dependent variables (log-cortisol and log α -amylase levels). Following the preliminary analyses, the independent variables (coping frequency and coping effectiveness) were entered, and then the covariates (race, sex, z-BMI, and age) were included in all models. As a means of examining the moderating effects of SBP and HR in the associations between the independent variables of coping and the dependent stress response variables, additional models were conducted with an SBP interaction term as well as a HR interaction term included in separate models.

Lastly, multivariable linear regression models were used to examine the association between coping frequency and effectiveness and the subjective psychological stress outcome (Δ acute stress). The same covariates used in the multilevel linear regression models were used in the multivariable regression models. An alpha level of α <0.05 was used to determine significance for all analyses.

RESULTS

Participant characteristics

A total of 60 participants completed the study. However, nine participants were excluded due to incomplete data and saliva samples that were insufficient to adequately complete the study analyses. As such, the analyses described in the results were completed for the remaining 51 participants who did not have missing data. Using the remaining 51 participants, the power to detect statistical significance at p<0.05 was 0.80 with a medium effect size (*f*=0.18). There were no significant differences in demographic variables between individuals who were included and excluded in the present analyses (p-values >0.05). Participant characteristics for the 51 participants included in these analyses are presented in Table 1. In summary, study participants were: 53% male (47% female), 45% African American (55% non-Hispanic white), 17.3 \pm 1.2 years of age, and had a mean BMI of 31.1 \pm 5.3 kg/m² with 47% of the adolescents classified as having overweight, and 53% as having obesity. *Insert Table 1 here.*

Confirmation of the stress response

Confirmatory analysis of physiological stress responses by stress and rest conditions and psychological stress response before and after the TSST is presented in Table 2. The confirmatory analysis supported the successful induction of a stress response during the stress condition. There were significant differences across conditions for stress reactivity (measured as the period before the TSST to the end of the TSST task) for both salivary cortisol (stress: $6.59\pm0.55 \ \mu g/dL \ vs \ rest: 3.52\pm0.30 \ \mu g/dL; \ p<0.01)$ and salivary α -amylase (stress: $3439.11\pm387.0 \ U/mL \ vs \ rest: 2661.73\pm279.7 \ U/mL; \ p=0.025)$, with a large effect size for cortisol reactivity (eta-square= 0.367) and a medium effect size for α -amylase reactivity (eta-square= 0.098). There was also a significant difference across conditions for stress recovery (end

of the TSST task to the end of the 1-hour recovery period) for salivary cortisol (stress: $10.70\pm0.93 \ \mu g/dL$ vs rest: $5.57\pm0.42 \ \mu g/dL$; p<0.01), with a large effect size (eta-square= 0.361). There was not however a significant difference across conditions for α -amylase stress recovery (stress: $5403.79\pm497.0 \ U/mL$ vs rest: $4759.12\pm490.0 \ U/mL$; p=0.18), with a small effect size observed (eta-square= 0.036). For acute psychological stress, there was a significant difference in self-reported psychological stress levels before and after the TSST (pre-TSST: 0.24 ± 0.292 vs post-TSST: 2.04 ± 0.328 ; p<0.01), with greater psychological stress levels post-TSST compared with pre-TSST. A large effect size was observed for acute psychological stress (eta-

square=0.233). Insert Table 2 here

Preliminary analyses of between- and within-person variance for physiological stress measures

Results from the unconditional (null) models with no predictors for the outcome variables were used to calculate the intraclass correlations coefficients (ICCs). The ICCs were then used to determine the between-person variance for the outcome variables during the stress and control conditions over the five-sample collection times for the stress and rest conditions. The ICCs for the unconditional models were; ICC_{stress α -amylase = .675, ICC_{stress cortisol}= .295, ICC_{control α -amylase = .721, ICC_{control cortisol}= .444. The residual variance for the variables, obtained by using the formula 1-ICC, indicated that approximately 32.5% of the variation in α -amylase output during the stress condition could be explained by within-person differences (i.e. level 1 covariates) and approximately 27.9% of the variation for α -amylase during the control condition could be explained by within-person differences, with approximately 55.6% of the variation for cortisol during the control condition could be explained by within-person differences, with approximately 55.6% of the variation for cortisol during the control condition could be explained by within-person differences. Overall, the ICCs from the unconditional models during the stress and control}}

conditions indicate that the physiological stress outcomes differ across individuals as well as within individuals for the present sample.

