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Salivary aldosterone and cortisone respond differently to high- and low-psychologically stressful soccer competitions

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ABSTRACT
Aldosterone and cortisone are released in response to physical and psychological stress. However, aldosterone and cortisone responses in children engaged in physical competition have not been described. We examined salivary aldosterone and salivary cortisone responses among Hong Kongese boys, aged 8-11 years, during (1) a soccer match against unknown competitors (N = 84, high psychological stress condition) and (2) an intrasquad soccer scrimmage against teammates (N = 81, low psychological stress condition). Aldosterone levels increased during the soccer match and intrasquad soccer scrimmage conditions, consistent with the view that aldosterone responds to physical stress. During the soccer match, winning competitors experienced larger increases in aldosterone compared to losing competitors, indicating that the degree of aldosterone increase was attenuated by match outcome. Cortisone increased during the soccer match and decreased during the intrasquad soccer scrimmage. Competitors on teams that resulted in a tie had larger cortisone increases compared to winners or losers. These findings highlight that the degree of cortisone change is related to boy's cognitive appraisal of the competitor type (i.e., teammates vs. unknown competitors) and the competitive nature of the game (e.g., tie). These results shed new light on adrenal hormone mediators of stress and competition during middle childhood.

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KEYWORDS
Aldosterone; cortisone; soccer; middle childhood; stress

1. Introduction

The adrenal gland produces hormones that regulate a number of important biological functions (Caretto et al., 2019; Silverman & Sternberg, 2012). Participation in sports and other forms of competition modulate these hormones, making activities like these a useful experimental paradigm to model the effect of physical and social challenges on adrenal activity (Flinn et al., 2012; Gatti & De Palo, 2011; Geniole et al., 2017). Cortisol is one adrenal biomarker that has been studied extensively as an indicator of psychosocial (e.g., Jezova et al., 2016; Korte, 2001; Liu et al., 2017) and physical stress in athletic competition (Casto & Prasad, 2017; Edwards et al., 2006) and in strenuous exercise (Anderson & Wideman, 2017; Thomas et al., 2009). Additionally, hypothalamic–pituitary–adrenal (HPA) axis activity is linked with competition outcome. Specifically, cortisol increases following defeat (e.g., Aguilar et al., 2013; Casto et al., 2014). However, research suggests that additional adrenal hormones may also play an important role in regulating a competitor's psychology, physiology, and behaviour within sports competitions (McHale et al., 2018, 2016). Aldosterone and cortisone are two hormones released from the adrenal cortex in response to HPA axis activation (Bollag, 2014; Del Corral et al., 2016; Zorbas et al., 2001), yet received far less attention in the stress literature. These hormones play an important and underappreciated role in the stress response system due to their high affinity for adrenal glucocorticoids, like cortisone, and mineralocorticoids like aldosterone in the brain through activation of mineralocorticoid receptors (Funder, 2009; for review see Kubzansky & Adler, 2010).

Aldosterone is produced in the zona glomerulosa of the adrenal cortex and increases during physically stressful contexts, such as exercise (Zorbas et al., 2001). It is involved in the regulation of blood pressure and fluid balance by acting on the kidneys to conserve salt and water by stimulating sodium and water reabsorption from the gut, and salivary and sweat glands, during physically demanding conditions (Bollag, 2014). Salivary aldosterone is closely associated with plasma aldosterone levels (Lichtenauer et al., 2016; Mcvie et al., 1979). Research on adults has shown that aldosterone levels significantly increase during physically taxing forms of exercise and competition, such as rally car races (Bollag, 2014; Del Rosso et al., 2016; Joëls & de Kloet, 2017; De Souza et al., 1989; Zorbas et al., 2001). Evidence also suggests that aldosterone may even decrease when both physical and psychosocial stress levels are low; among competitors in a non-physical, team, informal e-sports gaming competition, aldosterone significantly decreased following the game (Gray et al., 2018). These findings among adults imply that aldosterone is sensitive to intense physical stress; yet, the direction and magnitude of change may also be regulated by psychological stress (Apostolopoulou et al., 2014;

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Franklin et al., 2012; Hlavacova & Jezova, 2008a, 2008b; Segeda et al., 2017). More research is needed, however, to understand how aldosterone is regulated in competitive activities, like sports, during childhood.

Cortisone is an active metabolite of cortisol, due to its reduced glucocorticoid activity, and is also a precursor hormone that can be converted to cortisol (Mezzullo et al., 2018; Perogamvros et al., 2010). Free salivary cortisone levels are much higher than salivary cortisol due to the presence of 11 β-hydroxysteroid dehydrogenase type 2 in the salivary glands, which catalyzes cortisol conversion into cortisone (Shimojo et al., 1997). A few studies argue that salivary cortisone compared to salivary cortisol is a more accurate biomarker of total and free plasma cortisol (Del Corral et al., 2016; Perogamvros et al., 2010), indicating that salivary cortisone may be an underutilized biomarker when measuring the HPA axis stress response system. Recent work on adults demonstrates that cortisone increases due to the physical stress of exercise (Del Corral et al., 2016), and is highly sensitive to psychological stress, with its effects modulated by social conditions (Fancourt et al., 2015). For example, performers who sing during a high stress condition, operationalized as performing in front of an audience, have significant increases in salivary cortisone (Fancourt et al., 2015). Furthermore, significant cortisone decreases occur in performers during a low stress singing condition (i.e., without an audience present). Fancourt et al.'s (2015) interpretation of the latter result is that singing is in itself stress reducing. Thus, when no audience is present, singing causes cortisone levels to fall. Yet, it remains unknown the extent to which salivary aldosterone or cortisone levels may be sensitive to physical and psychosocial challenges in children.

