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ORIGINAL ARTICLE

Juvenile Children's Salivary Aldosterone and Cortisone Decrease during Informal Math and Table-Tennis Competitions

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Abstract

Objective Among adults, aldosterone and cortisone increases are reported in response to physically taxing forms of competition, enabling individuals to rapidly adapt to variable sociocompetitive contexts. Yet, aldosterone and cortisone responses in juvenile children engaged in less strenuous forms of competition have not been investigated. Here, we sought to measure aldosterone and cortisone responses in children who participated in math and table-tennis competitions. We hypothesized that the responses would significantly vary with respect to the type of competition.
Methods Pre- and post-match salivary aldosterone and cortisone were measured in Hong Kongese children, aged 8–11 years, during (1) a mixed-sex, team-based, math competition ($N = 45$) and (2) a dyadic, table-tennis competition against peers ($N = 22$).
Results In the math competition, aldosterone and cortisone levels decreased in boys and girls, while members on losing teams had greater match decreases in cortisone levels compared to individuals on winning teams. In the table-tennis competition, time of day led to significant diurnal differences in competitors' pre-match aldosterone and cortisone concentrations. As a result, each sample was analyzed independently according to match time (8:30 AM and 11:00 AM). Aldosterone levels decreased among the competitors who participated in the 11:00 AM table-tennis matches. Cortisone levels decreased for the majority of competitors, but only significantly decreased in the 8:30 AM sample.
Conclusion These findings highlight that juvenile competitors' hypothalamic-pituitary-adrenal (HPA) axis and renin-angiotensin-aldosterone system (RAAS) are sensitive to less physically strenuous forms of competition. Further, the differences in the competitive environment likely stimulate the direction of aldosterone (RAAS) and cortisone (HPA) reactive change.

Keywords Life-history theory · Aldosterone · Cortisone · Middle childhood · Competition · HPA axis

Introduction

For humans, it is hypothesized that the protracted juvenile stage of growth and development that spans ~6–11 years of age, also referred to as middle childhood, represents a critical developmental, behavioral, and endocrinological switch point (Benenson 2014; Bogin 1999; Crittenden et al. 2013; Del Giudice 2009; Del Giudice 2018). Behaviorally, middle childhood is characterized by increases in autonomy, social integration, and emergence and intensification of sex differences (Benenson 2014; Crittenden et al. 2013; Del Giudice 2015, 2018; however, see Lew-Levy et al. 2019). Endocrinologically, the juvenile transition is marked by adrenarche, a surge of androgen secretion by the adrenal glands that continues to rise until the mid-20s, while primary sex steroid production (e.g., testosterone, estrogens) remains low (Campbell 2011). Del Giudice (2009, 2018) presents a compelling evolutionary-developmental model of middle childhood wherein a surge of adrenal hormone production (e.g., adrenarche) is hypothesized to integrate genetic, environmental, and social information to promote and calibrate future life-history strategies. However, empirical support for this model, such as measuring adrenal hormone reactivity in children during naturalistic social challenges, rather than baseline hormone correlational studies, remains lacking.

With humans being a hyper-social species, natural selection is predicted to favor endocrinological and behavioral strategies that optimize limited energetic resources to appropriately respond to social challenges and stress across the life-course (Archer 2006; Del Giudice et al. 2009; Flinn et al. 2011; Gray et al. 2017). The human body is adapted to regulate psychosocial and physical stress in multifaceted ways. The major endocrine stress response comprises the renin-angiotensin-aldosterone system (RAAS) and the hypothalamic-pituitary-adrenal (HPA) axis (Ozunal et al. 2020; Saavedra and Benicky 2007; Gideon et al. 2019; Ponzi et al. 2020). The RAAS is central to the regulation of water and electrolyte balance, systemic vascular resistance, and blood pressure (Cartledge and Lawson 2000; Jackson et al. 2018; Joëls and de Kloet 2017). Activation of the RAAS starts with renin release (Connell and Davies 2005). Renin stimulates the formation of angiotensin in the blood and tissue, which promotes the release of aldosterone from the adrenal cortex (Gideon et al. 2019). Recently, the RAAS has been recognized to exert pleiotropic effects independent of cardiovascular homeostasis, including locally within the brain to affect cognition in response to psychosocial stress (Gideon et al. 2019; see review Jackson et al. 2018). Psychosocial stress likely activates the RAAS by first stimulating the sympathetic adrenal medullary (SAM) system, which induces secretion of catecholamines epinephrine and norepinephrine (Connell and Davies 2005). This process promotes renin synthesis which in turn activates the RAAS.

With regard to the HPA axis, activation promotes a cascade of hormone release in response to physical and psychosocial stress: corticotropin-releasing hormone (CRH) and copeptin from the hypothalamus and circulating adrenocorticotropic hormone (ACTH) stimulates the adrenal cortex to secrete androgens (e.g., testosterone, dehydroepiandrosterone (DHEA), androstenedione), mineralocorticoids (e.g., aldosterone), and glucocorticoids (e.g., cortisol, cortisone) (Bae et al. 2019; Gannon et al. 2019). Evidence suggests that HPA activity in early life leads to downstream changes in developmental patterns and adult phenotypes, a hallmark of human developmental plasticity (Ponzi et al. 2020; Flinn 2006).

Within a life-history framework, it is important to consider that juvenile children must compete for limited social resources, such as status, allies, reputations, and friends (Del Giudice 2015). Competition arises when individuals strive for a goal that cannot be shared (Magee and Galinsky 2008; Sapolsky 2004). The results of such contests often enhance social status in winners and decrease status in losers. Social competition may include physical and indirect forms of aggression (e.g., social exclusion) and prosocial behaviors, such as displaying skill and cooperative abilities (Del Giudice 2015; Hawley 2014). Such traits are visibly on display during sports competitions and other forms of dyadic (one vs. one) or polyadic (team) contests, such as an academic competition. Social success in middle childhood may lead to considerable advantages in adolescence, such as increased popularity, which has the potential for large increases in fitness in adulthood (Del Giudice et al. 2009). With increased status, individuals gain even greater access to limited energetic resources from their environment. For children, these inputs promote a fast life-history trajectory characterized by earlier pubertal development (Chang and Lu 2018). In adolescence and adulthood, greater access to resources translates into greater overall mating effort, including dominance, aggression, courtship and risk-taking behavior (Gray et al. 2017). Consequently, juvenile children and their stress response systems are predicted to be highly attuned to the sociocompetitive landscape (Del Giudice 2015; Flinn et al. 2011).