Predictors of the stress response

Table 3 displays the associations between the coping measures and the physiological outcomes at rest and during the stress condition. It was observed that coping effectiveness was associated with log-based α -amylase output during the stress condition (β = -0.025, p=0.018) as well as during the control condition (β = -0.030, p=0.005), with increased coping effectiveness associated with lowered α -amylase output. Coping effectiveness was not associated with cortisol across either condition (all ps>0.05). Coping frequency was not associated with α -amylase nor cortisol responses across both conditions. *Insert Table 3 here*.

Table 4 displays the associations between coping measures, α -amylase physiological outcomes, and the moderating effects of SBP and HR. The interaction models revealed that SBP at baseline moderated the association between coping effectiveness and log-based α -amylase (β =0.002, p=0.027) during the stress condition (Figure 1). The model suggested that for individuals with lower baseline SBP (1-standard deviation below the grand mean), higher coping effectiveness was associated with lower α -amylase output. A moderating effect of SBP on the association between coping effectiveness and α -amylase during the control condition was not observed (p>0.05). A moderating effect of HR was not observed during both the stress and control conditions (ps>0.05). *Insert Table 4 and Figure 1 here.*

Table 5 displays the associations between coping frequency and effectiveness with acute psychological stress. There was no association between coping frequency and acute psychological stress, nor was there an association between coping effectiveness and acute psychological stress (all ps>0.05). *Insert Table 5 here.*

DISCUSSION

The present study was an exploratory analysis examining the associations between the frequency and effectiveness of habitual stress coping strategies on both the acute psychological and physiological stress responses during an acute laboratory stressor in adolescents with overweight/obesity. It was observed that greater coping effectiveness was associated with lowered α -amylase responses, but not cortisol, during the stress and control conditions, and this relationship was moderated by resting SBP during the stress condition. Habitual coping strategy frequency was not associated with α -amylase nor cortisol responses during both conditions. Lastly, there were no significant associations between coping strategy frequency and effective use of stress coping strategies may help confer a dampening effect on sympathetic activity in adolescents with overweight/obesity. If confirmed in a large-scale longitudinal study, effective coping strategies may provide physiological resilience in an adolescent population at increased risk for cardiometabolic diseases.

Few studies in children and adolescents have explored the relationship between coping and α -amylase stress responses; findings have been mixed, and most have focused on the type of coping strategy employed. Katz and colleagues noted in a sample of adolescents that greater levels of disengagement coping (e.g. avoidance) predicted lower peak α -amylase activation, but not α -amylase reactivity or recovery following a social stressor (Katz et al., 2019). In another study conducted in preadolescents, Bendezu and colleagues observed that involuntary stress responses (a broad measure of one's immediate cognitive, behavioral, emotional, and physiological responses to stress) did not impact α -amylase reactivity. These involuntary stress responses did, however, influence α -amylase recovery, but it was dependent upon the type of

coping strategy a child was primed to use following the stressor (Bendezú et al., 2016). In the present study, greater coping effectiveness was associated with lower α -amylase output both at rest and during the stress condition. One possible explanation for these differing findings may relate to the moderating effects of SBP. Individuals in the present study with lower resting SBP (SBP at or below 1 SD of the grand mean) who used more effective coping strategies experienced a greater dampening of α -amylase output in comparison to those with average and higher SBP (at the grand mean and +1 SD) within the sample. Other investigators have also noted a relationship between effective coping and low resting SBP (Jones et al., 2018). In a cohort of 261 adolescents aged 13-16 years, Jones et al. observed lower resting SBP and smaller waist-to-hip ratios in adolescents that used more emotion regulation strategies (cognitive reappraisal) in response to chronic stress exposure. It is plausible that the lower SBP observed in the present study among some of the participants was an adaptive response to employing effective coping strategies to social-environmental stressors. As a result, individuals were "protected" from the heightened or dysregulated α -amylase response to acute stress, a profile often seen among individuals with overweight/obesity. On the other hand, it is also plausible that those with average or higher resting SBP may have had greater exposure to social-environmental stressors and thus the protective effects of coping were absent in this group of adolescents who may already have underlying dysregulated SNS activity as a function of their weight status (Tentolouris et al., 2006). Additional research is needed to better understand the physiological resilience observed in adolescents with overweight/obesity and low SBP.

