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Cortisol and Testosterone Coupling: Enhanced Hormone Reactivity to Intercollegiate Athletic Competition in Varsity Women Athletes

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# Cortisol and Testosterone Coupling: Enhanced Hormone Reactivity to Intercollegiate Athletic Competition in Varsity Women Athletes

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An abstract of a thesis submitted to the Faculty of Emory College of Arts and Sciences of Emory University in partial fulfillment of the requirements of the degree of Bachelor of Science with Honors

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# Abstract

# Cortisol and Testosterone Coupling: Enhanced Hormone Reactivity to Intercollegiate Athletic Competition in Varsity Women Athletes By Filip Durovic

In a variety of settings, cortisol and testosterone are positively "coupled." That is, within-person fluctuations of these hormones occur in parallel, with increases and decreases in one hormone associated with increases and decreases in the other. To explore the relationship between cortisol and testosterone coupling and hormone reactivity in the context of athletic competition, a dataset comprised of salivary cortisol and testosterone levels from varsity women athletes from six different Emory University sports teams (volleyball 2002, 2005, and 2008; softball 2004; tennis 2009; soccer 2013) assayed for saliva samples obtained on at least one neutral day (baseline) and various different stages of one or more intercollegiate competitions was assembled. For women who played, salivary levels of cortisol and testosterone rise significantly during the period of competition. C and T reactivity are significantly correlated – the higher an athlete's reactivity for one hormone, the higher her reactivity for the other. Whether for C or T, hormone reactivity is conserved across two competitions -- the most reactive individuals for the first competition tend to be the most reactive for the second. Whether for C or T, athletes for whom these two hormone were coupled, showed substantially higher hormone reactivity to competion that women for whom these hormones were uncoupled. These results suggest that C and T are parts of a coordinated and complementary response to the physical and/or psychological stress of athletic competition.

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#### **1. Introduction**

Cortisol (C) and testosterone (T) are steroid hormones that have profound effects on physiology and behavior. Given the importance of C in managing responses to a variety of stressors (Dickerson & Kemeny, 2004; Hellhammer et al., 2009; Smith & Vale, 2006) and links between T and social behaviors, especially those of a status-seeking nature (Archer, 2006; Booth et al., 1989; Casto & Edwards, 2016; Mehta et al., 2008; Welker & Carré, 2014), these hormones figure prominently in research having to do with the hormonal correlates of athletic competition. Levels of each hormone tend to go up in association with athletic competition (see Casto and Edwards, 2017 for a review), but little is known about the impact that these hormones have on each other in competitive athletic contexts. In variety of settings C and T are positively "coupled." That is, within-person fluctuations of one hormone are associated with corresponding increases and decreases in the other (Edwards & Turan, 2020; Harden et al., 2016; Johnson et al., 2013; Marceau et al., 2013; Turan et al., 2022). The present study is designed to explore the relationship between C/T coupling and hormone reactivity to intercollegiate athletic competition in women. Background information for the study follows.

# 2. Cortisol

# 2.1. What is Cortisol?

Cortisol is a glucocorticoid nested in the broad category of corticosteroids, which is included in the broader class of steroid hormones (Pelt, 2011). Steroid hormones act as "signaling molecules" that are transported from the site of synthesis to a wide variety of organs in the body, where they act to influence physiology and behavior (Nussey & Whitehead, 2001).

C is a steroid hormone produced in and secreted from the adrenal cortex of the adrenal gland in humans and many other vertebrate animals (Larsen et al., 2002). C binds to the glucocorticoid receptor (GR) as well as the mineralocorticoid receptor (MR), both of which are present in most vertebrate animal cells (Pelt, 2011).

