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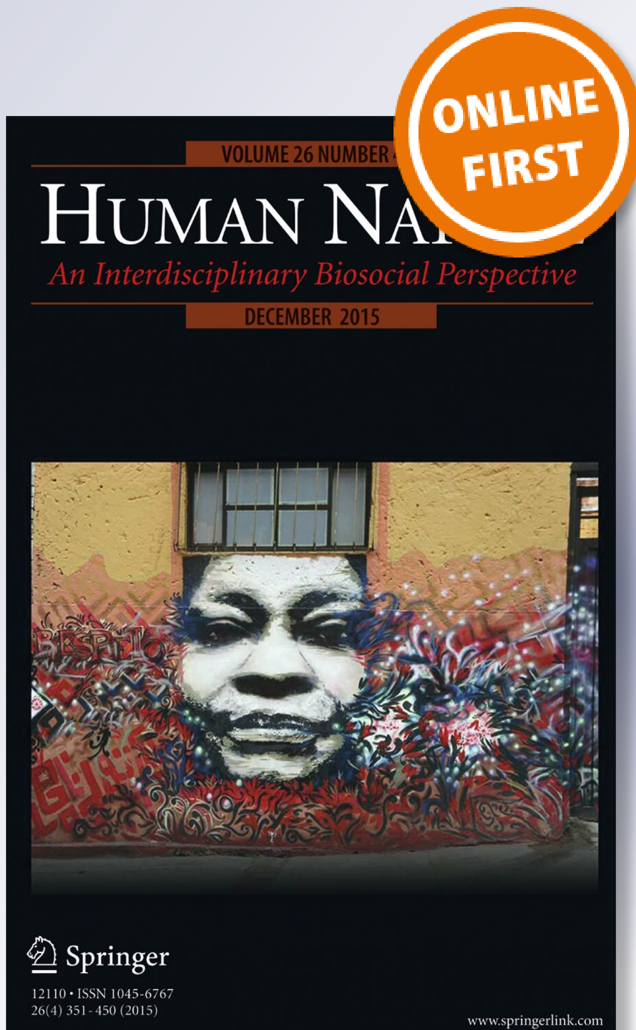

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Coalitional Physical Competition

Acute Salivary Steroid Hormone Responses among Juvenile Male Soccer Players in Hong Kong

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Abstract A large body of research links testosterone and cortisol to male-male competition. Yet, little work has explored acute steroid hormone responses to coalitional, physical competition during middle childhood. Here, we investigate testosterone, dehydroepiandrosterone (DHEA), androstenedione, and cortisol release among ethnically Chinese boys in Hong Kong (N = 102), aged 8–11 years, during a soccer match (n = 84) and an intrasquad soccer scrimmage (n = 81), with 63 participants competing in both treatments. The soccer match and intrasquad soccer scrimmage represented out-group and in-group treatments, respectively. Results revealed that testosterone showed no measurable change. DHEA increased during both treatments in the majority of participants and the degree of change had no relation to independent variables (e.g., performance, age, treatment, outcome) or covariate measures (Body Mass Index, Pubertal Development Scale). Most boys experienced androstenedione increases during match play, but no significant differences during the intrasquad soccer scrimmage competitions. The magnitude of change differed significantly between treatments and was positively associated with age. These latter findings suggest boys' androstenedione responses may be sensitive to competitor type (i.e., unknown competitors vs. peers). For most subjects, cortisol significantly increased during match play, decreased during the intrasquad soccer

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scrimmage, and differed significantly between treatments, suggesting each treatment promoted a different psychological state among competitors. Cortisol/DHEA molar ratio decreased during the intrasquad scrimmage, suggestive of a more relaxed mental state. These data shed new light on potential proximate mechanisms associated with coalitional competition among prepubescent boys, with relevance to adrenarche and life history theory.

Keywords Life history theory · Middle childhood · Competition · DHEA · Androstenedione · Testosterone

A large body of research has explored acute hormone responses to competition and aggression in human adults, with that work typically focusing on testosterone and cortisol (Casto and Edwards 2016; Geniole et al. 2017; Gray et al. 2017). Review of this literature has shown hormone changes are often context-dependent; they are contingent on individual competitors' motivation, performance, as well as cognitive appraisal of competition. For example, hormone responses can vary in winners vs. losers, individual vs. group competition, in-group vs. out-group opponents, and subjects' perceived individual performance (e.g., de Almeida et al. 2015; Flinn et al. 2012; Salvador and Costa 2009). However, little research has examined the relationship between prepubescent human juvenile aggression and baseline salivary steroid hormones (e.g., Azurmendi et al. 2016), with even less work exploring acute reactive effects of steroid hormones during different types of competition (Kamin and Kertes 2017; McHale et al. 2016).

The brain can metabolize testosterone independently of the gonads and is capable of metabolizing precursor hormones, such as dehydroepiandrosterone (DHEA) and androstenedione, centrally in response to aggressive encounters (Maninger et al. 2009; Soma et al. 2008). Comparative animal research recognizes that testosterone is not solely responsible for mediating aggression. For example, castration results in low levels of testosterone and yet does not reduce aggression in adult male prairie voles (*Mirotus ochrogaster*) (Demas et al. 1999). Further, in several seasonally breeding species, such as male song sparrows (*Melospiza melodia*) and Siberian hamsters (*Phodopus sungorus*), gonadal testosterone levels plummet during the nonbreeding season and are not associated with aggression (Pradhan et al. 2010; Scotti et al. 2008). Alternatively, acute rises in DHEA and androstenedione have been associated with the nonbreeding season during territorial challenges in male song sparrows (e.g. Pradhan et al. 2010). Scotti et al. (2008) suggest that high circulating levels of DHEA among Siberian hamsters during the nonbreeding season may be an important regulator of aggression by serving as a precursor for the conversion of androstenedione and testosterone.

Such novel, alternative mechanisms of hormone action may be of relevance to the human juvenile life history stage, at a time when reproduction is of no immediate consequence, testosterone production is low, and boys are faced with various social and competitive challenges. Our previous work among a small sample of boys in Las Vegas, Nevada, indicated that DHEA and androstenedione levels acutely increased in response to coalitional physical competition, whereas testosterone levels remained undetectable (McHale et al. 2016). Given the energetic and immunosuppressive costs of maintaining high baseline testosterone, organisms should limit testosterone release during the life course to the times it is most advantageous, such as when individuals are sexually mature and sexual partners are present (Muehlenbein 2006; Wingfield et al. 2001). These findings raise questions concerning human life history and the key factors influencing neuroendocrine responses to social competition prior to pubertal maturation.

The human juvenile transition represents the onset of middle childhood in biological terms, occurs on average between 6 and 7 years of age, and is traditionally viewed as an endocrinological marker of adrenarche, a time when production of adrenal androgens DHEA and its metabolite androstenedione increases (Campbell 2011a; Del Giudice 2014; Hornsby 2012). However, there is evidence to suggest that the timing of adrenal androgen release is variable and may start to increase in children as young as 3 years of age (e.g. Kushnir et al. 2010; Remer et al. 2005). DHEA and androstenedione are weak androgens that continue to rise through the mid-twenties (Campbell 2011b; Mouritsen et al. 2013). Adrenarche is shared with closely related great apes, and possibly some Old World monkeys, such as rhesus macaques, which speaks to conserved life history trajectories among humans and closely related nonhuman primates (Behringer et al. 2012; Bernstein 2017; Bernstein et al. 2012; Conley et al. 2011; Prall et al. 2015).