However, very little is known about the interaction and development of different adrenal hormones and neurobiological systems in response to social competition during childhood (Del Giudice 2018). There is a large adult literature on the social endocrinology of competition. HPA activity, as measured by cortisol change, a well-known biomarker of stress, is linked with baseline androgen levels (e.g., testosterone) and status followings victory or defeats (e.g., Carré et al. 2013; Mehta et al. 2008; Flinn et al. 2011). Recent findings on juvenile competitors suggest that HPA axis reactivity is context-dependent and responsive to athletic (e.g., sports) and non-athletic social challenges. For example, cortisol levels increase from pre-match levels in juveniles during high-stress, physically taxing athletic competitions, such as basketball, soccer, and taekwondo (Capranica et al. 2012; Mazdarani et al. 2016; McHale et al. 2016; McHale et al. 2018a), which parallels findings reported among adults (Casto and Edwards 2016; Heijnen et al. 2016). In contrast to adult competitors' general cortisol increases during competition, juvenile children's cortisol levels are reported to decrease during low-stress athletic and non-athletic forms of competition (McHale et al. 2018a, 2018b, 2018c). This growing body of literature illustrates that juvenile children's adrenal hormone stress responses to competition are not unidirectional and are likely attenuated by cognitive appraisal differences associated with the sociocompetitive environment. Understanding how juvenile children physiologically respond to adversity and acute social stress appropriately may be one indication of healthy psychosocial development. Identifying dysregulation of the RAAS and HPA axis, such as hyper- or hypoactivity, could lead to new intervention strategies intended to reduce the risk of physical and psychological problems (Del Giudice 2018; Edwards et al. 1999; Reilly and Gunnar 2019).

With the exception of cortisol (e.g., Capranica et al. 2012; Mazdarani et al. 2016), the activity of the HPA axis and the RAAS in response to acute stress remains understudied and poorly understood in children. Much of the literature that does exist focuses on adrenal androgens, which relates to HPA activity. The findings indicate that DHEA increases during strenuous forms of athletic contests (e.g., soccer) but does not significantly change during less physically demanding forms of competition (McHale et al. 2016; McHale et al. 2018a, 2018b, 2018c). Thus, acute increases in DHEA are speculated to be related to physical stress demands (e.g., energetic mobilization). Additionally, consistent androstenedione patterned increases in boys are reported during athletic competition but only when played against out-group competitors (McHale et al. 2016; McHale et al. 2018a, 2018b, 2018c). These findings imply that androstenedione increases in boys are responsive only to high-stress sociocompetitive conditions where aggression is likely a component (e.g., during physical competition). In support of this view, a combination of DHEA and androstenedione increase has been linked with mediating aggression in response to territorial aggression at a time when testosterone is low (e.g., male song sparrows: Pradhan et al. 2010; male Siberian hamsters: Scotti et al. 2008). Interestingly, testosterone and estradiol, two hormones traditionally associated with competition and aggression in men and women (e.g., Casto and Prasad 2017; Geniole et al. 2017), remain unresponsive in juvenile competitors irrespective of athletic or non-athletic forms of competition (McHale et al. 2016; McHale et al. 2018a, 2018b, 2018c).

Taken together, these cumulative findings raise the possibility that juvenile children may forgo the energetic and immunosuppressive cost of maintaining high levels of testosterone and estrogens (Folstad and Karter 1992; Muehlenbein and Bribiescas 2005) in favor of adrenal androgen-mediated stress pathways that are less costly (McHale et al. 2016; McHale et al. 2018a). Given that juvenile children are not in the reproductive state, adrenal androgen pathways potentially represent an adaptive life-history strategy (i.e., tradeoff) specific to middle childhood to mediate social challenges, aggression, energy output, and appropriate psychosocial development (Del Giudice 2009, 2018; Gray et al. 2020).

Aldosterone and Cortisone: Biomarkers of the RAAS and HPA Axis

Just as adrenal androgen pathways are speculated to be an import mediator of social challenges during childhood, aldosterone and cortisone are two hormones that have received even less empirical attention as surrogate markers of RAAS and HPA activity (Bae et al. 2019; Kubzansky and Adler 2010). Aldosterone is the major mineralocorticoid secreted by the adrenal cortex and is part of the RAAS (Cartledge and Lawson 2000; Joëls and de Kloet 2017). Among adults, components of the RAAS have been shown to be involved in regulating physical (Ozunal et al. 2020; Saavedra and Benicky 2007) and psychosocial stress (Gideon et al. 2019). Cortisone is an active metabolite of cortisol and is linked with HPA axis stress response (Bae et al. 2019; Mezzullo et al. 2018; Perogamvros et al. 2010). Cortisol rapidly is converted into cortisone by 11β-Hydroxysteroid dehydrogenase 2 in the saliva, leading to 2–6 times greater levels of salivary cortisone compared to salivary cortisol (Bae et al. 2016). Recent findings indicate that salivary cortisone may be a more precise measure of free and total plasma cortisol than salivary cortisol, especially in people who have low levels or undectable levels of cortisol (Bae et al. 2019; Debono et al. 2016; Del Corral et al. 2016, Perogamvros et al. 2010). This work highlights the untapped potential of salivary cortisone as a reliable measure of stress.

Research investigating brain mechanisms underlying psychological stress has identified that aldosterone and cortisone play an important and underappreciated role in the stress response system due to the high affinity for adrenal glucocorticoids (e.g., cortisol, cortisone) and mineralocorticoids (e.g., aldosterone) in the brain through activation of mineralocorticoid receptors (Funder 2009; Kubzansky and Adler 2010; Joëls and de Kloet 2017). Thus, assessing ontogenetic HPA axis and RAAS inputs and outputs in the context of varying socioecological competitive contexts is critical to illuminating our understanding of how both stress response systems shape life-history strategies and phenotypic plasticity (Del Giudice 2009, Del Giudice 2018; Ponzi et al. 2020; Roney 2016).

The adult literature provides insight into what types of social challenges stimulate aldosterone and cortisone changes and their corresponding biological functions. Aldosterone increases during physically demanding activities, such as exercise and sports, due to its involvement in the regulation of blood pressure and fluid and electrolyte balance (Bollag 2014; Cartledge and Lawson 2000; De Souza et al. 1989; Del Rosso et al. 2016; Joëls and de Kloet 2017; Zorbas et al. 2001). Research has also shown that aldosterone concentrations rapidly increase in response to acute psychosocial stress (Gideon et al. 2019) and decrease during a non-physical, low-stress, informal esports gaming competition, possibly due to the stress-reducing influence of the sociocompetitive environment (Gray et al. 2018). While the role of aldosterone during physically taxing activities is well defined, there is a dearth of research on aldosterone reactivity to less strenuous or non-physical forms of social competition.