## 2.2. HPA Regulation of Cortisol Secretion

C secretion is primarily regulated by the hypothalamic-pituitary-adrenal axis (HPA axis) (Larsen et al., 2002), a major neuroendocrine system, comprised of the hypothalamus, pituitary gland, and the adrenal gland (Larsen et al., 2002). Parvocellular neuroendocrine cells within the paraventricular nucleus of the hypothalamus (PVN) secrete corticotropin-releasing hormone (CRH) into the primary capillary plexus of the hypothalamo-hypophyseal portal system, an array of blood vessels connecting the hypothalamus and the anterior pituitary gland (Kovacs & Sawchenko, 1996). CRH is carried through these blood vessels to the anterior pituitary gland where it stimulates the corticotropic cells – corticotrophs - to secrete adrenocorticotropic hormone (ACTH) into the bloodstream (Kovacs & Sawchenko, 1996). ACTH acts on the adrenal cortex to stimulate the production and release of C into the bloodstream (Lovallo & Buchanan, 2017). High C levels can inhibit the secretion of CRH and ACTH, halting the secretion of C (Smith & Vale, 2006) so as to effectively prevent chronic hypersecretion of this hormone that could otherwise adversely affect physical and psychological health (Dickerson et al., 2009).

## 2.3. Physiological Effects of Cortisol

C has myriad physiological effects, most of which serve functions that have homeostatic implications. These can best be grouped by the receptors to which C binds, the mineralocorticoid

receptor (MR) and glucocorticoid receptor (GR). C has a higher binding affinity for the MR than the GR, and low (basal) circulating levels of C act on MRs in different organs to increase sodium retention, water retention, potassium excretion, and blood pressure (McKay & Cidlowski, 2003). Environmental stressors (defined as anything in the environment that might endanger or be perceived to endanger homeostasis) can stimulate the HPA axis, resulting in greatly increased levels of C (Herman et al., 2016) which can then bind to the GR to, among other things, stimulate glucose production in the liver. (Herman et al., 2022; MacDougall Shackleton et al., 2019; Laycock & Meeran, 2013; Sapolsky et al., 2000). In addition to its major role in energy regulation, C also influences the immune response to a stressor (Larsen, 2002), prevents the secretion of certain cytokines, such as interferon gamma (IFN-gamma), IFN-alpha, and tumor necrosis factor alpha (TNF-alpha), while upregulating others like interleukin 4 and interleukin 10 (Elenkov, 2004). This results in a shift to the Th2 response, which is particularly useful for combating bacterial infection and preventing an overactivation of the immune response (Elenkov, 2004).

## 2.4. Diurnal Changes in Cortisol Levels

The secretion of C is pulsatile, as secretions occur in bursts following a regular diurnal pattern (Herman, 2022). Generally, C levels in blood rise during the latter part of the night to peak level at early morning (right after waking up), and then slowly drop throughout the day to reach its lowest point around midnight. The fast and significant increase in C levels during the first hour after waking, aptly named the "cortisol awakening response" (CAR), provides energy for the start of the most active phase of an organism's functioning (Federenko et al., 2004).

## 2.5. Cortisol and Stress

While C is heavily implicated in the response to stressors, it constitutes just one component of an organism-wide response evolved to deal with current challenges and prepare the body for subsequent ones (Mac-Dougall Shackleton et al., 2019). Increases in C can be provoked by physical stressors (e.g., injury, intense exercise) and psychological stressors (e.g., singing in front of an audience) (Dickerson & Kemeny, 2004; Hill et al., 2008). C responses are elicited during situations in which, from a psychological point of view, goals pertinent to survival and social status are threatened (Blascovich & Tomaka, 1996; Carver & Scheier, 1999; Lazarus & Folkman, 1984). While it has long been known that an endangerment of the "physical self" can elicit a C response, a huge body of experimental research suggests that threats to the "social self" are also capable of stimulating C secretion (Dickerson & Kemeny, 2004). The Trier Social Stress Test (TSST) has long been used as a method of inducing sociopsychological stress in laboratory settings (Kirschbaum et al., 1993). It is composed of an anticipation period (10 min) in which the participant is required to prepare a free speech and a test period (10 min) in which the participant performs the speech (5 min) and mental arithmetic (5 min) in front of an audience (Kirschbaum, 1993). Both serum and salivary C typically increased 2- to 4-fold during the test, with serum C peaking first at the end of the test (20 minutes from start of stressor) while salivary C peaked 10 minutes after the end of the test (30 minutes from start of stressor) (Kirschbaum, 1993). A meta-analysis of 208 laboratory studies examining acute psychological stressors found that a bigger C response was elicited when participants performed tasks for which they were appraised by an audience (and/or videotaped) compared with studies that lack these elements (Dickerson & Kemeny, 2004).