1.1. The present research

In the present study, we evaluated pre- and post-competition salivary aldosterone and cortisone levels within an urban population of Hong Kongese children, aged 8–11 years, during two quasi-experimental conditions: (1) a team soccer match against unknown competitors (i.e., high physical stress, high psychological stress condition) and (2) an intrasquad soccer scrimmage among teammates (i.e., high physical stress, low psychological stress condition).

The present research offers several novel contributions. First, no studies to our knowledge have investigated salivary aldosterone or salivary cortisone responses in pre-pubescent children participating in athletic competition. Additionally, the behavioural endocrinology and competition literature is heavily skewed towards the regulation of cortisol and testosterone among adults (e.g., Casto & Prasad, 2017; Geniole et al., 2017), despite observations that juvenile children (~6-11 years of age) engage in a variety of strategies to compete with peers (e.g., reactive and proactive forms of aggression: Baker et al., 2008; bullying, prosociality, dominance: Hawley, 1999) and yet produce low concentrations of testosterone. With the exception of cortisol (e.g., Capranica et al., 2012; Mazdarani et al., 2016), we know very little about proximate adrenal hormone mediators of stress under HPA axis control during middle childhood development in the context of competition. Two recent studies have shown that children's adrenal hormones, dehydroepiandrosterone (DHEA) and androstenedione, in USA and Hong Kongese boys, significantly rise during soccer competition, while testosterone remains unresponsive (McHale et al., 2018, 2016). These findings raise the possibility that adrenal hormone mechanisms may play a larger role in mediating juvenile children's behaviour and physiology than currently appreciated. Thus, one of the primary goals of the present study was to evaluate two understudied candidate adrenal biomarkers of stress in children during physically taxing soccer conditions in which competitors participate in both high- and low-psychological stressful competition.

Second, the vast majority of studies detailing the physiology of stress draw from laboratory studies among adult college students in Western populations (Henrich et al., 2010). Previous work on hormones and competition find that naturalistic studies induce a greater hormone response between winners and losers compared to lab-based studies (Casto & Edwards, 2016; Geniole et al., 2017). This effect is likely due to the social context of competition, in which winning and losing is of real-world social significance for participants engaged in naturalistic competition, whereby competitors are more invested and motivated to perform well in front of their peers, activating a more pronounced HPA axis stress response to social challenges. Soccer (football) is one of the most common physical activities for Hong Kongese youth in primary school (Commission, C.S.C. of the S., 2009; Ha et al., 2010; Lau et al., 2015). Thus, soccer represents a culturally salient and meaningful competitive context well-suited to investigate aldosterone and cortisone responses to physically and psychologically demanding stressors of competition.

2. Hypotheses and predictions

In the present study we hypothesized that HPA axis activity, as indicated by pre- and post-competition salivary aldosterone and cortisone levels, would significantly vary with respect to the type of soccer competition. For studies 1 (soccer match) and 2 (intrasquad soccer scrimmage), boy's aldosterone levels were predicted to increase in both conditions due to high physical exertion demands. Further, HPA axis activity and glucocorticoid release are modulated by how individuals cognitively appraise a stressful situation (Kemeny, 2003). As such, competitors are likely to experience heightened psychological stress when competing against unknown competitors in a formal match setting compared to contests against teammates (peers). Consequently, cortisone is predicted to increase *only* during the soccer match condition and either not significantly change, or either significantly decrease, during the intrasquad soccer scrimmage, the latter of which would be consistent with a more relaxed psychological state of mind in competitors. Consistent with the interpretation put forth by Fancourt et al. (2015), soccer competition played among peers, in a low-stress, informal competitive setting, with few audience members present (i.e., the intrasquad scrimmage condition), is potentially stress-reducing, causing cortisone levels to fall.

3. Methods

We collected saliva samples from a total of 102 Hong Kongese boys' (aged 8–11 years) before and after (1) a soccer match against unknown competitors and (2) an intrasquad soccer scrimmage to assess salivary aldosterone and cortisone responses. Saliva samples were collected in 5-mL polypropylene tubes (VWR catalogue #16,465-262). The majority of participants played in both the soccer match and intrasquad scrimmage conditions. A few parents were always present for the intrasquad scrimmage conditions. There were more parents in attendance for the soccer match conditions. These experiments were part of a larger project that investigated testosterone, cortisol, dehydroepiandrosterone (DHEA), and androstenedione responses in soccer players (McHale et al., 2018).

At the end of each match participants were instructed to fill out two short questionnaires. The demographic questionnaire sought to elicit information on participants' age, team outcome and position played on the field, while the Pubertal Development Scale (PDS) was used to measure self-reported pubertal status (Petersen et al., 1988). The results indicated that none of the participants were undergoing pubertal maturation. Height and weight were recorded using an anthropometer and scale, allowing for calculation of participants' body mass index (BMI). For a more detailed description of the methods refer to McHale et al. (2018).