Salivary cortisone levels are also reported to change in response to physical (e.g., exercise) and psychosocial stress, and these responses are modulated by social conditions (Bae et al. 2019; Del Corral et al. 2016; Fancourt et al. 2015). For example, cortisone among singers increases when performing in front of an audience (i.e., high-stress condition) and decreases when no audience (i.e., low-stress condition) is present (Fancourt et al. 2015). Despite the reported findings among adults, it is unclear whether cortisone is responsive in children to social challenges.

The only study published investigating juvenile children's aldosterone and cortisone responses to physical competition shows that aldosterone levels increase during both high- and low-stress physically taxing soccer competitions (McHale et al. in press), likely due to energetic mobilization and cardiovascular homeostasis (Zorbas et al. 2001). Results further indicate that cortisone increases during the high-stress soccer match condition against an unknown team of competitors and yet decreases during a low-stress instrasquad soccer scrimmage against teammates (McHale et al. in press). These preliminary findings suggest that differential cortisone responses are capturing cognitive appraisal differences in juvenile competitors. It remains untested whether aldosterone is only responsive to general energetic demands associated with rigorous athletic competition in children and not the psychosocial competitive environment per se. It is also unclear whether aldosterone and cortisone are responsive to less physically demanding and less psychologically stressful forms of competition in boy and girl competitors, such as an academic competition (non-physical) or a table-tennis competition (low-physically taxing competition).

The Present Research

In the present study, we further explore HPA axis and RAAS stress pathways in children, represented by glucocorticoid (cortisone) and mineralocorticoid (aldosterone) change, during two competitive conditions. In contrast to recent findings of juvenile aldosterone and cortisone responses in physically taxing competition (McHale et al. in press), this study evaluates the factors that stimulate aldosterone and cortisone change during (1) a math competition and (2) a low-physically taxing, dyadic table-tennis competition. The present research offers several unique contributions to the literature. First, no studies have examined aldosterone and cortisone responses in juvenile children participating in non-physical competition, such as an academic competition, or during a dyadic sporting contest. Second, most behavioral endocrinology and stress research is conducted on Western populations (Henrich et al. 2010). In this study, we recruited a non-Western, urban sample of juvenile competitors aged 8–11 years.

Furthermore, the present research represents a naturalistic, ecologically valid design, as opposed to lab settings. Hong Kongese children face intense social pressure early in their academic lives to perform well in school, which is typified by their consistent rank as one of the highest-achieving student bodies in math proficiency worldwide (Chou 2012; Ingham 2007; Phillipson 2006). Students are attuned to the importance of academic success as an extension of social status and enhanced social mobility opportunities. In addition, one of the most common physical activities in Hong Kongese primary school is table-tennis participation (Community Sports Committee of the Sports Commission, Hong Kong 2009; Ha et al. 2010). Historically, the Chinese dominate in table-tennis competition, their national sport, on a global scale, such as in the Olympic Games (Besnier et al. 2018). Thus, math and table-tennis competitions represent a significant dimension of Hong Kongese children's socioecology. As a result, these conditions represent ecologically salient and culturally significant competitive contexts to investigate salivary aldosterone and salivary cortisone responses in juvenile competitors.

Hypotheses and Predictions

In the present study, we hypothesized that juveniles' aldosterone and cortisone levels will significantly vary with respect to the type of competition. For Study 1, the math competition, participants stood up to answer a series of math questions, in a rapid-fire succession, in front of ~30 classmate competitors. This competitive condition is expected to induce high psychological social stress among participants, similar to the validated stress-inducing effects of the Trier Social Stress Test (Liu et al. 2017). Thus, we predict that competitors will have significantly elevated aldosterone and cortisone post-match levels compared to pre-match levels.

In Study 2, the table-tennis competition, we do not predict that aldosterone levels will increase, due to the informal and minimally psychologically and physically stressful nature of the table-tennis exhibitions played with peers (Kondric et al. 2013). Given the reported cortisol decreases among juvenile table-tennis competitors, cortisone is predicted to decrease due to competing in a low-stress informal contest

(McHale et al. 2018c). This would be consistent with the speculation that the act of playing sports, such as table tennis, in an informal setting among peers, is stress reducing (McHale et al. 2018c).

Methods

Hong Kongese children's (aged 8–11 years) saliva samples were collected before and after (1) a team-based, mixed-sex math competition against classmates, and (2) two back-to-back dyadic boys table-tennis matches, to assess salivary aldosterone and cortisone responses. Participants played in either the math competitions or table-tennis contest. No parents (or audience) were present for the table-tennis or math competitions. A physical education coach was present for the table-tennis matches and a math teacher was present for the math competitions, along with 2–3 researchers for each study. Participant data collected for this study were part of a larger project that also measured testosterone, estradiol, cortisol, dehydroepiandrosterone (DHEA), and androstenedione responses in Hong Kongese children (McHale et al. 2018a, 2018b, 2018c).

For the math competition, participants were required to provide the number of questions answered correctly and incorrectly, a proxy measure for overall performance. If a participant failed to provide how many questions they answered correctly and/or incorrectly then the blank response(s) was coded as zero. Match outcome (win, loss) was also recorded for both studies. A scale and anthropometer were used to collect participant height and weight measurements, allowing for calculation of BMI. To ensure no participants were progressing through puberty, they completed the Pubertal Development Scale (PDS), a self-report measure of pubertal status (Petersen et al. 1988). The results indicated that none of the participants were undergoing pubertal maturation in either study (range: 1.00–2.20). For a more detailed description of the methods for each respective study refer to McHale et al. (2018b, 2018c).

Children on hormone medication were not allowed to participate. Participants were instructed not to consume food one hour prior to the start of each respective study. In an attempt to control for diurnal variation in steroid hormone levels all table-tennis and math salivary samples were provided in the morning (Lightman et al. 1981; Gröschl et al. 2003). All data were collected between October and December 2016. Study protocols, including parent and informed consent, were approved by the University of Nevada, Las Vegas and the University of Hong Kong Institutional Review Boards.