#### 2.6. Gender Differences in Cortisol Reactivity

Even though most studies show no significant differences in basal C between men and women, some have found that women have significantly higher morning C and morning – evening change in C than men (Wüst et al., 2000; Larsson et al., 2009). Men are typically reported to have a 1.5-2-fold higher increase in C compared to women in various versions of the Trier Social Stress Test (TSST) (Reschke-Hernández et al., 2016). This perhaps owes to the use of oral contraceptives (OC) in women, as OC users have a diminished C response to the TSST relative to non-users (Hernández et al., 2016; Gervasio et al., 2022).

# 2.7. Psychological Effects of Cortisol Administration

Putman & Roelofs (2011) review and summarize the results of studies of the effects of C administration on psychological functioning. While participant-reported unchallenged moods or emotions were found to be unchanged by varying doses of single-cortisol administration (5-40 mg), early cognitive processing of affectively significant information was influenced by C administration. Specifically, C inhibited processing of task-irrelevant emotional information (emotional stimuli that had a negative effect on task performance as they hijack attention systems in the brain) while it facilitated processing of task-relevant emotional information. Also, C administration appears to increase threat-avoidance in highly anxious individuals while it stimulates approach-related behaviors in low-anxious individuals. These findings suggest that C aids goal-directed behavior by inhibiting task-irrelevant emotional stimuli while enhancing task-relevant emotional stimuli.

#### 3. Testosterone

## 3.1. Overview

Testosterone is the primary sex hormone in men, belonging to the class of steroid hormones that exerts its effects by binding to androgen receptors (ARs). Androgens promote the embryological development of the primary male sex organs and the pubertal development of male secondary sex characteristics. In men, T is produced in and secreted by the testes, with additional amounts contributed by direct secretion from the adrenal cortex and the conversion of androgen precursors also secreted from the adrenal cortex. In adult women, circulating T is derived from three sources in approximately equal quantities: direct secretion from the adrenal glands; direct secretion from the ovaries; and indirect extraglandular (e.g., liver, kidney, muscle, fat, skin) conversion from T precursors secreted by the adrenal and ovary. (Handelsman et al., 2018, p. 806). Before puberty, men and women produce similar amounts of T and have similar amounts of circulating T. Due to a 20-fold increase in T production and secretion by the testes after the onset of puberty, T levels in adult men are substantially higher than in women (Handelsman et al., 2018).

## 3.2. HPG Regulated Secretion

The hypothalamic-pituitary-gonad (HPG) axis is the main secretory system that regulates the gonadal production and secretion of T (Swerdloff et al., 1992). Gonadotropin-releasing hormone (GnRH) is secreted into the hypothalamo-hypophyseal portal system by GnRH neurons of the hypothalamus (Millar et al., 2004). From there, GnRH is transported to the anterior pituitary where it stimulates pituitary gonadotrophs to release gonadotropins -- luteinizing hormone (LH) and follicle-stimulating hormone (FSH) -- into the bloodstream, via which these hormones travel to the gonads where they act on Leydig cells in male testes and theca cells surrounding the ovarian follicles in women to promote the secretion of T (Burger, 2002; Charlton, 2008). High T levels can inhibit the production/secretion of LH and FSH, effectively inhibiting its own production (Luetjens C.M. & Weinbauer, 2012). T can be synthesized from other circulating androgens (T precursors) in peripheral tissue (adipose tissue, skin, prostate etc.). In both sexes, T and its precursors are secreted by the adrenal cortex (Burger, 2002; Mooredian et al., 1987). In men, approximately 95 percent of T in the body is produced and secreted by the testes, with the remaining amount contributed by adrenal secretion or the peripheral conversion of adrenally- derived precursors whose production is mainly stimulated by ACTH (Mooredian et al., 1987). In women, 25% of T is circulation is produced by the ovaries, 25% comes from adrenal secretion, with the remaining 50% derived from the peripheral conversion of precursors (Burger, 2002).

# 3.3. Diurnal and Menstrual Cycle Changes in Testosterone Levels

In healthy men and women, T has a diurnal pattern of secretion, peaking at early morning (8 a.m.), then slowly falling until levels reach their lowest point at 9-10 P.M., after which they remain relatively stable until midnight when they start increasing again (Resko & Eik-Nes, 1966; Ankarberg & Norjavaara, 1999). When measured at the same time of day, individual differences in T levels are relatively stable even over a temporal span of up to one year (Granger et al., 2004). In women, T levels vary in relation to stage of menstrual cycle, with peak levels occurring around the time of ovulation (Dabbs & La Rue, 1991).