Children on hormone medication were excluded from this study. Parents were instructed to prevent their child from consuming food one hour prior to the start of each respective study. Parent and Child Informed Consent Forms available in Chinese and English and signed prior to data collection. Attempts were made to address diurnal variation in steroid hormone levels by ensuring all soccer salivary collections occurred in the late afternoon or early evenings (Gröschl et al., 2003; Lightman et al., 1981). This methodology ensured the within-group hormone responses for those who participated in both the soccer match and intrasquad scrimmage conditions were comparable. All data were collected between October and December 2016. Study protocols were approved by the University of Nevada, Las Vegas and the University of Hong Kong Institutional Review Boards.

3.1. Hormone analysis

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 analysing multiple steroids simultaneously and is considered the most technically sophisticated and accurate salivary steroid hormone analyses available (Shackleton, 2010). Interference issues resulted in unmeasurable aldosterone values for 65/84 pre- and post-soccer match and 32/81 pre- and post-soccer scrimmage values. Out of the available samples for which we have data, 5/19 pre-soccer match, 2/19 post-soccer match, 18/49 pre-soccer scrimmage, and 12/49 post-soccer scrimmage aldosterone samples were below assay sensitivity (< 3.3 pg/mL). These low concentrations are considered valuable data points even though exact measures are below the reliability of the assay. Thus, 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. 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%.

4. Soccer match and intrasquad soccer scrimmage competitions

4.1. Participants

Hong Kongese boys ($N = 102$) were recruited from four teams from the youth Hong Kong Soccer Association: an under 12 (U12) soccer club from Tai Po ($n = 29$), U12 soccer club from Sha Tin ($n = 29$), under 10 (U10) soccer club from Tai Po ($n = 25$), and a U10 soccer club from Sha Tin ($n = 19$). Data were collected on 84 boys who participated in the soccer match, 81 who participated in an intrasquad soccer scrimmage, and 63 boys who participated in both the soccer match and soccer scrimmage conditions. BMI ($N = 94$; $M = 16.56$ kg/m^2), age ($N = 101$; $M = 9.98$ years), PDS ($N = 98$; $M = 1.46$) and outcome (soccer match: $n = 84$; intrasquad scrimmage outcome: $n = 77$) were reported for nearly all participants. The frequency distribution of pubertal categories (PDS) for participants of the soccer matches (1.00–1.99 = Prepubertal, 86%; 2.00–2.66 = Early pubertal, 14%) and the instrasquad soccer scrimmages (1.00–1.99 = Prepubertal, 84%; 2.00–2.99 = Early pubertal, 13%; 3.00–3.60 = Midpubertal, 3%) were also assessed.

4.2. Soccer procedures

Each participating team agreed to play in one soccer match and one intrasquad soccer scrimmage scheduled on separate days. Operationally, the intrasquad scrimmage represented the low psychological stress condition in which players from the same team were divided into smaller teams consisting of teammates. The soccer match was engineered to be played on a neutral field (i.e., no home field advantage: Fothergill et al., 2017) against an unknown team of competitors and thus represented the high stress condition. The soccer match and intrasquad scrimmage consisted of two 12-min halves separated by a 5-min halftime. Participants were required to play the full duration of time during both conditions to minimize potential confounds in hormone reactivity related to physical exertion (Gouarné et al., 2005; Morgan et al., 2004). Approximately 3 mL of saliva were collected via passive drool ~10 mins prior to the team warm-up for both the soccer scrimmage and intrasquad soccer scrimmage and ~10 mins after the completion of the contests. Each saliva collection event lasted ~5 mins on average. The team warm-up (stretching, passing the ball, running) typically lasted ~15-20 mins prior to the start of the soccer contests.

4.2.1. Soccer match

The U10 soccer matches began at 7:15 PM. The U10 Tai Po team had 24 participants while the U10 Sha Tin team had 15. Players were placed into three teams, resulting in two matches of 8 vs. 8 and one 7 vs. 7. The U10 Tai Po team had more participants. Thus, eight additional U10 Sha Tin players who were not part of the study competed in the matches. The U10 Sha Tin team won all three matches (7–0; 3–1; 2–0).

For the U12 soccer matches, 21 participants from the U12 Tai Po Soccer Club competed against 25 of the U12 Sha Tin Soccer Club's players. Coaches randomly divided the players into three smaller teams consisting of two matches of 8 vs. 8 and one match of 9 vs. 9. Four players from the U12 Tai Po Soccer Club did not participate in the experiment but were allowed to play in the soccer match. All three matches began simultaneously at 7:30 PM. The U12 Sha Tin team won two of the matches (3–0; 1–0) and tied the third match (2–2).