As it relates to cortisol, no significant associations were observed between coping effectiveness and cortisol responses. These findings are in contrast to those of Drake and colleagues who in a prior study observed a beneficial effect of coping efficacy on diurnal cortisol

patterns (steepness in slopes) (Drake et al., 2015). Similarly, the same group of researchers in a different study also observed that when adolescents perceived greater than usual stress, greater cortisol levels are only seen among individuals who have low (below average) coping efficacy (Sladek et al., 2016). Differences in study findings between Drake, Sladek, and the present study may be due in part to differences in study populations (healthy weight vs overweight/obese). Excess weight has been previously shown to be associated with dysregulated cortisol activity (Incollingo Rodriguez et al., 2015), thus adolescents with overweight/obesity may be experiencing HPA axis dysregulation, and as a result, may not demonstrate the "protective effect" of coping effectiveness on HPA axis functioning. Evidence from other investigators have supported this hypothesis (e.g. (Hillman et al., 2012; Pervanidou et al., 2015; Ruttle et al., 2013). For example, Hillman and colleagues had previously observed that cortisol reactivity was associated with adiposity, suggesting a differential response to stress may be dependent or related to weight (Hillman et al., 2012). Thus, it may be that the beneficial effect of coping on cortisol responses in adolescents may not be seen due to underlying cortisol dysregulation related to weight status. Additional research is needed with a healthy weight control group to verify these hypotheses.

Previous research examining coping frequency and stress reactivity are mixed. For example, Sladek and colleagues observed that perceiving greater stress than usual was associated with elevations in cortisol (relative to diurnal patterning) only during situations when adolescents reported greater frequency of coping than usual (Sladek et al., 2016). In another study, Foland-Ross and colleagues observed that among adolescent girls with familial risk for depression (i.e. mothers diagnosed with depression) who more frequently used involuntary relative to voluntary coping strategies, had significantly greater overall diurnal cortisol release (Foland-Ross et al., 2014). Similarly, other investigators have observed that greater use of adaptive coping strategies

in such forms as problem-solving and problem-focused coping have been associated with beneficial cortisol responses on stress-tasks in children and older adolescent college-students (Matheson & Anisman, 2009; Slatcher et al., 2015). It is plausible that differences among the present study findings and that of previous studies may be due in part to differences in the type of coping strategies measured, particularly as a global measure of coping frequency was presently used as opposed to specific types of coping strategies. Empirical evidence suggests that adolescents with overweight/obesity more frequently cope using avoidant strategies in response to the increased stress they experience as a result of their weight (Himmelstein & Puhl, 2019; Puhl & Luedicke, 2012). Thus, adolescents with overweight/obesity may be engaging in other habitual coping strategies that were not captured by the SCSI. Together, these studies point to the continued need for examining the type of coping strategy employed by adolescents beyond that of assessing the frequency and effectiveness of general measures of coping.

In our present study, neither coping frequency nor coping effectiveness was associated with changes in acute psychological stress responses. The lack of association between habitual coping strategies and acute psychological stress responses in the present study may be a result of the temporal mismatch between measures of coping and stress. It is important to note that coping is a dynamic process, and the temporal nature of our measurements (habitual vs. acute) may have prohibited our ability to observe a significant relationship between stress coping and psychological stress. Moreover, the use of a single-item measure of acute psychological stress is a notable limitation that may have also hampered our ability to observe a significant relationship. It is also plausible that the lack of an association between coping strategies and acute psychological stress responses observed presently may indicate that the physiological resilience experienced by participants with low SBP is independent of the negative affect experienced

during an acute stressor. From a developmental perspective, timing of puberty may have contributed to the physiological vulnerability observed in participants with normal to higher SBP. Early menarche is associated with an overweight profile during childhood, and increased risk for cardiovascular diseases such as hypertension (Ahmed et al., 2009; Canoy Dexter et al., 2015; Day et al., 2015). Investigators have previously observed that adolescent girls with early puberty had higher systolic blood pressure compared with those with later maturation at baseline and after a one and a half-year follow-up. A similar pattern was observed in boys, although the difference was not statistically significant at follow-up (Chen & Wang, 2009). Other studies exploring early timing of puberty have found similar results (e.g. (Berentzen et al., 2017; Chen & Wang, 2009; Remsberg et al., 2005), pointing to an increased vulnerability for hypertension and cardiovascular disease with early pubertal development. Early pubertal maturity may also affect social relationships with peers and family members (Holmbeck, 2002), which in turn may influence psychological stress levels. Peer acceptance and "fitting in" is one of the primary aims of youth; those who develop earlier or are physically distinguished from their peers, and may have a more challenging time fitting in and maintaining friendships due to accelerated development. Given the empirical evidence that suggests that overweight/obesity is associated with earlier pubertal development (e.g. Kaplowitz, 2008; Lee et al., 2016), adolescents with lower SPB in comparison with those with normal to prehypertensive counterparts, may have physiological resilience independent of the negative affect experienced during an acute stressor. Future research should examine stress reactivity and adolescent coping strategies across the developmental stages of puberty to better understand the physiological resilience observed in the present study.