## 3.4. Physiological and Behavioral Effects of Testosterone

Both dominance-related and aggressive behavior are associated with T (Mazur & Booth, 1997). In men, normal sexual behavior is dependent on adequate levels of T (Davidson et al., 1979; Davidson et al., 1982). Sexual arousal and activity in men is linked with T, with higher levels of the hormone being associated with periods of more pronounced sexual activity (Roney et al., 2003). Men exposed to scents of an ovulating female subsequently show higher levels of T than men exposed to non-ovulating females or a control scent (Maner & Miller, 2009). T may also contribute to the capacity for genital sexual arousal in women (Traish et al., 2002). Higher basal levels of T are linked to increased sexual arousal in women; T levels are higher before and after sexual activities compared to non-sexual ones in women (Anders et al., 2007). Sexual thoughts also increase T levels in women (Goldey & Anders, 2011).

#### 3.5. Dominance, Status and Non-Athletic Competition

In humans, as well as many other animals, levels of T are related to dominance, that is behaviors that are meant to preserve and gain high status within a group (Mehta et al., 2008). Generally, in both men and women, individuals with higher basal T were found to be more dominant, had higher social status, and were more aggressive than individuals with lower basal T (Archer, 2006; Joseph et al., 2003; Mazur & Booth, 1998). Greater persistence in completing high-difficulty competitive tasks has also been linked to higher levels of basal T in men (Welker & Carré, 2014). Competition-related environmental factors (e.g., home venue vs away venue or winning vs losing) might impact T levels, but laboratory studies paint a slightly inconsistent picture, with some studies finding that victories or defeats in male sports competition (tennis and chess) influence T levels for subsequent competitions, with losers having lower and winners having higher levels of T, while other studies have shown a completely opposite effect or no effect at all (See Casto & Edwards, 2017 for review).

### 3.6. Testosterone Administration Studies

T is an anabolic steroid and long-term supplements of T can increase muscle mass and strength in men, particularly if combined with resistance training (Bhasin et al., 1996). Similar effects are seen in women (Handelsman et al., 2018). Individual differences in salivary T levels predict persistence in men as measured in a laboratory setting (Welker and Carré, 2014). A single dose study has reported that supplemental T (typically administered via a gel applied to the skin) in men can increase persistence in a laboratory-conducted competition with others (Kutikova et al., 2021).

### 4. Exercise Effects on Cortisol and Testosterone Levels

Acute resistance exercise has been found to increase C levels in both men and women (Hill et al., 2008; Mulligan et al., 1996). Total and free testosterone in plasma increase during aerobic exercise in proportion to the intensity/and or duration of the workout (e.g., Viru, 1992), perhaps due to a combination of factors including a decrease in metabolic clearance rate (Cadoux-Hudson et al., 1985).

#### 5. Testosterone, Cortisol, and Athletic Competition

The effects of athletic competition on C and T have been extensively reviewed elsewhere (Casto & Edwards, 2016). There is a general consensus that, across a wide variety of sports,

athletic competition is typically associated with an increase in C and T in men and women, with the greatest increases seen during the period of actual play (Casto & Edwards, 2016).

### 6. Testosterone and Cortisol Coupling

In a variety of settings, C and T are positively "coupled." That is, within-person fluctuation of these hormones occurs in parallel, with increases and decreases in one hormone associated with increase and decreases in the other (Edwards & Turan, 2020), Positive diurnal coupling between C and T has been found in studies of adolescent boys and girls (Marceau et al., 2013), incarcerated adolescent boys (Johnson et al., 2013), and a mixed-sex lifespan sample of participants aged 11-81 years (Harden et al., 2016). C and T coupling was also shown in the context of a stress-inducing socio-evaluative threat, as C and T were found to covary in prepubertal boys and girls and adult men participating in the TSST (Turan et al., 2015). Significant C and T coupling has been reported for a combined sample of intercollegiate women volleyball and soccer players (Edwards & Turan, 2020) and a sample of undergraduate women performing a musical recital in front of an audience (Turan et al., 2022). In the latter study, positive coupling of C and T appeared to predict testosterone reactivity to the performance phase of recital, with coupled individuals showing substantially higher increases in T than uncoupled individuals.