Although the function of adrenarche remains poorly understood, Campbell (2011a, b) speculates that it may serve an underappreciated biological role in facilitating neural development and behavioral plasticity during middle childhood. Comparative evidence suggests that adrenal androgens may promote prolonged development of the human prefrontal cortex, an area of the brain associated with complex cognitive tasks, learning, and that has been implicated to influence social behavior (Campbell 2011b). In addition, Byrne et al. (2017) contend that adrenarche is a sensitive period in which adrenal hormones, environmental factors, and neurobiological development interact to promote psychosocial development.

The timing of adrenarche in humans corresponds with a protracted period of juvenile development, intense social learning, and has been hypothesized to represent a critical cognitive switch point in a child's social development (e.g., Bogin 1997; Del Giudice et al. 2009; Meehan and Crittenden 2016). Cross-culturally, the juvenile transition is a time of greater self-reliance and independence which often coincides with emergent sex-specific play behaviors (Benenson and Markovits 2014; Crittenden et al. 2013; Geary 2010; Konner 2010). These data have led some evolutionary theorists to speculate that an extended juvenile period may be necessary to prepare, practice, and refine complex social competencies before entering the highly competitive social world of adolescence and adulthood (Flinn and Ward 2005; Flinn et al. 2011), all of which may be related to increased adrenal androgen release. Despite these observations, little research has investigated the activational effects steroid hormones have in response to competitive social behaviors in boys, such as coalitional competition, and the extent to which psychosocial factors affect acute steroid hormone release.

Most research investigating physical activity has focused on Western populations, such as Canada, Australia, and the United States, where sport is traditionally highly valued (Tsai 2005). Fewer analyses have been conducted in sociocultural contexts that extend beyond Western populations, such as Hong Kong, where physical activity and sports achievement are typically less valued (Ha et al. 2010; Tsai 2005). Despite this, nearly all (95.6%) Hong Kong children 7–12 years of age participate in organized sports, the highest of any age group (Community Sports Committee of the Sports Commission, Hong Kong 2009). Soccer (football) is rated as the most popular among Chinese children (Lau et al. 2015), suggesting this activity represents a salient cultural sociocompetitive sport to assess Hong Kongese boys' hormone responses to competition.

We investigated steroid hormone responses to male-male, coalitional, physical competition (soccer) among 102 ethnically Chinese, Hong Kongese boys, aged 8–11 years. Saliva samples were collected before and after two experimental treatments: (a) a soccer match against an unknown team of competitors and (b) an intrasquad soccer scrimmage played among teammates (peers). The treatments represented out-group and in-group competitive contexts, respectively. The aim of the experimental design was to evaluate steroid hormone responses in juvenile male athletes when faced with naturalistic and physically taxing coalitional competitions under two different experimental conditions. This study also explored the relationship between steroid hormone change and additional factors: match play vs. intrasquad soccer scrimmage, age, self-report measure of performance, goal scorer, and outcome of contest. Thus, simultaneous measures of salivary testosterone, DHEA, androstenedione, and cortisol were assessed in juvenile male soccer players while controlling for Body Mass Index (BMI), physical duration of play, and pubertal development. Cortisol/DHEA molar ratio was also evaluated, which has been proposed as a more accurate indicator of overall hypothalamic-pituitary-adrenal (HPA) axis activity (Kamin and Kertes 2017).

Hypotheses and Predictions

Here we tested two hypotheses: (1) boys will experience acute changes in DHEA, androstenedione, and cortisol, rather than testosterone, while playing in a soccer competition, and (2) the magnitude and direction of hormone change will be moderated by additional factors: match play vs. intrasquad scrimmage, age, self-report measure of performance, goal scorer, and outcome of contest. We predicted DHEA, androstenedione, and cortisol will increase during match play whereas androstenedione and cortisol will exhibit no statistical change during the intrasquad scrimmage. DHEA release is likely sensitive to physical exertion effects, which has been shown to occur among adults (Collomp et al. 2015) and has been reported to increase during soccer matches and soccer training in our previous research among boys, whereas androstenedione was shown to rise only during soccer match play in comparison (McHale et al. 2016). The HPA axis stress response to the competition is predicted to promote an increase in cortisol during match play. Further, we predicted boys will exhibit a stronger magnitude of increase in DHEA, androstenedione, and cortisol when competing in a soccer match in comparison to the intrasquad soccer scrimmage. Competition against unknown competitors, with the presence of spectators, may activate more pronounced steroid hormone responses that promote heightened coalitional competitive effort, focus, and intensity during male-male team competition. This phenomenon has been reported in the literature on adult males as individuals typically elicit a stronger magnitude of salivary testosterone increase when competing against out-group members in dyadic and team competitions (e.g., Flinn et al. 2012; Oxford et al. 2010). Additionally, we predicted winners and players who score at least one goal, a proxy measure of individual performance, will experience a larger magnitude of increase in DHEA and androstenedione, and decreases in cortisol, relative to pre-match and pre-scrimmage levels. Age is predicted to be positively correlated with the magnitude of DHEA and androstenedione increases, possibly due to higher baseline levels of bioavailable adrenal androgens and/or heightened cognitive sensitivities to male-male competition among older juvenile boys. We predicted cortisol will not change during the intrasquad scrimmage, whereas testosterone concentrations will remain low during all conditions. Lastly, the cortisol/DHEA molar ratio is predicted not to differ significantly before and after the soccer match, but to decrease substantially during the intrasquad scrimmage, indicative of a more relaxed psychological state in accordance with previous findings (McHale et al. 2016).

Methods

Participants

Participants were 102 boys from Hong Kong, aged 8–11 years, recruited from four teams: under 12 (U12) Tai Po Soccer Club ($n = 29$), U12 Sha Tin Soccer Club ($n = 29$), under 10 (U10) Tai Po Soccer Club ($n = 25$), and U10 Sha Tin Soccer Club ($n = 19$). Each club regularly practices twice per week and is part of the highly competitive youth Hong Kong Soccer Association. The teams are from the Tai Po and Sha Tin districts of the Hong Kong Special Administrative Region of the People's Republic of China, with each team having between 25 and 30 players. 84 boys participated in a soccer match, 81 boys participated in an intrasquad soccer scrimmage, and 63 of them participated in both the soccer match and scrimmage. The benefit of within-subject comparison is that error variance associated with individual physiological differences is reduced. Children on hormone medication were excluded. Parent and Child Informed Consent Forms were available in Cantonese and English and were signed by each participant and at least one parent or legal guardian. The study protocol was approved by the University of Nevada, Las Vegas and the University of Hong Kong Institutional Review Boards.