4.2.2. Intrasquad soccer scrimmage

The intrasquad soccer scrimmages consisted of four teams, randomly split into smaller teams, allowing for competition against teammates. All players for each respective team played in the scrimmages irrespective if they participated in the study. The U10 Sha Tin's intrasquad scrimmage saliva collection began at 4:30 PM (n = 16; two matches of 5 vs. 5; scores: 1–1, 4–1). The U10 Tai Po's data collection began at 7:00 PM (n = 25; two matches of 7 vs. 7; scores: 1–1, 7–1). The U12 Sha Tin's data collection began at 6:30 PM (n = 22; two matches of 7 vs. 7; scores: 5–1, 3–2); and the U12 Tai Po's data collection occurred at 8:00 PM (n = 19; two matches of 5 vs. 5; scores: 4–1, 7–1).

4.3. Statistical methods: Soccer match and intrasquad soccer scrimmage

All analyses were conducted using SPSS version 25. The raw cortisone and aldosterone pre- and post-soccer match and pre- and post-soccer scrimmage hormone data were non-normally distributed. With the exception of pre- and post-soccer scrimmage aldosterone concentrations, log base 10 transformations achieved normality for all pre- and post-soccer match and pre- and post-soccer scrimmage aldosterone and cortisone values (Shapiro-Wilk's test, $p > 0.05$). A Wilcoxon signed-rank sum test was performed on the raw pre- and post-scrimmage aldosterone concentrations to compare pre-match and post-match aldosterone change.

Linear regressions were performed to assess whether BMI, age, or PDS predicted the change in hormone concentrations during the soccer match and soccer scrimmage. If a significant relationship was identified then BMI, age, and PDS were included in an analysis of covariance of change (ANCOVA) when appropriate to determine the possible main effects of the covariates (control variables: BMI, age, PDS) on pre- and post-match cortisone, pre- and post-scrimmage aldosterone, and pre- and post-scrimmage cortisone. Additionally, a one-way ANOVA was employed to test whether changes were related to outcome (0 = lose, 1 = win, 2 = tie). If all covariates and outcome variables were unrelated to the dependent variable, then paired-samples t-tests were employed.

Additionally, we computed an alternative dependent variable by dividing the raw post-soccer match hormone concentrations by the raw pre-match concentrations, creating a ratio. This method was also applied to the scrimmage hormone data. Creating a ratio avoids negative values and preserves directionality of change, which resulted in successful log base 10 transformations for normality (e.g., dependent variable: "cortisone soccer match change").

Finally, we modelled the combined effects of the match and scrimmage on hormone change utilizing "aldosterone change" and "cortisone change" dependent variables. This method allowed "condition" (0 = match; 1 = scrimmage) to be included as an independent variable and test whether hormone change significantly varied as the result of playing in a soccer match compared to playing in a soccer intrasquad scrimmage. The "total participant sample" includes all participants irrespective if they competed in one or both conditions. This method was further applied to the "within-subject sample" of 63 subjects who played in both the soccer match and intrasquad scrimmage conditions, and benefits from reduced error variance between individuals since the subjects act as their own control. These analyses resulted in between-subject and within-subject ANCOVAs for comparisons. All tests are two-tailed (α =.05). For all ANOVA and ANCOVAs homogeneity of variance was met.

4.4. Results: Soccer match and intrasquad soccer scrimmage

Descriptive statistics are provided in Table 1. BMI, age, and PDS were not significant predictors of aldosterone soccer match

Table 1. Descriptive data for participants and raw pre- and post-soccer match and intrasquad soccer scrimmage salivary aldosterone and cortisone concentrations, N = 102.

Variables	Mean	SD	Minimum	Maximum
Soccer Match (n = 84)				
Age (years)	9.98	1.21	7.89	11.92
BMI (kg/m^2)	16.56	2.67	12.43	24.59
Pubertal Development Score	1.46	0.38	1.00	2.66
Soccer Scrimmage (n = 81)				
Age (years)	9.86	1.19	7.89	11.87
BMI (kg/m^2)	16.47	2.71	11.80	24.59
Pubertal Development Score	1.50	0.47	1.00	3.60
Hormone Concentrations				
Soccer Match				
Pre-match Aldosterone pg/mL **(n = 19)**	10.89	11.75	1.65	40.80
Post-match Aldosterone pg/mL	**20.27***	21.18	1.65	93.30
Pre-match Cortisone ng/mL **(n = 84)**	4.57	3.03	1.00	16.50
Post-match Cortisone ng/mL	**5.43***	3.87	0.50	22.60
Soccer Scrimmage				
Pre-scrimmage Aldosterone pg/mL **(n = 49)**	6.16	4.87	1.65	19.60
Post-scrimmage Aldosterone pg/mL	**13.33****	13.94	1.65	52.80
Pre-scrimmage Cortisone ng/mL **(n = 81)**	4.68	2.90	0.80	17.50
Post-scrimmage Cortisone ng/mL	**4.16***	2.83	0.80	15.70

Note. Hormone concentrations represent raw, untransformed values. For convention, means, standard deviation (SD), minimum, and maximum values are displayed. Raw pre- and post-soccer scrimmage aldosterone concentrations were assessed via the Wilcoxon signed-rank sum test. Log transformed pre- and post-soccer scrimmage cortisone values employed a paired-samples t-test. Log transformed pre- and post-soccer match aldosterone and cortisone values were assessed via an ANCOVA. **$^*p < 0.05$, $^{**}p < 0.001$** represents a significant post-match change.

change, intrasquad soccer scrimmage change, or cortisone scrimmage change ($p > 0.05$). Competition outcome was unrelated to change in aldosterone and cortisone during the intrasquad scrimmage condition and was unrelated to cortisone match change.