The present study is designed to explore the relationship between C and T coupling and hormone reactivity in the context of athletic competition. To this end, a dataset comprised of salivary C and T levels from varsity women athletes from six different Emory University sports teams assayed for saliva samples obtained on at least one neutral day and various different stages of one or more intercollegiate competitions was assembled. Within-individual correlations between C and T allowed for the assignment of individuals as being either "coupled" or "uncoupled," a designation that was then used to determine the relationship between C and T coupling and competition-related hormone reactivity. Details follow.

# 7. Methods

#### 7.1. Selecting Competitions and Participant Samples for Analysis

For this retrospective study of women athletes, the 94 participants were the consenting members of the Emory University (Atlanta, GA) women's 2002, 2005, and 2008 varsity volleyball teams (16, 18, and 14 participants respectively), the 2004 varsity softball team (16 participants), the 2009 tennis team (5 participants), and the 2013 women's soccer team (25 participants). For purpose of this research, participants were included for data analysis only if they had given at least one neutral-day saliva sample as well as before- and after-competition saliva samples for at least one of the analyzed competitions for which their team contributed samples. The dataset does not include values for five soccer players, one softball player, and one volleyball player, eliminated because they did not meet one or more of these criteria. Nor does it include data for two volleyball players who suffered injuries serious enough to prevent or limit competitive play. The study includes data for three different volleyball teams. Three volleyball players ontributed saliva samples during two different seasons of volleyball play. For purposes of analysis, only the hormone values for saliva samples contributed during their senior season of competition were included.

As described elsewhere (Edwards & Casto, 2013. p. 155), Emory University is a Division III (no athletic scholarship) school. Intercollegiate competitions are taken seriously by the participants, who played with intensity and an obvious passion for their sport. Circumstances were such that players expected that the outcomes for the games/matches for which saliva samples were obtained saliva samples would have a bearing on league standing and/or opportunities for post-season play. Each of the studies was approved by Emory's Institutional Review Board. Potential participants were informed about the purpose of the study as well as the method and frequency of saliva sampling, and athletes gave written informed consent prior to participation. Although not queried about their undoubtedly varied use of prescription/nonprescription medications, as part of the consent procedure women were specifically asked about hormone contraceptive use. For the study with soccer players, each woman was asked to respond "yes" or "no" to the following question: "Are you currently using oral contraceptives?" For all the other studies, women were asked to respond "yes" or "no" to this question and another: "Are you currently using any injected, implanted, or patch-delivered hormone contraceptive?" With the exception of the tennis team, saliva samples for at least two competitions were collected. For teams that played more than two matches (volleyball 2002, softball 2004, volleyball 2005), for the purposes of describing the hormone correlates of athletic competition, the two contests that were the most competitive based on outcome for analysis and presentation were selected.

Depending on team/sport, each participant gave multiple saliva samples including some given on one or more neutral days and in connection with one or more competitions. For purposes of analysis, for each participant a baseline (neutral-day) value was calculated for each hormone by averaging the values for all the samples obtained on neutral days. This could be on the day of consent or before one, two, or three practice sessions depending on team/sport.

For all the competitions this report draws upon, saliva samples were obtained at multiple times that varied according to team. For all teams except the 2004 softball team and the 2013 soccer team, saliva samples were obtained before and after one or two practice session during a

neutral day. For soccer players (2013) a single neutral day sample was obtained at the time of consent. Softball players (2004) gave samples before three practice sessions, but not after. For competition-days, saliva collection was done immediately prior to warm-up which began approximately 1 h before the start of play. This was done for every team and every competition. For some teams (volleyball 2008, tennis 2009, soccer 2013), saliva samples were also collected mid/after warmup. For every team and every competition, saliva samples were also obtained immediately after the end of competition. Saliva was sampled in the same manner in all situations according to procedures describe in detail elsewhere (Edwards & Casto, 2013). Briefly, participants were provided with a piece of sugar-free gum (Trident®, original flavor) to stimulate saliva production and a 20 ml polypropylene vial which they were asked to fill to a 5 ml line marked on the side. Participants chewed for a timed 2-minute period before starting to fill the vial. Including the pre-delivery chewing period, collection of a single saliva sample typically took 4–7 min.