Experimental Procedures

Treatments Because of the known behavioral and physiological effects of home field advantage documented for adult males (Fothergill et al. 2017), efforts were made to ensure all soccer match exhibitions were played at neutral sites. In addition, each team agreed to arrange one intrasquad soccer scrimmage during a regularly scheduled practice. The soccer match exhibition represented the out-group treatment in which participants competed against an unknown team of similar-age competitors. The intrasquad soccer scrimmage represented the in-group treatment, in which players competed against teammates (peers). The soccer match and intrasquad soccer scrimmage each consisted of two 12-min halves separated by a 5-min halftime with a coach acting as the referee. Duration of physical exercise has been found to influence the magnitude of hormone reactivity (e.g., Collomp et al. 2015; Hayes et al. 2015). Thus, each participant was required to play the entire duration of the match and/or scrimmage to ensure equal playing time. On average, 50 players were in attendance during each of the U12 and U10 soccer match data collections. Before the soccer match and intrasquad soccer scrimmages, the coaches informed the players that it was a formal competition that they should take seriously. Most of the players' parents were in attendance during the matches, with fewer in attendance during the intrasquad scrimmages.

To address circadian rhythms and nutrition-related factors (Gröschl et al. 2003), efforts were made to standardize times of matches and scrimmages. All soccer matches and scrimmages began in the late afternoon or early evening. Participants' parents were instructed to prevent players from consuming food for one hour prior to the start of the warm-up period. Additionally, participants were told to avoid drinking sugary sports drinks and eating during each event. Players did not begin their warm-up until after they provided a pre-match and pre-scrimmage saliva sample. All data were collected between October and December 2016. Player positions (e.g., offense, defense, and goalkeeper), outcome, and final scores were recorded by researchers. A few participants played part of the contest as a goalkeeper and the remaining time as a player on the field (match: $n = 7$; scrimmage: $n = 11$). Since hormone responses from these participants were consistent with the overall trends observed by players, goalkeepers were included in the overall analysis.

For the U12 match, 21 participants from the U12 Tai Po Soccer Club competed against 25 of the U12 Sha Tin Soccer Club's players on October 28, 2016. To ensure equal playing time for all participants, coaches divided the players into three smaller teams, resulting in two matches of 8 vs. 8 and one match of 9 vs. 9. The U12 Tai Po Soccer Club had four remaining players who did not participate in the experiment but played 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). The U10 matches began simultaneously at 7:15 PM on November 11, 2016. The U10 Tai Po team consisted of 23 participants; the U10 Sha Tin team had 15 participants. Players were broken up into three smaller teams, resulting in two matches of 8 vs. 8 and one match of 7 vs. 7. The U10 Tai Po team had more participants, so eight additional U10 Sha Tin players who were not participants in the study competed in the match. The U10 Sha Tin team won all three matches (7–0; 3–1; 2–0). In total, 84 participants competed in a soccer match, with 13 participants scoring one or more goals.

Coaches separated players into smaller teams prior to the intrasquad soccer scrimmages. This allowed for two intrasquad soccer scrimmages to be played simultaneously, consisting of four teams total, during each scrimmage treatment. Players who did not participate in the study were allowed to play in the scrimmages. The U10 Sha Tin's team scrimmage occurred on November 19, 2016. Saliva collection began at 4:30 PM ($n = 16$; two teams of 5 vs. 5; scores: 1–1, 4–1). The U10 Tai Po's team scrimmage occurred on November 18, 2016, at 7:00 PM ($n = 24$; two teams of 7 vs. 7; scores: 1–1, 7–1). The U12 Sha Tin's team scrimmage occurred on November 22, 2016, at 6:30 PM ($n = 22$; two teams of 7 vs. 7; scores: 5–1, 3–2); and the U12 Tai Po's team scrimmage occurred on December 11, 2016, at 8:00 PM ($n = 19$; two teams of 5 vs. 5; scores: 4–1, 7–1). Twenty-one of the 81 participants scored one or more goals during the scrimmages.

Independent Variables and Covariates Participants who scored at least one goal were coded as "goal scorer," a proxy measure of individual performance. Age, treatment, outcome of contest, and goal scorer were assessed in each model as independent variables.

Participants completed the Pubertal Development Scale (PDS), a validated self-report, noninvasive five-question measure of pubertal maturation (Petersen et al. 1988). Participants' scores (M = 1.45, range: 1.00–3.6) indicated none had undergone puberty. Participants' height and weight were recorded using an anthropometer and scale at initial assessment, enabling calculation of participants' BMI. Ages were recorded for 101 of the 102 participants, BMI for 94 of the 102 participants, and PDS for 98 of 102 participants.

Saliva Collection

Saliva samples were collected 10 min before the start of the warm-up period and 10 min after the completion of the competition for each of the soccer matches and intrasquad soccer scrimmages. Each warm-up period typically lasted ~15–20 min prior to the competition. Every participant provided ~3 mL of passive drool saliva. Players' saliva samples collected before warm-up represent "before match" and "before scrimmage" hormone concentrations (Edwards and O'Neal 2009). Saliva collection during field research is a noninvasive and relatively stress-free alternative to serum and plasma (Gatti and De Palo 2011). Salivary samples were immediately stored at −20° C upon collection and shipped to ZRT Laboratory in Beaverton, Oregon.

Hormone Determination

Salivary samples were assayed by ZRT Laboratory using liquid chromatography–tandem mass spectrometry (LC-MS/MS), the most accurate and sophisticated method of hormone analysis available (Büttler et al. 2016; Handelsman and Wartofsky 2013). For testosterone, 310 of 338 total saliva samples (91.7%) were below the detection limit of the assay (< 3.2pg/mL) and therefore no statistical analysis was conducted. 103 of 338 saliva samples of DHEA (30.4%) were below the detection limit (17.1 pg/mL), with two additional values missing from the lab assay results. The low values of DHEA are considered valuable data points representing low hormone concentrations even though exact measures were below sensitivity. Thus, each measure below sensitivity was assigned a value that is one half of the minimum detection limit, resulting in 103 values of 8.55 pg/mL of DHEA. All 338 samples of cortisol and androstenedione were detectable. One of the values of cortisol was not included in our analyses due to the extreme nature of the outlier (SD > 3.5). The intra-assay coefficient of variation for all analytes tested range from 2.7 to 15.7% over the following hormone concentrations: testosterone (9.8–83.5 pg/mL), DHEA (35.6–567 pg/mL), androstenedione (21.3–343 pg/mL), and cortisol (400–1370 pg/mL). Inter-assay precision over the same hormone concentrations range between 4.3 and 18.7%. Raw data are provided in the ESM.

Statistical Methods

The raw match and scrimmage hormone data were non-normally distributed. Preliminary analyses employed Spearman's Rank-Order Correlation to explore the relationships among raw pre-competition DHEA, androstenedione, and cortisol hormone levels, and competition hormone change across the soccer matches and intrasquad scrimmages. The Wilcoxon Signed-Rank Sum Test was performed on the raw hormone concentrations to test "before" and "after" hormone changes for each treatment. Log ratio measures were employed to reflect the difference between log-transformed log values. Log transformations are appropriate for hormone concentrations given that dose-response values often, even if not uniformly, follow a log function, depending on rates of receptor binding.