A one-way ANOVA was conducted to test whether soccer match outcome was related to aldosterone match change. Change in aldosterone from pre- to post-match significantly differed in winners and losers, $F(1, 17) = 6.12$, $p = 0.024$, $R^2 = 0.27$, a moderate effect. Players on winning teams experienced greater increases in aldosterone match change on average ($n = 10$; $M = 0.54$, $SD = 0.41$) compared to players on losing teams ($n = 9$; $M = 0.09$, $SD = 0.39$). However, a post-hoc power analysis using G*Power 3.1.97 (Faul et al., 2009), with an effect size $R^2 = 0.27$, $\alpha = 0.05$, produced a $1- \beta = 0.20$. In order to achieve power of $1- \beta = 0.80$ with the observed effect it would be necessary to collect data from a total of 110 subjects. To test whether pre- and post-match aldosterone levels changed, we conducted an ANCOVA in which match outcome was included as a fixed factor (control), pre-match aldosterone as a covariate, and post-match aldosterone as the dependent variable. The results indicated that match outcome was not related to post-match aldosterone ($p > 0.05$). Yet, there was an increase in aldosterone from pre- to post-match levels, $F(1, 16) = 6.62$, $p = 0.02$, partial $\eta^2 = 0.29$, a large effect, which produced a $1- \beta = 0.67$. In total, 15 out of 19 participants demonstrated increases in aldosterone during the soccer match while 4 experienced decreases, resulting in an 86% mean increase in raw pre-match (10.89 pg/mL) to post-match (20.27 pg/mL) aldosterone levels.

Likely due to the high number of aldosterone cases coded as 1.65 pg/mL (pre-scrimmage = 18/49; post-scrimmage = 12/49), transformations did not achieve normality. A Wilcoxon signed-rank test determined a statistically significant median increase in post-scrimmage aldosterone concentration ($Mdn = 6.80$ pg/mL) compared to pre-scrimmage levels ($Mdn = 5.10$ pg/mL), $z = -3.64$, $p < 0.001$. Out of 49 soccer scrimmage participants, 29 had aldosterone increases, 11 had decreases, with 9 experiencing no change. Players had a 116% mean increase in pre-scrimmage (6.16 pg/mL) to post-scrimmage (13.33 pg/mL) raw aldosterone levels. For a summary of pre- and post-aldosterone change during the soccer match and intrasquad soccer scrimmage conditions see Figure 1.

Possible placement

Player's age significantly predicted cortisone soccer match change, accounting for 9.1% of the variance, $F(1,82) = 8.24$, $p = 0.005$, $R^2 = 0.09$. Thus, age and pre-match cortisone were included in the ANCOVA as covariates, with post-match cortisone as the dependent variable. After adjustment for age there was an increase in post-match cortisone levels compared to pre-match cortisone levels, $F(1, 81) = 3.41$, $p < 0.001$, partial $\eta^2 = 0.43$, a large effect, and produced a $1- \beta = 1.00$. In other words, within-individual post-match cortisone significantly increased from baseline, pre-match levels. Additionally, age was significantly related to post-match cortisone, $F(1, 81) = 0.29$, $p = 0.025$, partial $\eta^2 = 0.06$, a small effect, indicating that of the 8–11 year old boys, older competitors experienced larger increases in cortisone.

Lastly, a paired-samples t-test revealed a significant mean decrease of 0.057 ($SE = 0.003$; raw mean decrease = 0.52 ng/mL) when comparing pre- and post-scrimmage cortisone, $t(80) = 2.093$, $p = 0.039$, $d = 0.23$, a small effect size. Specifically, 53/81 participants had decreases in cortisone, 26 had increases, and two participants' cortisone levels did not change. A post-hoc power analysis was used to assess achieved power, with an effect size $d = 0.23$, $\alpha = .05$, and produced a $1- \beta = 0.67$ (Faul et al., 2009). In order to achieve power of $1- \beta = 0.80$ with the observed effect it would be necessary to collect data from a total of 116 subjects. For a summary of pre- and post-cortisone change for the soccer match and intrasquad soccer scrimmage see Figure 2.