Hormone Analysis

All salivary samples were stored at −20 °C upon collection and sent to ZRT Laboratory in Beaverton, Oregon for analysis. ZRT Laboratory utilized liquid chromatography–mass spectrometry (LC-MS/MS). This method benefits from analyzing multiple steroids simultaneously and is the most accurate steroid hormone analysis available (Shackleton 2010). The lab had issues with interference for some of the aldosterone samples, such that 5/45 pre- and post-math competition values were unmeasurable.

Four out of 22 table-tennis post-match aldosterone, 2/40 pre-math competition aldosterone, and 3/40 post-math competition aldosterone levels were below sensitivity of the assay (< 3.3 pg/mL). These low concentrations are considered meaningful data points even though exact measures are below the reliability of the assay. As a result, each aldosterone measure below 3.3 pg/mL was assigned a value that is one half of the minimum detection limit, 1.65 pg/mL, which is the most common statistical strategy for biomarkers that fall below the minimum detection limit (Hu et al. 2018). The intra-assay coefficient of variation for all analytes tested range from 9.3–56.3 pg/mL (aldosterone) and 2.0–9.3 ng/mL (cortisone). Inter-assay precision over the same hormone concentrations range from 3.1% to 5.0%.

Math Competition

Participants

Boys ($n = 18$) and girls ($n = 27$) from two elite Chinese primary schools in Kowloon, Hong Kong, aged 9–10 years, participated in an in-class, mixed-sex, team math competition ($N = 45$). Three classrooms consisting of ~30 students in each, took part in a math competition. All student participated in the math competition even if they elected not to participate in the study.

Math Competition Procedures

All math contests occurred in the mornings during regularly scheduled math class. Pre-match saliva samples were collected 10 min before the start of the math competition and post-match samples were immediately collected after it was completed. Two of the match competitions were simultaneously conducted in two separate classrooms, from 8:25 AM to 8:50 AM, $n = 24$. The third math competition occurred from 10:30 AM – 10:55 AM, $n = 21$.

Teachers divided the students into randomly assigned mixed-sex teams containing four students per team, with each team sitting with their teammates only and facing the front of the classroom. The majority of teams had four players, with one team consisting of three players. Each classroom had relatively equal numbers of boys and girls. The first two classrooms to participate had 7 teams. The third classroom had 8 teams. All teams participated against one another simultaneously in their respective classrooms. Before the start of the math contest, the teachers reviewed the rules of the math competition with their students. Furthermore, students were informed that the winning team would be awarded a prize provided by the research team. The prizes consisted of a wrapped present filled with food and sweets.

An 80-question PowerPoint presentation was created by one of the math teachers containing a different math question per slide written in Chinese. The slide presentation was used to ask each math question in succession. For consistency, the same slide deck was used in all three classrooms.

Every team started the competition with 100 points. For each correct answer, 20 points were awarded to the answering team's score. Incorrect responses resulted in a loss of 10 points per team. When a new question was displayed on the slide deck the first student to stand up was called upon to answer the question on behalf of their team. Students who were called upon to answer had no more than 3 s to answer the question. If a student was called upon and failed to answer the question in under 3 s, or if they answered incorrectly, that student's team lost 10 points. Students were only given one chance to answer the question correctly and then the next question was presented to the classroom in a rapid-fire succession in an effort to engineer a psychologically stressful, meaningful, competitive contest. A research assistant kept track of team scores so that it was visible on the board in front of the classroom for all participants. Due to the naturalistic experimental design in a primary school classroom, only one team from each of the three respective classrooms could win each contest. Thus, an unbalanced number of winners ($n = 7$) and losers ($n = 38$) is reported.

Statistical Methods: Math Competition

Pre- and post-match aldosterone and cortisone concentrations were non-normally distributed. To achieve normality, we applied square root transformations to pre- and post-match aldosterone and cortisone measures. However, this strategy did not produce normal distribution curves for pre- and post-match cortisone. As a result, log 10 transformations for pre- and post-match cortisone were utilized to achieve normality. Independent samples t-test confirmed that (baseline) sqrt pre-match aldosterone and log pre-match cortisone did not statistically differ between the 8:30 AM ($n = 24$) and 10:30 AM ($n = 21$) samples, $p > .05$. Among the total sample, exploratory analyses investigated potential relationships among aldosterone change (post-match aldosterone minus pre-match aldosterone), covariates (BMI, age, PDS, sex), and independent variables, match outcome (0 = loss; 1 = win), and individual performance (number of correct answers – incorrect answers). This analysis was repeated for cortisone. All difference variables (post-match hormone concentration minus pre-match hormone concentration) were normally distributed. Sex, BMI, PDS, age, and performance measures were unrelated to aldosterone change. Thus, paired sample t-tests were employed to compare pre- and post-match aldosterone levels. Match outcome was the only covariate included in the ANCOVA to model pre- and post-match cortisone change because it was the only covariate significantly related to cortisone match change. The ANCOVA satisfied homogeneity of variance testing ($p > 0.05$).

Results: Math Competition

Descriptive statistics are presented in Table 1. BMI, age, PDS, sex, and performance were unrelated to pre- and post-match aldosterone and cortisone change. Match outcome was only significantly related to cortisone change.

A significant decrease was observed when comparing pre-match ($M = 4.38$, $SD = 1.52$) to post-match ($M = 3.49$, $SD = 1.41$) aldosterone levels, $t(39) = 4.44$, $p < .001$ (Fig. 1, for ease of interpretation raw values are depicted). Thirty out of 40 participants had post-match decreases in aldosterone, 9 had increases, along with 1 participant having no change.

Next, we conducted a one-way ANOVA which indicated that match outcome was significantly related to competition change in cortisone (pre-match cortisone minus post-match cortisone), such that competitors on losing teams ($n = 38$; $M = -3.05$ ng/mL) had significantly greater cortisone decreases compared to competitors on winning teams ($n = 7$; M change $= -1.17$ ng/mL), $F(1, 43) = 6.44$, $p = .015$ (Fig. 2). To explore how match outcome effected change in cortisone from pre to post match we performed an ANCOVA

Table 1 Descriptive characteristics on raw pre- and post-math competition salivary aldosterone and cortisone concentrations, age, BMI, and PDS, $N = 45$