Many important strengths and limitations of this study should be noted. Strengths of the study include the use of a validated, empirically supported laboratory stressor with high internal validity. Secondly, the use of a comprehensive assessment of acute physiological stress responses (cortisol and α -amylase) during both a stress and control condition. Lastly, the inclusion of a racially diverse sample of an "at-risk" target group are notable strengths of this study. Despite these strengths, the findings from this study should be interpreted in light of its limitations. Firstly, the cross-sectional study design limits inferences on the directionality of the associations between coping strategies and stress reactivity. Additionally, the use of the SCSI originally validated in children and not adolescents, without differentiation between primary and secondary coping, is another limitation. Moreover, the lack of a healthy control group prevented any conclusive assumptions regarding the protective effect of coping on α -amylase stress responses. Future studies examining these associations should include a healthy control group. Furthermore, the operationalization of acute psychological stress via a single-item measure may have hampered our ability to comprehensively examine the effects of habitual coping strategies on acute psychological stress. Future studies should utilize a more comprehensive measure of acute psychological stress to properly capture the relationship between habitual stress coping and psychological stress. Lastly, this was an experimental study conducted in a laboratory setting with small sample size and thus cannot be readily generalized to real-world settings. Despite these limitations, it was observed that greater coping effectiveness was associated with lower αamylase responses at rest and during an acute stressor in adolescents with overweight/obesity.

CONCLUSION

Adolescence is a period of heightened stress exposure, appraisal, and reactivity. Adolescents with overweight/obesity are particularly susceptible to the deleterious effects of

stress. Coping strategies are a modifiable target to reduce the negative impact of cognitive, social, and environmental stressors often experienced by adolescents with overweight/obesity. The present findings provide preliminary evidence that suggests effective habitual coping strategies are associated with decreased acute physiological stress responses among adolescents with overweight/obesity and lower blood pressure profiles. If replicated in a larger, longitudinal study that utilizes a control group, effective habitual coping strategies may help provide physiological resilience to adolescents with overweight/obesity, a condition associated with dysregulated sympathetic nervous system activity (Tentolouris et al., 2006). With the increased stress and mental health challenges observed among adolescents in the twenty-first century (Bor et al., 2014), along with the heightened and often overlooked burden of weight stigma on physical and mental wellbeing (Puhl et al., 2020), the importance of finding approaches to help reduce stress and mitigate its psychological and physiological health implications among youth is vital. Strategies such as a family-based multicomponent behavioral program equipping adolescents and caregivers with tools to manage maladaptive stress coping behaviors (Llabre et al., 2020), or modifying environments to provide youth with healthy alternative activities (e.g. physical activity) are viable options (Carr & Epstein, 2020). Nevertheless, in light of the many insignificant findings of the present study, these findings should be confirmed in a large-scale longitudinal study among adolescents of varying weight status to be conclusive.

		1											
Variables	Mean (SD)		2	3	4	5	6	7	8	9	10	11	12
1. Coping frequency	30.23 (8.34)	1											
2. Coping effectiveness	35.55 (9.97)	0.61*	1										
3. Sex (% female)	47%	0.2*	-0.03	1									
4. Race (% Non- Hispanic white)	55%	-0.34*	-0.14	-0.14	1								
5. Age (years)	17.3 (1.2)	-0.2*	-0.20*	-0.05	0.35*	1							
6. BMI (kg/m ²)	31.1 (5.3)	0.16	-0.15	0.20*	-0.28*	-0.24*	1						
7. BMI%ile	94.0 (4.4)	0.09	0.02	0.05	-0.39*	-0.29*	0.82*	1					
8. BMIz	1.72 (0.5)	0.19*	-0.01	0.10	-0.35*	-0.25*	0.89*	0.91*	1				
9. Systolic blood pressure (mmHg)	113.3 (13.6)	-0.14	-0.01	-0.40*	-0.14	-0.04	-0.05	0.16	0.06	1			
10. Diastolic blood pressure (mmHg)	71.0 (11.4)	-0.16	-0.14	-0.21*	-0.16	0.01	0.24*	0.37*	0.33*	0.69*	1		
11. Resting heart rate (bpm)	79.9 (12.8)	0.05	-0.16	0.11	-0.09	-0.11	0.38*	0.23*	0.31*	0.19*	0.28*	1	
12. Perceived Stress	24.0 (6.8)	0.2*	0.01	0.22*	0.03	-0.20*	0.17	0.12	0.12	-0.28*	-0.30*	-0.05	1

Table 1. Participant characteristics and zero-order correlations. Statistics are in mean and SD. BMI, body mass index, SBP, systolic blood pressure, DBP, diastolic blood pressure, bpm, beats per minute. *p<0.01. Percentages for dichotomous variables.