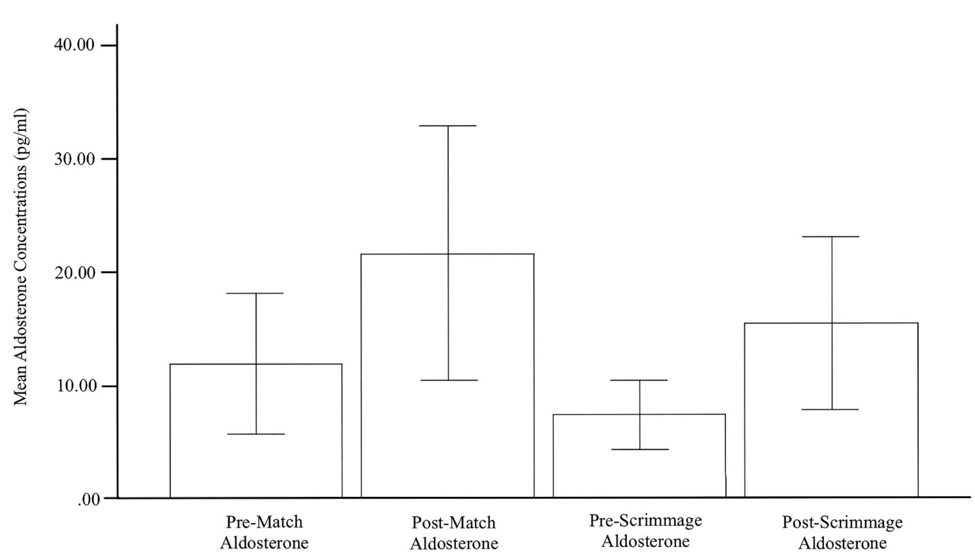

Figure 1. Raw pre- and post-soccer match ($n = 19$; $p = 0.020$) and pre- and post-intrasquad soccer scrimmage ($n = 49$; $p < 0.001$) increases in aldosterone concentrations (error bars represent 95% confidence intervals).

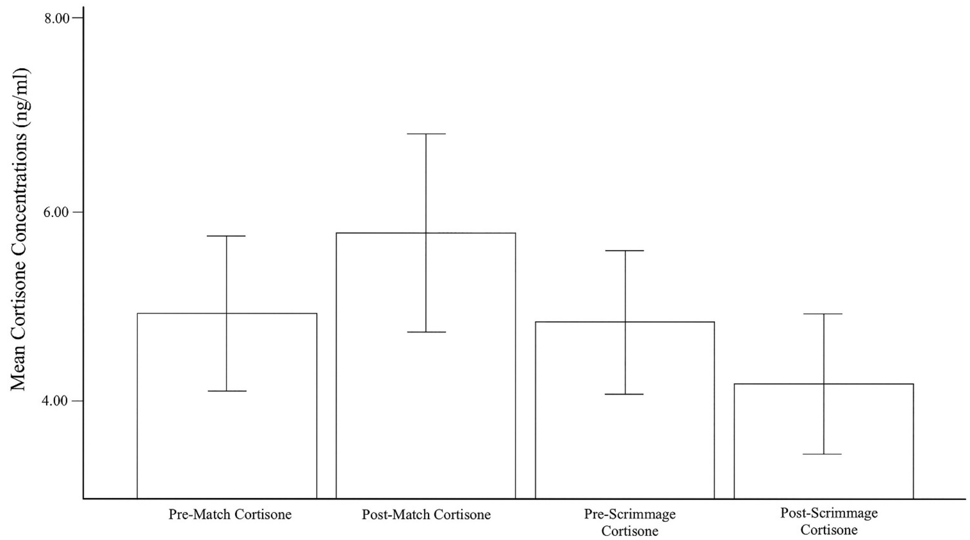

Figure 2. Raw pre- and post-soccer match increase (n = 84; p < 0.001) and pre- and post-intrasquad soccer scrimmage decrease (n = 81; p = 0.039) in cortisone concentrations (error bars represent 95% confidence intervals).

Possible placement

4.4.1. Total participant sample: Modelling hormone changes between soccer match and soccer scrimmage conditions

Preliminary analyses were conducted to test the effects of condition (coded: 0 = soccer match, 1 = soccer scrimmage), BMI, age, PDS, and outcome (coded: 0 = lose, 1 = win, 2 = draw) on the *total change* in aldosterone (e.g., soccer match aldosterone change + soccer scrimmage aldosterone change = total aldosterone change) and total change in cortisone, dependent variables. Total aldosterone change (N = 68) and total cortisone change (N = 165) were normally distributed. BMI, age, PDS, outcome, and condition were unrelated to total change in aldosterone (p > 0.05). Specifically, the magnitude of aldosterone increase did not statistically differ when comparing the soccer match and intrasquad soccer scrimmage, and aldosterone change was unrelated to BMI, age, PDS, and outcome among the total combined sample of participants.

Linear regression analyses established that BMI, F(1, 153) = 4.97, p = 0.027, R^2 = 0.03, and age F(1, 162) = 6.102, p = 0.014, R^2 = 0.04, positively predicted total cortisone change, while PDS did not (p > 0.05). A one-way ANOVA showed that competition outcome had a significant, yet small effect, on total cortisone change, F(2, 157) = 3.17, p = 0.045, and R^2 = 0.04. Post hoc comparisons using the Tukey HSD test showed that the mean change in cortisone among competitors who tied (n = 19, M = 0.13, SD = 0.26) had significantly larger cortisone increases compared to those who lost (n = 67, M = −0.03, SD = 0.25), p = 0.035. However, comparing cortisone change among those who tied to winning competitors (n = 74, M = −0.004, SD = 0.26) did not reach conventional levels of statistical significance compared, p = 0.088 (see Figure 3).

Further, soccer condition was significantly related to total cortisone change, F(1, 163) = 7.44, p = 0.007, R^2 = 0.04, a small effect. After adjustment for BMI and age, a two-way ANCOVA was conducted to compare the main effects of competition outcome and soccer condition on total cortisone change. All effects were non-statistically significant at the 0.05 significance level except for the soccer condition factor, F(1, 143) = 5.75, p = 0.018, partial η^2 = 0.04. On average, players' cortisone levels increased during the soccer match and decreased during the intrasquad soccer scrimmage, after controlling for age and BMI, leading to a significant difference between competitors' cortisone responses between the soccer conditions.