Secondly, in designs containing quantitative covariates, one standard method of analysis involves analysis of covariance of change (ANCOVA) from pre-match concentrations using the difference between post-match and pre-match concentrations as the dependent variable (van Breukelen 2015). Thus, absolute change in raw match and scrimmage concentrations were calculated. The absolute change data could not be normalized for DHEA because of the high frequency of 8.55 pg/mL values and the presence of negative values. To accommodate this distribution, a ratio was created as an alternative method of analysis for each dependent variable, with the "after match" hormone concentrations divided by the "before match" concentrations. This method was applied to the scrimmage data as well. Utilizing the ratio data avoided negative values but maintained directionality of change to be observed, which resulted in successful log base 10 transformations for normality for all dependent variables. Here, log ratio values represent a measure of hormone concentration change that occurred during each treatment. To determine the possible main effects of each independent variable and covariates, log ratio match and scrimmage hormone data were used for statistical modeling when appropriate.

Lastly, we modeled the combined totals of the log ratio match and scrimmage data for each dependent variable. This method allowed "treatment" (0 = match; 1 = scrimmage) to be included as an independent variable for further hypothesis testing. This method was applied to the 63 subjects who played in both the soccer match and scrimmage, which reduced the error variance between individuals given that subjects act as their own control. These analyses resulted in between-subject and within-subject ANCOVAs for comparison. All tests are two-tailed ($\alpha = .05$) and were carried out using SPSS statistical software.

Results

Table 1 presents descriptive characteristics of age, BMI, PDS, and match and scrimmage salivary hormone concentration data. We also included correlational analyses to explore potential links between acute adrenal hormone changes during competition among juvenile boys to better assess overall HPA axis activity (Appendix). We used multiple statistical techniques in an effort to provide a complementary analysis of the raw hormone values and the transformed values, the latter of which allowed us to assess how the independent variables (e.g., performance, treatment, age) potentially influenced the degree of hormone changes.

Hormones and Control Variables

Correlations between raw before-competition hormone concentrations, hormone change, BMI, and PDS are presented in Tables 2 (match) and 3 (intrasquad scrimmage)

Table 1 Descriptive characteristics on raw pre- and post-match and pre- and post-scrimmage salivary hormone concentrations, ratio log data, molar ratios, age, BMI, and PDS.

Variables	*M*	*SD*	Min	Max
Age (years)	9.99	1.16	8.00	11.92
BMI (kg×m−2)	16.44	2.58	11.80	24.59
Pubertal development score	1.49	0.45	1.00	3.60
Hormone concentrations (pg/mL)				
Pre-match DHEA	36.31	48.09	8.55	388.50
Post-match DHEA	58.19*	88.67	8.55	616.60
Pre-match androstenedione	9.38	6.05	2.40	38.70
Post-match androstenedione	12.44*	10.04	2.30	75.90
Pre-match cortisol	492.78	641.41	100.00	4500
Post-match cortisol	613.25*	693.66	0.00	5100
Pre-scrimmage DHEA	28.09	24.34	8.55	98.40
Post-scrimmage DHEA	40.48*	49.07	8.55	298.30
Pre-scrimmage androstenedione	8.45	5.52	1.40	33.40
Post-scrimmage androstenedione	8.95	5.49	1.40	33.40
Pre-scrimmage cortisol	532.10	548.14	100	3900
Post-scrimmage cortisol	458.02*	571.15	100	3200
Cortisol and DHEA molar ratios				
Pre-match DHEA (nmol/L)	0.13	0.17	0.03	1.35
Post-match DHEA (nmol/L)	0.20*	0.31	0.03	2.14
Pre-match Cortisol (nmol/L)	1.36	1.77	0.28	12.42
Post-match Cortisol (nmol/L	1.69*	1.91	0.00	14.07
Pre-match Cortisol/DHEA	20.03	26.90	0.00	155.25
Post-match Cortisol/DHEA	18.02	27.75	0.00	165.67
Pre-scrimmage Cortisol/DHEA	25.96	34.16	1.04	202.33
Post-scrimmage Cortisol/DHEA	18.23*	27.57	1.08	193.00

*$p < 0.05$ between pre- and post-competition levels. DHEA, androstenedione, cortisol, and cortisol/DHEA molar ratio analyses used the Wilcoxon signed-rank sum test.

(see Appendix for more information summarizing the correlational results and interpretations of these data). All hormone measures and control variables passed visual inspection of the scatterplots determining a monotonic relationship existed.

Soccer Match

DHEA Our results show that, on average, participants' DHEA concentrations significantly increased from their "before match" (*Mdn* = 22.6 pg/mL) to "after match" (*Mdn* = 35.35 pg/mL) values, $z = -4.938$, $p < 0.001$. However, of the 84 individuals who participated in one of the soccer matches, 16 participants experienced a decrease in DHEA and 12 participants saw no change. Still, a majority of participants, 56 of them, experienced an increase in DHEA.

Cortisol Forty-two of 83 participant samples experienced increases in cortisol during the soccer matches, 25 displayed decreases, and 16 experienced no change. Cortisol levels increased for the majority of participants when their before (*Mdn* = 300 pg/mL) and after (*Mdn* = 400 pg/mL) concentrations are compared, $z = -2.516$, $p = 0.012$.

Androstenedione Fifty-six of 84 match participants experienced increases in androstenedione during the soccer match, 25 experienced decreases, while 3 experienced no change. Androstenedione therefore increased for the majority of participants: before (*Mdn* = 7.75 pg/mL), after (*Mdn* = 9.85 pg/mL), $z = -4.53$, $p < 0.001$.

Cortisol/DHEA Forty-six of 84 participants' cortisol/DHEA molar ratios decreased, 34 increased, and 4 did not change. No significant change was observed between before (*Mdn* = 10.07) and after (*Mdn* = 9.28) levels, $z = -1.28$, $p = 0.199$.

Intrasquad Soccer Scrimmage

DHEA Forty-two of 80 participants' DHEA increased during scrimmage play, 21 experienced a decrease, and 17 had no change. Thus, most participants experienced DHEA increases between before (*Mdn* = 19.05 pg/mL) and after (*Mdn* = 25.65 pg/mL), $z = -3.139$, $p = 0.002$.

Androstenedione Forty-six of 81 boys experienced an increase in androstenedione during the intrasquad scrimmage, 32 exhibited a decrease, and 3 revealed no change. No significant difference was observed between before (*Mdn* = 7.40 pg/mL) and after (*Mdn* = 8.20 pg/mL) androstenedione levels, $z = -1.370$, $p = 0.171$.

Cortisol Forty-seven of 81 participants' cortisol levels decreased during the scrimmage, 23 increased, whereas 11 did not change. Cortisol decreased for the majority of boys: before (*Mdn* = 400 pg/mL) and after (*Mdn* = 300 pg/mL), $z = -2.53$, $p = 0.012$.

Cortisol/DHEA Fifty of 80 participants experienced decreases in their cortisol/DHEA molar ratios, 25 increased, and 5 did not experience any change. A significant overall decrease was revealed between before (*Mdn* = 13.79) and after (*Mdn* = 9.28) levels, $z = -2.62$, $p = 0.009$.