¥ 7.	mean±	SE	p-value	eta-square
Physiological stress response	Stress	Rest		
Cortisol reactivity (µg/dL)	6.59±0.55	3.52±0.30	< 0.01	.367
Salivary α -amylase reactivity (U/mL)	3439.11±387.0	2661.73±279.7	0.025	.098
Cortisol recovery (µg/dL)	10.70±0.93	5.57±0.42	< 0.01	.361
Salivary α -amylase recovery (U/mL)	5403.79±497.0	4759.12±490.0	0.18	.036
Psychological stress response	Pre-TSST	Post-TSST		
Self-reported acute psychological stress	$0.24 \pm .292$	$2.04 \pm .328$	< 0.01	.233

Table 2. Confirmatory analysis of physiological stress responses by stress and rest conditions, and psychological stress response preand post-TSST.

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•	Log α-amyl condit		Log α-amylase (control condition)		Log-cortisol (stress condition)		Log-cortisol (control condition)	
Fixed effects	Est.	SE	Est.	SE	Est.	SE	Est.	SE
Intercept:	4.30**	.149	4.06**	.152	-2.01**	.119	-2.42**	.132
Coping frequency	002	.013	.003	.014	006	.011	.012	0.12
Coping effectiveness	025*	.011	030*	.011	001	.009	008	.010
Gender	.187	.171	.278	.175	.053	.136	.096	.152
Age	143*	.072	129	.074	011	.057	.090	.064
z-BMI	.212	.173	.346	.177	023	.137	085	.153
Race	321	.189	126	.193	027	.150	035	.168
Time of day	.001	.001	000	.001	001	.001	004**	.001
Random effects								
Within-person (L1) variance	.199**	.021	.185**	.020	.322**	.034	.268**	.028
Between-person (L2) variance	ce .259**	.063	.274**	.066	.123**	.040	.181**	.050

Table 3. Regression coefficient estimate with standard error (SE). Outcomes calculated from log based salivary cortisol and α -amylase values. *p<0.05; **p<0.01

•	•	Log α-amylase (stress condition)		Log α-amylase (control condition)		Log α-amylase (stress condition)		Log α-amylase (control condition)	
Fixed effects	Est.	SE	Est.	SE	Est.	SE	Est.	SE	
Intercept:	4.32**	.159	4.01**	.171	4.25**	.156	4.02**	.165	
Coping frequency	.001	.013	.004	.014	.012	.015	.010	.016	
Coping effectiveness	029**	.010	031**	.011	042**	.014	039**	.015	
Gender	.135	.182	.317	.195	.226	.169	.304	.180	
Age	175*	.071	138	.076	173*	.072	152*	.077	
z-BMI	.218	.167	.365*	.180	.365*	.178	.433*	.190	
Race	304	.189	081	.203	243	.190	071	.202	
Time of day	.001	.001	000	.001	.001	.001	000	.001	
Coping effectiveness *SBP	.002*	.001	.000	.001					
Coping effectiveness *HR					0.001	.001	.000	.001	
Random effects									
Within-person (L1) variance	.202**	.022	.187**	.020	.198**	.022	.187**	.020	
Between-person (L2) variance	.232**	.058	.276**	.067	.232**	.059	.271**	.067	

Table 4. Regression coefficient estimate with standard error (SE). Outcomes calculated from log-based salivary α -amylase values. SBP=systolic blood pressure; HR=heart rate*p<0.05; **p<0.01. Two separate models were conducted, one with SBP, the other with HR as interaction terms with coping effectiveness.

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Psychological stress	β	SE	p-value
Acute stress (standardized Δ Pre- & Post-TSST)			
Coping frequency	023	.024	0.35
Coping effectiveness	.019	.019	0.34

Table 5. Associations between coping strategy frequency, effectiveness, and acute psychologicalstress. All models included and were controlled for gender, age, z-BMI, and race.

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