Variables	Mean	SD	Minimum	Maximum
Math Competition				
Age (years)	10.04	0.59	9.00	10.90
BMI (kg/m^2)	17.52	4.22	12.96	33.78
Pubertal Development Score	1.25	0.25	1.00	2.00
Hormone Concentrations				
Total sample				
Pre-match Aldosterone pg/mL ($N = 40$)	21.42	13.32	1.65	64.40
Post-match Aldosterone pg/mL	**14.11*** **	11.65	1.65	54.80
Pre-match Cortisone ng/mL ($N = 45$)	8.51	3.14	4.60	17.60
Post-match Cortisone ng/mL	**5.75*** **	2.09	2.00	11.70
Boys				
Pre-match Aldosterone pg/mL ($n = 16$)	19.31	10.62	5.50	43.00
Post-match Aldosterone pg/mL	12.05	8.61	1.65	32.10
Pre-match Cortisone ng/mL ($n = 18$)	8.31	3.13	5.20	16.10
Post-match Cortisone ng/mL	5.58	2.09	2.80	11.70
Girls				
Pre-match Aldosterone pg/mL ($n = 18$)	22.82	14.90	1.65	64.40
Post-match Aldosterone pg/mL	15.48	13.29	1.65	54.80
Pre-match Cortisone ng/mL ($n = 27$)	8.64	3.22	4.60	17.60
Post-match Cortisone ng/mL	5.87	2.12	2.00	10.20

Hormone concentrations represent raw, untransformed values. For convention, means, minimum, and maximum values are displayed. Square root transformed pre- and post-match aldosterone was assessed by paired-samples t-test. Comparisons of log transformed pre- and post-cortisone, controlling for individual performance and outcome, utilized an ANCOVA

***$p < 0.001$ represents a significant post-match increase

and specified match outcome as the covariate. A highly significant decrease and large effect was observed between pre- ($M = 0.90$) and post-match ($M = 0.73$) cortisone, $F(1,42) = 83.81$, $p < 0.001$, partial $\eta^2 = 0.67$ (see Fig. 3; raw values are depicted for ease of interpretation). Out of 45 competitors, 43 had cortisone decreases, 1 had an increase, and 1 competitor's cortisone levels did not change. Match outcome also had a significant effect on post-match cortisone levels, $F(1,42) = 8.00$, $p = .007$, partial $\eta^2 = 0.16$, whereby competitors on losing teams ($n = 33$; $M = 0.72$), had lower post-match cortisone concentrations compared to winning competitors ($n = 7$) ($M = 0.79$).

Discussion: Math Competition

Boy and girl math competitors' aldosterone and cortisone levels significantly decreased during the math competition, which was contrary to our prediction. The math competition was designed to be psychologically stressful but due to our observations (e.g., lots of laughing, cheering, and smiling by math competitors) it led us to believe that it actually was not, and this is likely reflected in the direction of hormonal change.

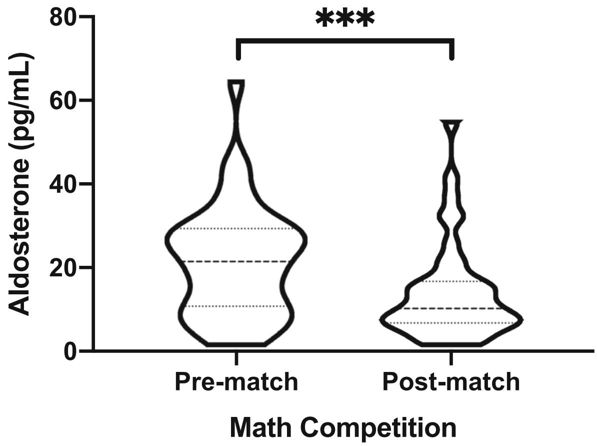

Fig. 1 Comparison of raw pre- and post-match aldosterone change during the math competition ($N = 40$). Statistical analysis relied upon square root transformations of pre- and post-match aldosterone concentrations, $p < .001$. The violin plot combines elements of the box plot and density trace (or smoothed histogram) to show the structure of the data (Hintze and Nelson 1998). Median (in black) and quartiles (in gray) are represented by the horizontal dotted lines. The top and bottom of the plot show the range

Further, the decrease in cortisone likely reflects the biopsychological effect of an informal competition with peers, without an audience present, which conceivably promotes a lower-stress competitive environment. In a recent study among juvenile boy soccer players, salivary cortisone increased during a high-stress soccer match played against an unknown team of competitors and decreased during a low-stress, instrasquad soccer competition played against teammates, despite similar rates of physical exertion in participants (McHale et al. in press). Taken together, these findings

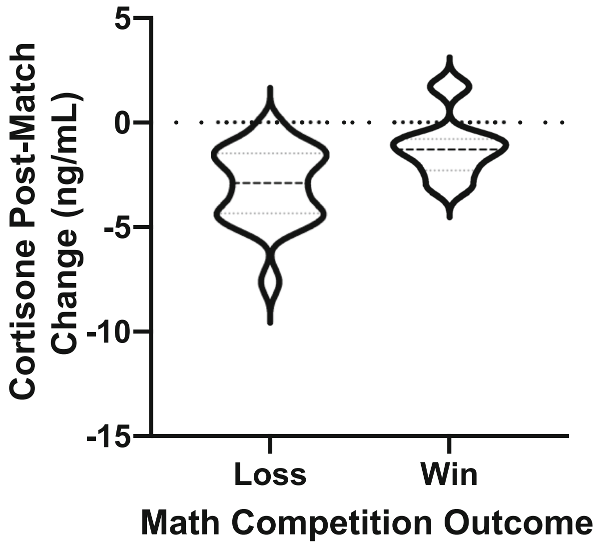

Fig. 2 Comparison of raw mean cortisone change (post-match minus pre-match) for losing ($n = 38$) and winning ($n = 7$) competitors, $p = .015$.

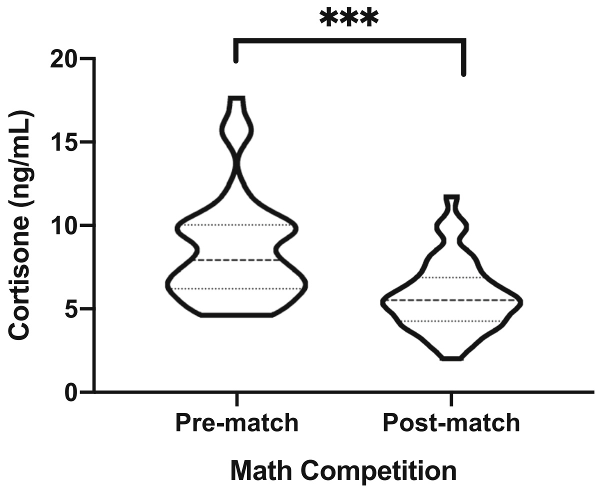

Fig. 3 Comparison of raw pre- and post-match cortisone change during the math competition ($N = 45$). Statistical analysis relied upon log 10 transformations of pre- and post-match cortisone, $p < .001$.n

highlight that juvenile competitors' cortisone responses are sensitive to both physically taxing (soccer) and non-physical (math competition) forms of team competition and that differences in the competitive environment stimulate the direction of reactive cortisone change. Future work would benefit from including self-report measures of pre- and post-match competitive stress in order to assess whether cortisone decreases represent a decrease in psychological stress for competitors (e.g., Cox et al. 2003).