ANCOVA: Soccer Match and Intrasquad Scrimmage

Log ratio match and scrimmage data were normalized for all analyses as assessed by the Shapiro-Wilk's test ($p > 0.05$). Exploratory analyses were performed using a one-way ANOVA to test the effects of independent variables and covariates on the dependent variables in both treatments. Significant independent variables and covariates were then included in each model for statistical analysis.

DHEA Match Prior to conducting the log transformations on the match and scrimmage DHEA ratio data, participants who had concentrations below detection for *both* of their "before" and "after" match values (i.e., coded as 8.55 pg/mL for before and after) were removed from analyses. One extreme outlier was also removed from the DHEA difference data before log transformation, resulting in a normal distribution of DHEA match data. This resulted in 13 participants being excluded from the remaining analyses (n = 71). Preliminary analyses revealed no significant effects between outcome of match (p = 0.16) and goal scorer (p = 0.22) on changes in DHEA log ratio match levels. Similarly, none of the covariates were significantly associated with DHEA log ratio match ($p > 0.05$), all of which suggest DHEA increase during match play is not moderated by the independent variables or covariates.

Androstenedione Match Androstenedione match ratio data had two extreme outliers removed prior to conducting the log transformation (n = 82). Outcome of contest approached significance (p = 0.053) as a predictor of change in androstenedione log ratio match, whereas goal scorer was not a significant predictor (p = 0.614). Age (p = 0.004) was also a significant independent variable. There was no main effect of outcome of contest on the change in androstenedione log ratio match data after controlling for age, $F_{2,\ 77} = 1.40$, $p = 0.253$, $\eta^2 = 0.035$. Additionally, age did not have a significant effect in the model, $F_{1,\ 77} = 3.56$, $p = 0.063$, $\eta^2 = 0.044$. There was homogeneity of variances, as assessed by Levene's test of homogeneity of variance (p = 0.829).

Cortisol Match One cortisol value was 0.00 pg/mL, which prevented a ratio transformation and was coded as undefined and removed from subsequent analyses (n = 82). Preliminary analysis revealed outcome of contest approached significance (p = 0.050) as a predictor of change in cortisol ratio log match. Further, age (p = 0.038) demonstrated a significant effect. Thus, an ANCOVA was applied to examine the main effect of outcome of contest on cortisol log ratio match after controlling for age. The outcome of the match did not show a statistically significant difference in cortisol log ratio match, $F_{2,\ 77} = 2.32$, $p = 0.105$, partial $\eta^2 = 0.057$, after controlling the effects of age. Further, age was not significant in the model, $F_{1,\ 77} = 1.57$, $p = 0.215$, $\eta^2 = 0.020$. The Levene's test of homogeneity of variance verified the samples had equal variances (p = 0.819).

DHEA Scrimmage The inclusion criteria described above for *DHEA match* were applied to normalize the DHEA scrimmage data, resulting in the removal of 15 participants (n = 65). Preliminary analysis found no significant relationships between the independent variables or covariates on the change in DHEA log ratio scrimmage data ($p > 0.05$).

Androstenedione Scrimmage All androstenedione ratio values were normalized following the log transformation (n = 81). No significant associations were revealed following exploratory analyses between the independent variables and covariates ($p > 0.05$) on androstenedione log ratio scrimmage data.

Cortisol Scrimmage All cortisol ratio values were normalized following a log transformation (n = 81), with no significant relationships emerging after conducting exploratory analysis on each independent variable and covariates ($p > 0.05$) on the cortisol log ratio scrimmage data.

ANCOVA: Total Participant Sample

Exploratory analyses were performed using a one-way ANOVA to test the effects of "treatment," independent variables, and covariates on potential changes to the total log ratio data (log ratio match + log ratio scrimmage). If a significant effect was identified, an ANCOVA was performed. Total log ratio data were normalized for DHEA and androstenedione as assessed by Shapiro-Wilk's test ($p > 0.05$). Cortisol total log ratio data passed visual inspection of normality but violated Shapiro-Wilk's test of normality ($p = 0.030$). Because of the robustness of the ANCOVA, we decided to proceed with the analyses for cortisol, which was supplemented with Levene's test to check for homoscedasticity.

DHEA Preliminary analyses revealed no significant differences for treatment, independent variables, or covariates with change in DHEA total log ratio data ($N = 131$).

Androstenedione Treatment ($p = 0.009$) and age ($p = 0.015$) were the only two variables that showed statistically significant relationship with change in androstenedione total log ratio data ($N = 162$). After adjustment for age, a statistically significant effect in the change in androstenedione total log ratio data between "treatments" remained, $F_{1,\ 159} = 7.00$, $p = 0.009$, $\eta^2 = 0.042$. Post-hoc analysis was performed with a Bonferroni adjustment. Androstenedione total log ratio data were statistically significantly greater in the match ($M = 0.101$, $SE = 0.021$) than the scrimmage ($M = 0.022$, $SE = 0.021$, 95% CI [0.020, 0.138]; Fig. 1). Age was significantly related to change in androstenedione total log ratio data, $F_{1,\ 159} = 9.84$, $p = 0.002$, $\eta^2 = 0.058$ (Fig. 2). The Levene's test of homogeneity of variance was confirmed ($p = 0.250$).

Cortisol Treatment was the only variable observed to have a highly significant effect on the change in cortisol total log ratio data ($N = 163$), $F_{1,\ 161} = 13.53$, $p < 0.001$, $\eta^2 = 0.078$, such that cortisol total log ratio increased during the match ($M = 0.10$, $SE = 0.04$), and decreased during the scrimmage ($M = -0.11$, $SE = 0.04$), resulting in a statistically significant mean difference between treatments of 0.21, CI [0.093, 0.310] (Fig. 3). Levene's test of homogeneity of variance was confirmed ($p = 0.230$).

ANCOVA: Within-Subject Sample

Methods employed above on the total sample were performed on the 63 participants who competed in both a soccer match and a scrimmage. Total log ratio data for the within-subject samples were normalized for DHEA and androstenedione. Cortisol passed visual inspection of normality on the histogram and was supplemented with Levene's test to check for homoscedasticity but violated Shapiro-Wilk's test of normality ($p = 0.020$). Results revealed that the within-subject sample hormone responses parallel findings described above, strengthening the reliability of these data.

DHEA The inclusion criteria described in above for *DHEA Match* was utilized for all cases (n = 103). Exploratory analyses revealed no significant association between DHEA total log ratio data, independent variables, or covariates ($p > 0.05$).

Androstenedione Treatment (p = 0.013) and age (p = 0.007) were the only two variables that showed statistically significant effects on changes in androstenedione total log ratio during preliminary analyses (n = 124). A statistically significant effect was identified on change in androstenedione total log ratio levels between treatments, $F_{1,\ 121} = 6.77$, $p = 0.010$, $\eta^2 = 0.053$, after controlling for age. Age was also significantly related to change in androstenedione total log ratio, $F_{1,\ 121} = 8.03$, $p = 0.005$, $\eta^2 = 0.062$. Levene's test of homogeneity of variance was confirmed (p = 0.701). Post-hoc analysis was performed with a Bonferroni adjustment. Androstenedione total log ratio change was statistically significantly greater in the match (M = 0.089, SE = 0.025) than the scrimmage (M = −0.002, SE = 0.021), 95% CI [0.022, 0.161].