Possible placement

4.4.2. Within-subject sample: Modelling hormone changes between soccer match and soccer scrimmage conditions

Consistent with the results reported among the total sample of participants, the within-subject sample (n = 63) BMI, F(1, 120) = 5.85, p = 0.017, R^2 = 0.05, and age F(1, 124) = 4.30, p = 0.04, R^2 = 0.03, positively predicted total cortisone change. PDS did not predict cortisone change (p > 0.05). Additionally, soccer condition was significantly related to total cortisone change, F(1, 124) = 5.49, p = 0.021, R^2 = 0.05. In contrast to previous reported findings among the total sample, competition outcome was not significantly related to total cortisone change for the within-subject sample (p = 0.173). We proceeded to assess the effect of condition on cortisone change, controlling for BMI and age. BMI and age did not have a significant effect, p > 0.05, while the soccer condition, F(1, 118) = 4.75, p = 0.031, partial η^2 = 0.04, had significant effect on cortisone change, further confirming that the total and within-subject samples of participants experienced differences in cortisone production during the soccer match compared to the intrasquad soccer scrimmage.

Within-subject aldosterone data were only available for 17 players who competed in both conditions. Total aldosterone change, condition, outcome, BMI, age, and PDS were unrelated (p > 0.05). Therefore, the magnitude of aldosterone increase did not statistically differ between the soccer match and intrasquad soccer scrimmage.

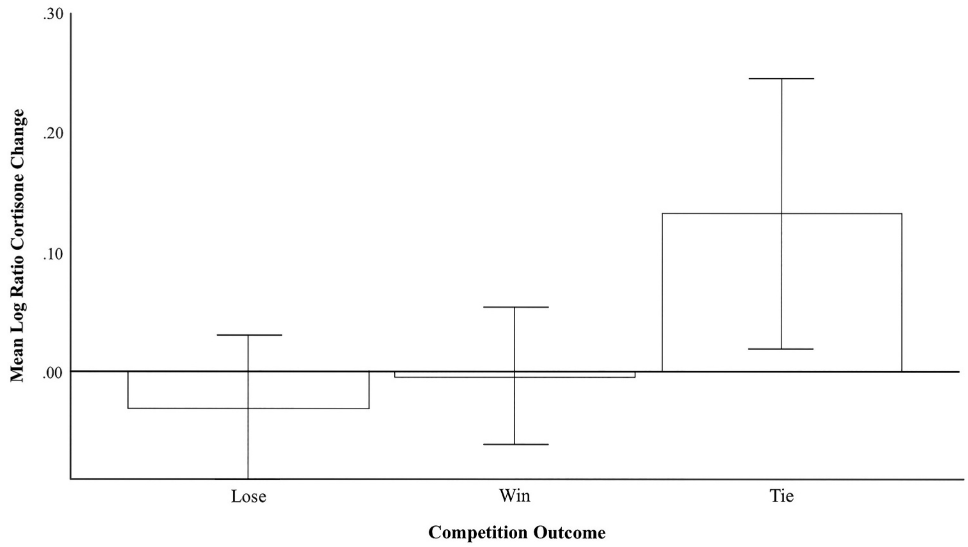

Figure 3. The effect of competition outcome on mean log ratio cortisone change was significant ($N = 160$; $p = 0.045$) when comparing cortisone change among the total participant sample (i.e., soccer match + intrasquad soccer scrimmage mean cortisone change), indicating that competitors in games that resulted in a tie ($n = 19$) had a significantly higher level of cortisone change increase compared to losing competitors ($n = 67$), $p = 0.035$. However, comparing cortisone change between competitors who tied to those who won ($n = 74$) did not reach conventional levels of statistical significance, $p = 0.088$. Error bars represent 95% confidence intervals.

5. Discussion

In keeping with our hypotheses, the findings indicate that boys', aged 8–11 years, aldosterone levels significantly increased during both the soccer match and intrasquad soccer scrimmage conditions. Our results parallel findings from the adult aldosterone literature (Bollag, 2014; Del Rosso et al., 2016), where it has been found that aldosterone is responsible for the regulation of blood pressure and fluid balance. Here, we found that boys' aldosterone levels increased during both the soccer match and the intrasquad soccer scrimmage conditions. Thus, acute aldosterone increases likely functioned to help regulate the physically taxing demands of soccer competition. When assessing aldosterone change among the total participant sample (i.e., soccer match aldosterone change + soccer scrimmage aldosterone change), the magnitude of aldosterone increase did not statistically differ between conditions, and aldosterone change was unrelated to age, BMI, PDS, and outcome. However, the magnitude of aldosterone change differed between winners and losers during soccer match play only, such that players on winning teams had significantly greater increases in aldosterone compared to losing competitors. This finding raises the possibility that when boys are invested in more meaningful forms of athletic competition, such as competition against out-group members, aldosterone production is attenuated by perceptions of stress caused by winning or losing. This latter interpretation should be met with caution given the small sample of soccer match aldosterone data we have available for match play ($N = 19$). Nonetheless, a winner-loser effect is consistent with observations among adult soccer competitors' steroid hormone responses (Slimani et al., 2017).