The observed decrease in aldosterone may also be due to the psychosocial effects of low-stakes competition (Gray et al. 2018). Additionally, aldosterone is known to affect the body's ability to regulate blood pressure (Bollag 2014); therefore, stress-reducing activities can lower blood pressure and deactivate the RAAS, which may stimulate decreases in aldosterone concentrations that were reported in boy and girl math competitors (Kubzansky and Adler 2010; Rainforth et al. 2007). Similarly, aldosterone decreases were reported among a small sample of college-aged male esports gaming competitors during a non-physical, team-based, informal, match against peers (Gray et al. 2018). Requiring competitors to wear heart monitors to measure pre- and post-match heart rate change would strengthen the functional interpretation that aldosterone decreases are capturing cardiac activity. Nonetheless, this novel finding provides preliminary evidence that non-physical forms of juvenile competition can cause aldosterone levels to significantly decrease.

Lastly, we examined several potential covariates. We observed that boys and girls did not have significant differences between pre-match aldosterone and cortisone levels. Aldosterone and cortisone change were also unrelated to BMI, age, and performance measures. However, match outcome was significantly related to the degree of cortisone change and post-match cortisone levels. Specifically, losing competitors had a significant greater decrease in pre- to post-match cortisone levels and losing competitors had lower post-match cortisone concentrations overall compared to winners. There is a large adult literature on human and non-human animals linking cortisol and testosterone reactivity to winner and loser effects that influence post-competition behavior (e.g., willingness to compete again) (Archer 2006; Mehta et al.

2008; Oliveira et al. 2002; Salvador and Costa 2009; van Anders and Watson 2006). This finding further suggests that the magnitude of cortisone decrease is attenuated by psychosocial variables of competition, for which match outcome is salient in juvenile competitors. However, given the small sample size of winners ($n = 7$) compared to losing competitors ($n = 38$), these preliminary results should be interpreted with caution and require replication.

Table-Tennis Competition

Participants

Boys ($N = 22$) from two Chinese primary schools in Kowloon, Hong Kong, consisting of 14 and 8 participants respectively from each school, competed in two back-to-back, table-tennis matches. Nearly all competitor's BMI ($N = 18$), age ($N = 22$), PDS ($N = 21$), and outcome ($N = 21$) were documented. Four competitors left after the table-tennis matches without providing height and weight measurements and one competitor did not fill out his PDS and outcome on the questionnaire.

Table-Tennis Procedures

Table-tennis competitions began at 8:30 and 11:00 AM. Saliva samples were collected immediately before a 10-min warm-up (rallying the ball back and forth) and after the second rounds of matches. The coach from each school prearranged their players to compete against players with similar skill levels during the first round of matches in an effort to increase the competitiveness of the games. The first player to score 11 points by a 2-point margin or more won the game. The first player to win 2 games won the match. For the second-round matches, winning players were paired against one another, while losing players were paired against losing challengers. Each round lasted ~7–8 min on average, totaling ~15 min of competitive match play, for a total of ~25 min of table-tennis play (warm-up period plus match play).

Statistical Methods: Table-Tennis

Log 10 transformation of pre- and post-match aldosterone levels achieved normality. Pre- and post-match cortisone concentrations were normally distributed. Exploratory analyses were performed to assess associations between BMI, age, PDS, match outcome (0 = lost both matches; 1 = lost one match and won one match; 2 = won both matches) and aldosterone and cortisone match change. Because of differences in collection times, pre-match (baseline) aldosterone and pre-match cortisone levels were compared between the 8:30 AM ($n = 8$; aldosterone $M = 28.98$ pg/mL; cortisone $M = 10.21$ ng/mL) and 11:00 AM ($n = 14$; aldosterone $M = 11.49$ pg/mL; cortisone $M = 6.19$ ng/mL) samples via an independent-samples t-test, revealing that the 8:30 AM concentration of pre-match aldosterone ($p = .005$) and pre-match cortisone ($p = .011$) were significantly higher compared to the 11:00 AM samples. Consequently, collection time was included as a covariate (control) in the ANCOVA when assessing pre- and post-match change in hormone concentrations. Paired-samples t-tests were used to compare pre- and post-match hormone change separately for the 8:30 AM and 11:00 AM samples. All tests satisfied homogeneity of variance testing ($p > 0.05$).

Results: Table-Tennis

Descriptive statistics are presented in Table 2. BMI, age, PDS and match outcome were unrelated to pre- and post-match aldosterone and pre- and post-match cortisone change ($p > .05$).

A significant change was observed when comparing pre- to post-match aldosterone after adjustment for 'match time', $F(1,18) = 32.34$, $p < 0.001$, partial $\eta^2 = 0.642$, demonstrating a large effect. Match time was also significantly related to post-match aldosterone levels, $F(1,18) = 6.59$, $p = .019$, partial $\eta^2 = 0.268$, such that the 8:30 AM competitors had significantly higher concentrations of post-match aldosterone ($M =$ 1.51; $SD = 0.23$) compared to the 11:00 AM competitors' concentrations ($M = 0.76$; $SD = 0.45$). The interaction between match time and pre-match aldosterone (8:30 AM pre-match concentrations: $M = 1.39$; SD = 0.31; 11:00 AM concentrations: $M = 0.95$; SD = 0.32) did not meet conventional levels of statistical significance, $F(1,19) = 3.65$, $p = .072$, $\eta^2 = 0.168$.

Because we established a significant change in aldosterone according to match time, we further examined the extent to which it changed within the 8:30 AM and 11:00 AM participant samples separately. We observed a meaningful decrease in the larger sample of 11:00 AM competitors' pre-match aldosterone ($M = 0.95$, $SD = 0.32$) levels compared to post-match levels ($M = 0.76$, $SD = 0.45$), $t(13) = 2.96$, $p = .011$, for which 9/14 competitors' levels declined (Fig. 4, for ease of interpretation raw values are depicted). The difference between 8:30 AM pre-match ($M = 1.39$, $SD = 0.31$) and post-match ($M = 1.51$, $SD = 0.23$) aldosterone samples, did not achieve conventional levels statistical significant, $t(7) = -1.96$, $p = .091$.