Cortisol Exploratory analysis identified "treatment" as the only variable that showed a statistically significant effect on cortisol total log ratio data (n = 124), $F_{1,\ 122} = 10.383$, $p = 0.002$, $\eta^2 = 0.078$, whereby the average cortisol total log ratio increased during the soccer match treatment (M = 0.07, SE = 0.05) and decreased in the soccer scrimmage treatment (M = −0.14, SE = 0.04), with a mean difference of 0.21, CI [0.098, 0.30]. Levene's test of homogeneity of variance was confirmed (p = 0.077).

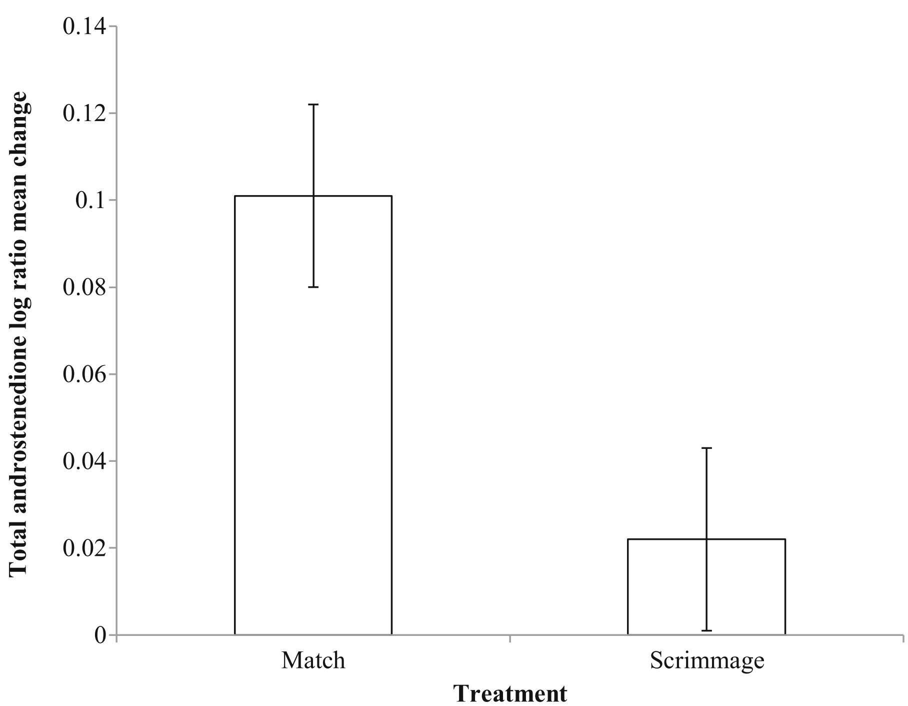

Fig. 1 Total androstenedione log ratio mean change difference across the two treatments, $p = 0.009$ ($N = 162$). Error bars represent ± 1 SE from means

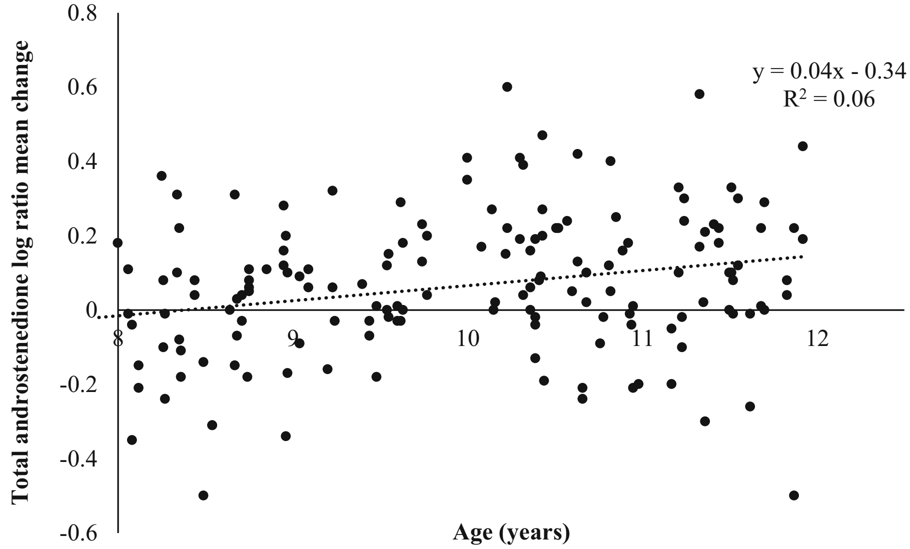

Fig. 2 Total androstenedione log ratio mean change plotted against subjects' age, $p = 0.002$ ($N = 162$). The dotted line represents the line of best fit

Discussion

The results generally support the hypotheses and predictions. Testosterone levels were generally below the detectable range in the majority of Hong Kongese boys, aged 8–11 years. These results reflect adrenal hormone responses to competition rather than testicular responses in juvenile boys. DHEA increased during the soccer match and intrasquad soccer scrimmage for the majority of participants, but these increases were not moderated by the independent variables (e.g., match play vs. intrasquad scrimmage, age, self-report measure of performance, goal scorer, and outcome of contest) or covariates (BMI, PDS), which contrasts with our predictions. The latter null findings

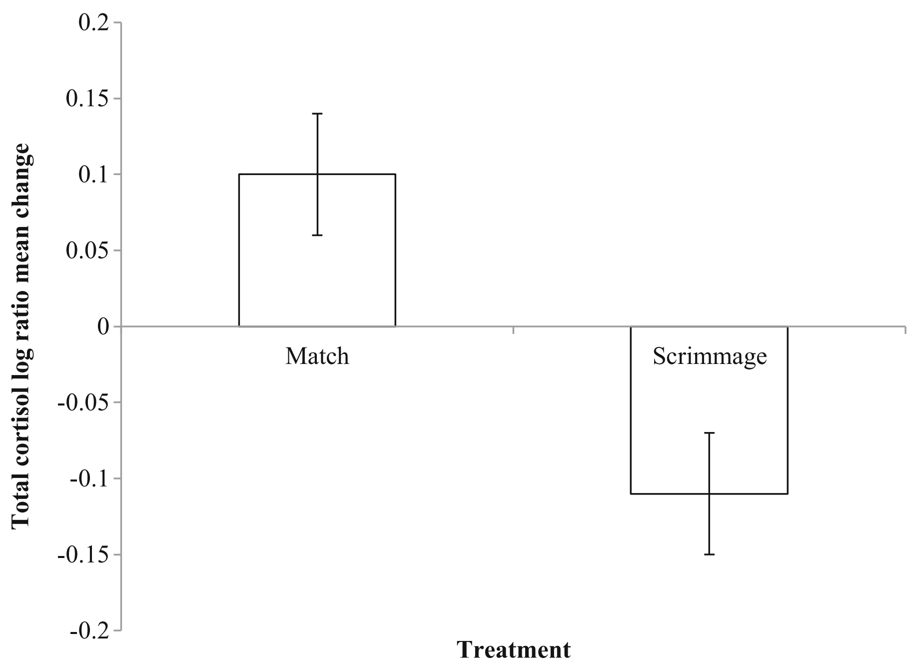

Fig. 3 Total cortisol log ratio mean change difference across the two treatments, $p < 0.001$ ($N = 163$). Error bars represent ± 1 *SE* from means

indicate DHEA rise may be related to energetic demands of physical exertion or male-male coalitional competition more generally.