Cortisone levels significantly increased during the soccer match, significantly decreased during the intrasquad soccer scrimmage, and significantly differed between conditions while controlling for BMI and age. These findings support the view that juvenile competitors' HPA axis stress response is highly sensitive to cognitive appraisal differences, where unknown competitor type and/or audience effects likely promote heightened psychological stress during match play and low perception of psychological stress during the intrasquad scrimmage condition. Consistent with the competition and hormone literature on adults, the intrasquad scrimmage condition is presumed to activate the psychology of in-group membership, while competition against unknown competitors is expected to evoke more intense coalitional competitive effort against out-group competitors in boy competitors (Flinn et al., 2012; Geary & Byrd-Craven, 2003; Oxford et al., 2010). This would further support the view that the HPA axis reactivity is adaptive, context dependent, flexible, and highly sensitive to the psychosocial context of competition as early as middle childhood.

Additionally, competitors' age was positively related to cortisone change during the soccer match condition. This finding may reflect higher adrenal gland activity in older juvenile competitors, leading to larger increases. This interpretation is consistent with the observed positive relationship between baseline glucocorticoid concentrations and age among children and adults (Eyal et al., 2016; Lavretsky & Newhouse, 2012). Among the total participant sample, competitors in games that resulted in a tie had significantly larger cortisone increases compared to winning or losing competitors. This relationship may be caused by differences in the competitive nature of the soccer games, such that a tie (draw), compared to wins or losses, would reflect two evenly matched teams and the highest psychological stress in competitors. Consistent with interpretations put forth detailing hormone mediators among adult soccer athletes, differential cortisone responses in boy athletes highlight the importance of considering match outcome and competitive level (e.g., close match versus blowout; high pressure soccer match vs friendly intrasquad soccer scrimmage) as key moderating variables of HPA axis activity that are relevant for assessing psychophysiological relationships during middle childhood development (Burk et al., 2019; Jiménez et al., 2020; Slimani et al., 2017).

Our study has several strengths. It is the first study to assess salivary aldosterone and salivary cortisone changes during physically taxing, athletic competitions among juvenile boys utilizing LC–MS/MS methodology, while controlling for age, BMI, and PDS. LC–MS/MS is the most efficient and accurate hormone assay technique available and benefits by avoiding cross-reactivity issues (Mezzullo et al., 2018). These findings contribute to the endocrinology of competition, childhood development, and stress physiology literature by demonstrating that salivary aldosterone and salivary cortisone responses are context-dependent and sensitive to physical and psychosocial stressors of athletic competition in a non-Western urban sample of Hong Kongese boys (i.e., non-WEIRD). Lastly, all hormone data were collected in the late afternoon or early evenings, thus minimizing potential confounding effects related to diurnal changes of baseline adrenal hormone concentrations.

However, some limitations of the study need to be underscored. Firstly, aldosterone concentrations were only available for 19/84 soccer match, and 49/81 intrasquad soccer scrimmage participants, which may have constrained the ability to detect smaller effects of independent variables on aldosterone change. The participants' age and relationship with adrenal activity likely resulted in the high number of aldosterone cases coded as 1.65 pg/mL, which represents levels below the sensitivity of the assay (< 3.3 pg/mL). Furthermore, despite ensuring each participant played the same duration during each condition, individual variation in physical effort and physical intensity could affect adrenal hormone release. Despite efforts to control for circadian rhythm effects, differences in start times for the intrasquad soccer scrimmages could modulate the degree of hormone change. As a result, future studies would benefit by ensuring each competition occurs at the same time of day, while including more precise measures of physical exertion (e.g., fit bit; accelerometers), such as tracking heart rate and blood pressure as controls. Additionally, requiring participants to provide self-report measures of emotional investment in winning, perceived stress, and cognitive appraisal of out-group versus in-group competition, would allow more nuanced interpretations of future studies. Lastly, the low number of winners and losers for the soccer match condition for which there is available aldosterone data means that outcome effect in the soccer match should be treated with some caution.

6. Conclusions

This study demonstrates that salivary aldosterone and salivary cortisone responses significantly change in Hong Kongese juvenile boys during physically taxing, naturalistic, quasi-experimental, soccer competitions. The results show that early in human development aldosterone and cortisone responses are context-dependent, sensitive to psychosocial variables of competition, such as team outcome, and vary with respect to high- and low-psychological stressful soccer conditions. Findings shed new light on the role of two understudied adrenal biomarkers of HPA axis stress reactivity in regulating investment in competition during middle childhood. Extending this methodology to underrepresented, rural (non-urban), indigenous communities, would enhance our present understanding of the nature of the HPA axis stress response for individuals who experience nutritional and disease ecologies (e.g., nutritional insecurity, high pathogen load) that fundamentally differ compared to urban populations, and all of which likely play a significant role in shaping adrenal activity during middle childhood development and across the life course (Hodges-Simeon et al., 2019; Hodges-Simeon et al., 2017).

Disclosure statement

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