After controlling for 'match time', a significant effect was observed between pre- and post-match cortisone, $F(1,19) = 16.50$, $p = 0.001$, partial $\eta^2 = 0.47$, indicating a large effect. Follow up analysis identified that 15/22 competitors had post-match cortisone decreases. Among the 8:30 AM sample, 7/8 competitors' cortisone decreased (pre-match: $M = 10.21$ ng/mL, $SD = 1.32$ ng/mL; post-match: $M = 8.81$ ng/mL, $SD =$ 1.52 ng/mL), $t(7) = 3.44$, $p = .011$, which was statistically significant (Fig. 5). Despite 8/14 competitors having declines in cortisone during the 11:00 AM matches, pre-match ($M = 6.19$ ng/mL, $SD = 3.92$ ng/mL) and post-match ($M = 5.83$ ng/mL, $SD =$ 2.78 ng/mL) cortisone levels did not statistically differ, $t(13) = 0.47$, $p = .646$.

Discussion: Table-Tennis

Time of day was an important factor that led to significant diurnal differences among the 8:30 AM and 11:00 AM competitors' pre-match aldosterone and cortisone concentrations. As a result, each sample was analyzed independently according to match time. Research shows that both aldosterone and cortisone are related to cortisol, which

Table 2 Descriptive characteristics on raw pre- and post-match table-tennis salivary aldosterone and cortisone concentrations, age, BMI, and PDS

Variables	Mean	SD	Minimum	Maximum
Table-tennis Competition (*N* = 22)				
Age (years)	9.72	0.86	8.10	11.10
BMI (kg/m^2)	16.55	2.63	13.14	21.18
Pubertal Development Score	1.50	0.41	1.00	2.20
Hormone Concentrations				
8:30 AM Sample (*n* = 8)				
Pre-match Aldosterone pg/mL	28.98	14.17	6.30	45.90
Post-match Aldosterone pg/mL	36.25	19.03	13.70	76.60
Pre-match Cortisone ng/mL	10.21	1.32	8.40	12.00
Post-match Cortisone ng/mL	**8.81***	1.51	6.70	10.90
11:00 AM Sample (*n* = 14)				
Pre-match Aldosterone pg/mL	11.49	8.59	3.30	30.00
Post-match Aldosterone pg/mL	**9.39***	9.85	1.65	33.40
Pre-match Cortisone ng/mL	6.19	3.92	2.40	17.60
Post-match Cortisone ng/mL	9.39	9.85	1.65	33.40

Hormone concentrations represent raw, untransformed values. For convention, means, minimum, and maximum values are displayed. Significance was obtained by comparing pre- and post-match hormone changes for the 8:30 AM and 11:00 AM samples separately via paired-samples t-tests. *$p < 0.05$, represent a significant post-match change

demonstrates a clear diurnal pattern. Episodic patterns of aldosterone release are tightly linked with the cortisol awakening response (Charloux et al. 1999), while cortisone fluctuations vary in parallel with cortisol concentrations (Gouarné et al. 2005).

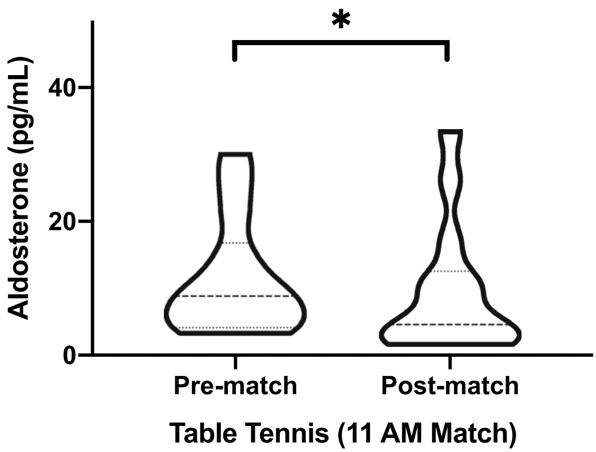

Fig. 4 Comparison of pre- and post-match aldosterone change among the 11:00 AM participant sample (n = 14). Statistical analysis relied upon log 10 transformations of pre- and post-match aldosterone concentrations, p = .011.

The findings partially support our hypotheses. Aldosterone and cortisone levels significantly decreased in table-tennis competitors, yet the hormone changes were not uniform across the total sample of participants. In contrast to one of the original predictions, aldosterone levels significantly decreased, rather than remained unchanged, among competitors who participated in the 11:00 AM table-tennis matches, likely capturing cognitive appraisal of a low-stress contest promotes acute decreases during an informal match against peers.

In keeping with our second prediction, cortisone decreased for the majority of competitors (15/22), but only reached statistical significance for the 8:30 AM sample. This finding is consistent with previous published findings that show cortisol significantly decreased among the same sample of competitors playing table-tennis (McHale et al. 2018c), which may be capturing the physiology of low cognitive appraisal of competition, where dyadic table-tennis competition is stress-reducing for boys when played among peers in a low-stakes environment, with no audience present.

Future work would benefit by collecting saliva hormone measures in the afternoon to prevent confounds in observations with morning circadian decline. Thus, the effect of 'competition-event' in the present study is potentially confounded by time effects across the morning. Also, the light activity of each competition, and resulting blood flow, could be metabolizing concentrations of these hormones from the waking related peak.