Androstenedione increased during match play for the majority of competitors, but not in the intrasquad scrimmage. The degree of androstenedione change significantly differed between treatment types and was positively correlated with age in the total and within-subjects samples, the latter of which provides the most reliable statistical results concerning the independent variables' effects on acute androstenedione change. The aforementioned findings of testosterone, DHEA, and androstenedione are consistent with previous work measuring boys' hormone responses to soccer training (practice) and a soccer match among a small sample of juvenile boys from the United States (McHale et al. 2016). These findings suggest gonadal testosterone may play a reduced role in regulating male-male coalitional competition among juvenile boys when compared with adolescent or adult males' physiological responses to competition (Geniole et al. 2017). These data further support the view that boys' acute DHEA and androstenedione increases are related to coalitional physical competition.

Consistent with studies on athletic competition and exercise that link cortisol activation with the neuroendocrine stress responses (e.g., Casto and Edwards 2016; Hayes et al. 2015), the majority of participants' cortisol levels increased during match play and decreased during the intrasquad soccer scrimmage. The latter observation was not predicted. Further, cortisol differed significantly between treatment types: the majority of participants experienced increases during the match and decreases during the scrimmage. Our prediction that the cortisol/DHEA molar ratio would decrease significantly during the intrasquad scrimmage but not during the match was supported. When relaxed, individuals often report a decrease in cortisol and a DHEA increase, resulting in a cortisol/DHEA ratio decrease (Izawa et al. 2008; Kamin and Kertes 2017; Marceau et al. 2014).

Cortisol likely dropped during the scrimmage because the event caused little perception of stress. Although formal participant observations measures were not obtained, it was evident that athletes' dispositions were starkly different when competing in the intrasquad soccer competition in comparison with the soccer match. In particular, players appeared much more relaxed and were often observed laughing during the scrimmages and serious (no laughing), intensely focused, and displayed a more physical style of game play during the soccer matches. Match play likely evoked a different psychological state among competitors than the intrasquad soccer scrimmage, such that participants were more stressed by coalitional competition against out-group competitors and the expectation to perform well in front of spectators.

The results presented here and in our previous work (McHale et al. 2016) demonstrate that adrenal responses to coalitional physical competition are not uniform for androstenedione and cortisol, and they likely reflect cognitive appraisal differences related to competitor type, spectator effects, and motivation to perform well during a more meaningful competition. However, it is difficult to discern causal roles of competitor type, heightened stress of match play, or a combination of both factors, in a naturalistic study design testing effects of adrenal androgen change.

This is the first study to identify that prepubescent boys' androstenedione responses may be moderated by in-group/out-group effects of coalitional competition and become more pronounced with age, likely as a result of having more bioavailable androstenedione and/or the HPA axis becoming more sensitive to male-male competition as boys mature. Humans likely evolved cognitive and physiological mechanisms to temper or heighten the intensity of competitive responses depending on the relationship among competitors (e.g., Flinn et al. 2012). Despite competitive play and aggression representing two distinct phenomena, they also likely share certain elements that potentially are activated during more meaningful team competitions, where androstenedione release appears to be most sensitive.

Neuroimaging and social science studies have shown that specific neural activation occurs when encountering in-group members (Van Bavel et al. 2008), and this effect is strongest among men (Yamagishi and Mifune 2009). The extent to which adrenal androgens are converted to testosterone and dihydrotestosterone directly in the brain, such as in the amygdala, where it interacts locally with androgen receptors in juvenile boys to influence aggression, remains poorly studied across the life course in humans. A longitudinal sample of males between 4 and 22 years of age, utilizing magnetic resonance imaging, has shown that DHEA shapes amygdala-dependent cortical plasticity and may enhance cortical functions, such as attention and working memory (Nguyen 2018; Nguyen et al. 2013, 2016). Further, adrenal androgens, such as DHEA, have been shown to be stress reactive (e.g., Phan et al. 2017). These findings lend support for the view that adrenal androgens exert pleiotropic effects during middle childhood development. Juveniles conceivably would benefit by avoiding the immunosuppressive and energetic costs of maintaining high levels of testosterone with a distinct, yet functionally equivalent, adrenal hormone response to regulate competitive behavior across differing contexts in preparation for the adaptive challenges one is expected to face in adulthood.

One life-history concern for childhood physical activity is whether this could delay the onset of puberty. The timing of pubertal maturation is multifaceted, characterized by dynamic sexually dimorphic changes in body size and the development of secondary sexual characteristics. Heritability, nutritional status, health, and psychosocial stress are all linked to accelerated or delayed maturation in both boys and girls (Euling et al. 2008; Perkins et al. 2016; Sørensen et al. 2010; Wohlfahrt-Veje et al. 2016; Worthman and Trang 2018). In post-industrialized societies, mean age of menarche tends to be delayed upwards of 3–4 years among elite, high-performance prepubescent female athletes and ballet dancers in comparison with non-athletes (Cumming et al. 1994; Rogol et al. 2000). Leanness and low body fat have also been argued to be strong etiological factors promoting delayed menarche and slower growth velocities. In contrast, boys who participate in sports tend to have normal growth rates and normal or advanced rates of pubertal maturation (Malina 1994). However, in sports that require high-intensity physical exertion, such as endurance running, the extent to which physical exertion effects pubertal timing among males remains unclear (Rogol et al. 2000). Accordingly, the timing of pubertal maturation in humans exhibits striking plasticity and reflects life history trade-offs between competing energetic demands for growth, maintenance, and development.

This study has several strengths. The data were collected under naturalistic competitive conditions, which in the adult literature are more likely to evoke greater and more natural hormone responses to physical and psychological stress in comparison with lab settings (Geniole et al. 2017). All U12 and U10 soccer matches were conducted at the same time of day, with scrimmages also conducted at similar times of day. Unlike typical hormone and competition research on adults, this study simultaneously used LC-MS/MS technology to measure DHEA and androstenedione, in addition to testosterone and cortisol, among children. These steroid hormones were assessed across the two treatments in a relatively large sample (N = 102), including 63 subjects for whom within-subject comparisons were performed. Lastly, reference values for steroid hormones during adrenarche in prepubertal ethnically Chinese boys are now available for future research.