General Discussion and Conclusion

The reported findings are the first to demonstrate that salivary aldosterone and salivary cortisone acutely change during less strenuous forms of competition in juvenile children. Specifically, pre- to post-match salivary aldosterone and salivary cortisone levels decreased in a sample of Hong Kongese children, aged 8–11 years, during (1) a mixed-sex, team-based math competition and (2) a dyadic table-tennis competition

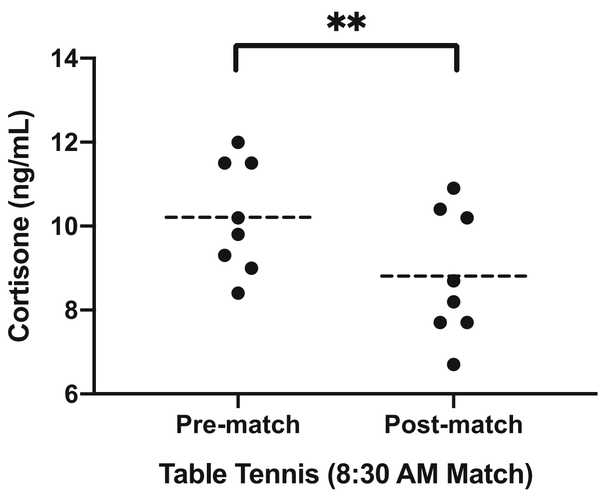

Fig. 5 Comparison of pre- and post-match cortisone change among the 8:30 AM participant sample ($n = 8$, $p = .011$). Due to the small sample, a scatterplot is used to aid visualization of the data

against peers. For study 1 (math competition), hormone levels did not differ between genders. Furthermore, aldosterone and cortisone levels decreased, contrary to predictions, with losing team members having greater decreases in pre- to post-match cortisone levels compared to winners. For study 2 (table-tennis competition), time of day was an important factor that led to significant diurnal differences between the 8:30 AM and 11:00 AM competitors' pre-match aldosterone and cortisone concentrations. As a result, each sample was analyzed independently according to match time. Aldosterone levels significantly decreased among the 14 competitors who participated in the 11:00 AM table-tennis matches. Cortisone levels decreased for the majority of competitors in both match times but only decreased significantly among the 8:30 AM sample.

Adult aldosterone increases have been interpreted in the exercise and competition literature for of its role in regulating blood pressure and fluid and electrolyte balance (Bollag 2014; Cartledge and Lawson 2000; Del Rosso et al. 2016; Zorbas et al. 2001). Surprisingly, although aldosterone is also released via HPA axis activation, it traditionally has not been considered important in regulating psychosocial stress until recently (Gideon et al. 2019; see review Jackson et al. 2018). Whether stress-reducing activities deactivate the RAAS and the HPA axis, which in turn stimulates rapid aldosterone and cortisone decreases in adults or children, remains largely unexplored (Gray et al. 2018; McHale et al. in press). In our study, we observed that aldosterone and cortisone concentrations decrease when psychosocial and physical stressors of competition are relaxed in juvenile children. These results lend credence to the view that both stress systems play a dynamic and underappreciated role in regulating middle childhood social stress, psychosocial development, and homeostatic equilibrium (Funder 2009; Kubzansky and Adler 2010; Joëls and de Kloet 2017).

In order to make sense of the inputs and socioecologies which shape developmental plasticity, life-history strategies, and healthy psychosocial development, an integrative developmental-evolutionary approach of middle childhood is required (Del Giudice 2018). Investigations on brain mechanisms underlying psychosocial stress indicate that corticosteroids (e.g., aldosterone, cortisone, cortisol) induce chemical changes that alter neural pathways, which influences which behavioral outcomes are more likely (Carré et al. 2013; Kubzansky and Adler 2010; Mehta et al. 2008; Flinn et al. 2011). However, most of this work has focused on adolescents or adults. A growing body of literature on endocrine responses to social competition (McHale et al. 2016; McHale et al. 2018a, 2018b, 2018c; McHale et al. in press) suggest that adrenal stress-mediated pathways emerge during the juvenile transition in order to calibrate the appropriate within- and between-group competitive social behavior prior to adolescence and adulthood at a time when primary sex steroid production of testosterone (boys) and estradiol (girls) is low.

Behaviorally, juvenility is a time to compete for social status and to learn complex social skills and social attachment strategies, coping mechanisms, and emotional regulation (de Veld et al. 2012; Del Giudice 2009, 2018; Del Giudice et al. 2009). Thus, examining how a developing child's brain and behaviors are shaped by RAAS and HPA axis activation in response to adaptive social challenges is critical. In keeping with Del Giudice's (2009, 2018) evolutionary-development model of middle childhood, mapping adrenal-hormone stress pathways to early life acute and chronic stressors and their relationship with ecological inputs (e.g., nutritional insecurity, social neglect) would provide clues on identifying factors that promote healthy psychosocial development and factors which lead to deficits in stress regulation. With approximately 13.4% of children and adolescents suffering from stress-related mental health issues worldwide (Polanczyk et al. 2015, Perou et al. 2013; Reardon et al. 2017), researchers would benefit by investigating further the relationship between the RAAS and HPA axis and how adrenal-hormone stress pathways coordinate morphological, physiological, and behavioral traits.

Limitations and Future Directions

The present studies have several limitations, which provide avenues for future research. First, additional research including larger sample sizes, female participants (particularly in table-tennis competitions), and self-reported measures of pre- and post-competition stress and mood would strengthen the reliability and interpretations of the findings reported here. In addition, assessing juvenile competitors' RAAS and HPA axis responses to higher-stakes forms of competition, such as a regional math or table-tennis tournaments with an audience present, would provide a high-stress competitive arena as a complement to the informal, low-stress math and table-tennis competitive conditions in this study.

Second, pre-competition aldosterone and cortisone levels differed between the 8:30 AM and 11:00 AM table-tennis match times, which reduced the overall sample size and power. Future work should ensure that match times begin at the same times of day to control for this potential confound. Given the sensitivity of baseline adrenal hormones to diurnal shifts, it would be of interest to compare whether diurnal fluctuations in baseline (pre-match) adrenal hormone levels may promote differential aldosterone and cortisone reactivity when confronted with sociocompetitive stressors in morning, afternoon, and evening samples.

Lastly, this body of research would benefit by broadening the investigation of juvenile children's adrenal hormone responses to social challenges in socioecologies that exist beyond traditional Western or urban populations, such as in communities where children are exposed to high pathogen load or food insecurity or experience early life family stressors (e.g., trauma, social and/or parental neglect). Such factors are known to affect baseline adrenal hormone levels during ontogeny and into adulthood (Flinn 2006; Hodges-Simeon et al. 2017; Ponzi et al. 2020). In doing so, this work would fill a large gap in the behavioral endocrinology, middle childhood development, and life-history theory literature. These efforts also have the potential to provide intervention strategies during middle childhood that address dysregulation of the appropriate stress response (Del Giudice 2018; Edwards et al. 1999; Reilly and Gunnar 2019). Integrating stress regulation to acute social challenges and the impact of early and late childhood life stressors would provide a more comprehensive synthesis of human developmental plasticity and life-history strategies (Flinn 2006; Ponzi et al. 2020; Salvador 2005).

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Compliance with Ethical Standards

Conflict of Interest The authors declare they have no conflict of interest with the contents of this manuscript.

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