The study is subject to limitations. Several participants' DHEA levels did not change during either the match or scrimmage conditions, which is unusual in steroid hormone research on competition among adults. The participants' ages and the relationship between DHEA release and adrenarche likely resulted in the high number of 8.55 pg/mL values, which represent levels of DHEA below the sensitivity of the assay. Additionally, participant fitness levels, competition experience, and psychological indices of mood and motivation likely influence the adrenal system's capacity to respond to stress and may account for the individual variation observed in the hormone responses reported in this study. Further, despite attempts to control for playing time, use of more sophisticated indicators of physical exertion would have allowed for more precise measures, such as heart monitors or accelerometers (Urlacher et al. 2017). Given the low numbers of goal scorers and wins, losses, and ties, the ability to detect potential differences in hormone responses related to performance and outcome was constrained. Finally, the composition of teams competing during the intrasquad scrimmages differed slightly from that of the matches, which was unavoidable owing to the variable numbers of subjects who participated in each team. The potential adaptive interpretation of boys' steroid hormone responses during coalitional competition requires further integrative research to rigorously evaluate. While this Hong Kong sample extended the cultural scope of research in hormones and competition, further work could benefit from testing relationships between baseline and reactive steroid hormones among children living in hunter-gatherer and other small-scale societies as well as among girls and in different types of competition besides soccer.

Conclusion

This study shows that the adrenal hormones, DHEA, androstenedione, and cortisol change rapidly among a non-Western population of boys engaged in physical, coalitional competition during middle childhood, and that adrenal responses differ depending on the context of the coalitional competition. Testosterone levels were low and not measurably responsive to the same kinds of competitive behavior previously observed in adult and adolescent males (Geniole et al. 2017). Findings shed new light on potential proximate mechanisms associated with male-male coalitional physical competition among boys and the ontogeny of HPA axis activity during ecologically salient, socially competitive contexts. Also, these findings further support the view that endocrine responses to male-male coalitional competition likely shift across the life course depending on an individual's developmental stage. These data revealed novel relationships among pre-competition androgens and competition-induced adrenal hormone changes across two coalitional competitive treatments, which warrant further exploration in order to provide a more nuanced interpretation of the factors underpinning HPA axis reactivity during middle childhood. These findings can be situated in broader discussions of human life history theory, ontogeny, middle childhood behavioral endocrinology, and adrenarche, while also highlighting reactive hormone changes in response to coalitional competition as a complement to studies testing relationships between baseline steroid hormones, aggression, growth and development.

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Appendix

Correlations between Raw Pre-Competition Hormone Concentrations, Hormone Change, BMI, Age, and PDS across the Soccer Match and Intrasquad Soccer Scrimmage Treatments

This study identified several intriguing and novel exploratory relationships among baseline adrenal hormones, competition-induced hormone change, and covariates among this sample of boys. The results are reported in Table 2 (soccer match) and Table 3 (intrasquad soccer scrimmage). Consistent with reported increases in adrenal androgen production during juvenile development (i.e., adrenarche), pre-competition DHEA and pre-competition androstenedione concentrations were positively correlated across both treatments and positively associated with age and BMI. Pre-scrimmage DHEA, but not pre-scrimmage androstenedione, was also associated with age. Interestingly, pre-competition cortisol and pre-competition androstenedione were positively correlated, while pre-competition cortisol and androstenedione change were highly negatively correlated across both competitive treatments.

Pre-scrimmage androstenedione was negatively correlated with the androstenedione change during the instrasquad soccer scrimmage only, which further confirms the relationships between androstenedione baseline levels and androstenedione acute reactive effects differ across competitive treatments. DHEA, androstenedione, and cortisol match change were significantly positively associated with age, but this relationship was not found during the intrasquad soccer scrimmage treatment. In other words, during match play only older boys experienced a greater degree of adrenal hormone increases. Perhaps, boys' HPA axis responses tend to become more sensitive with age at times in which male-male coalitional competitions are highly meaningful and against unknown competitors.

Further, cortisol and androstenedione competition change were highly positively correlated during match play and the intrasquad soccer scrimmage. DHEA change was positively correlated with androstenedione change and cortisol change across both treatments. These findings reveal a significant relationship among acute DHEA change, androstenedione change, and cortisol change among a sample of prepubescent boys engaged in physical coalitional competition.

Similar results have been reported among a small sample of ethnically Chinese juvenile boys, aged 8–11 years, competing in a moderately physical table-tennis exhibition ($N = 22$), and in both boys ($N = 18$) and girls ($N = 27$), aged 9–10 years, during a non-physical math competition (McHale et al. 2018a, b). Therefore, competition induced cortisol and androstenedione change were positively correlated across all three competitive settings (soccer, table-tennis, and math competitions), irrespective of differences in energetic demands across competitions. No relationship was found among DHEA change and either cortisol or androstenedione change in the latter two studies. In the adult human competition and hormone literature, the dual-hormone hypothesis posits that a consistent relationship exists between cortisol as a predictor of testosterone change in response to physical and psychological stressors (e.g., Mehta and Josephs 2010; Mehta and Prasad 2015; Sherman et al. 2016). These findings may be of relevance for interpreting juvenile adrenal hormone responses to competition, where a consistent link is emerging between cortisol and androstenedione, rather than testosterone, during athletic and non-athletic competition during middle childhood.

Table 2 Correlations between raw pre-match hormone concentrations (pg/mL), hormone change (pg/mL), BMI, Age, PDS

	Pre-match			Change (Δ)					
	DHEA	AE	Cortisol	DHEA	AE	Cortisol	BMI	Age	PDS
Pre-match DHEA		.52**	.04	.20	–.01	.09	.23*	.28*	.05
Pre-match AE			.28*	.16	–.11	.08	.35**	.35**	.02
Pre-match Cortisol				–.13	–.46**	–.26*	–.23*	–.32*	.07
Δ DHEA					.42**	.30*	.14	.31*	.02
Δ AE						.65**	.16	.38**	.14
Δ Cortisol							.11	.22*	.05
BMI (kg×m−2)								.46**	–.12
Age									–.21*

AE Androstenedione; *BMI* Body Mass Index; *PDS* Pubertal Development Scale

*$p < 0.05$ (two-tailed)

**≤ 0.002

Table 3 Correlations between raw pre-scrimmage hormone concentrations (pg/mL), hormone change (pg/mL), BMI, Age, PDS

	Pre-scrimmage			Change (Δ)					
	DHEA	AE	Cortisol	DHEA	AE	Cortisol	BMI	Age	PDS
Pre-scrimmage DHEA		.57**	.15	–.10	–.17	–.17	.28*	.24*	.05
Pre-scrimmage AE			.25*	–.06	–.28*	–.14	.26*	.21	–.03
Pre-scrimmage Cortisol				–.10	–.36**	–.51**	–.22†	–.44**	.12
Δ DHEA					.34**	.30*	.21	.00	.09

Table 3 (continued)

	Pre-scrimmage			Change (Δ)					
	DHEA	AE	Cortisol	DHEA	AE	Cortisol	BMI	Age	PDS
Δ AE						.61**	.07	.11	.12
ΔCortisol							.11	.01	.10
BMI (kg×m−2)								.46**	–.12
Age									–.21*

AE Androstenedione; *BMI* Body Mass Index; *PDS* Pubertal Development Scale

*p < 0.05 (two-tailed)

**≤ 0.002

† = 0.052

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