The games chosen for each team and the associated saliva samples assayed for those games (baseline, before warm-up, and after end of competition) that were used for the purposes of analyzing the effects of athletic competition on C and T levels are explained in detail below. Only one analysis, the zero-order Pearson correlation between C and T and subsequent assigning of "coupled" vs "uncoupled" status, was done using all available saliva samples for all games that saliva samples were previously taken for (explained in more detail in the cortisol/testosterone coupling section)

For the 2002 volleyball team, saliva samples were obtained before and after one practice session. The before-practice hormone values constituted baseline values for this team. Saliva samples were also obtained immediately before warm-up and immediately after the third and

fourth (of a total of four) matches played as part of a home volleyball tournament held over the course of a weekend in mid-October. The third match (a 3–1 win) began at 8:00 PM and was completed by 9:50 PM. The fourth match (a 3–1 win) began at 3:00 PM the next day and was completed a few minutes before 5:00 PM.

For the 2004 softball team, hormone values for samples obtained before three practice sessions as well as samples obtained immediately before warmup and after completion of two home games were analyzed. The average of the three before practice sessions constituted baseline for this team. For the players that had hormone values for fewer than 3 practice sessions, the mean of the two they had (if they had two) or only one (if they had one) represented baseline values. The first match (a 2–0 defeat) was played in mid-March. The match started at 3:00 PM and finished at 4:35 PM. The second (a 2–1 victory) was played in early April. The game started at 3:00 PM and finished at 4:15 PM.

For the 2005 volleyball team, hormone values for samples obtained before and after a single practice session, as well as for samples obtained immediately before warm-up and after two home games played over the course of a single weekend in late October were analyzed. The before practice hormone value constituted baseline values for this team. The first match, a 2–3 loss, was played at 7:00 PM and was finished at 9:20 PM. The second match, a 0–3 loss, was played one day later at 7:00 PM and was completed at 8:20 PM.

For the 2008 volleyball team, hormone values for saliva samples obtained before and after two practice sessions as well as samples obtained before warm-up and after completion of two home games played one day apart on October 31st and November 1st were analyzed. The mean before practice hormone values constituted baseline values for this team. If a participant gave a saliva sample only for one practice sessions, before practice hormone values for that session constituted baseline. The first game (a 3–0 victory) began at 7:10 PM and was completed at 8:30 PM. The second game (a 0–3 loss) began at noon and was completed at 1:10 PM.

For the 2009 tennis team participants gave saliva samples for a single intercollegiate match played outdoors as well as before and after a single outdoor practice session. The match started with doubles competitions which were then followed by singles matches. Saliva samples were given before warm-up for the doubles matches, immediately after the end of each doubles match, approximately 1 h later, and then again after their singles match. Before practice hormone values constituted baseline or this team. Only hormonal data from baseline, before warm-up, and after the end of the singles matches were used in our analysis of the hormonal correlates of athletic competition.

For the soccer 2013 team, saliva samples were given on the day of consent, before warmup, immediately after, and 30 minutes after two intra-conference NCAA soccer competitions, 1 week apart, one at home (1-0 loss) and the other away (2-0 victory). Saliva samples obtained on the day of consent constituted baseline for this team.

After collection, samples were stored at -20 or -80 °C. Frozen samples were later sent to the Biomarkers laboratory of the Yerkes Primate Center in Atlanta, Georgia for hormone assay. Salivary C was assayed in duplicate using enzyme immunoassay kits from either Salimetrics (State College, PA) or Diagnostic Systems Laboratories (Beckman Coulter, Webster TX) with a range of analytical sensitivity of .025–10 µg/dl for test volumes of 25 µl. Salivary T was assayed in either duplicate or triplicate using a modification of the Diagnostic Systems Laboratories radioimmunoassay kit with a range of analytical sensitivity of 2–500 pg/ml for volumes of 200  $\mu$ l. All intra-assay coefficients of variation for the various assays were < 8%; all inter-assay coefficients of variation were < 20%.

#### 7.2. Statistical Analyses

The SPSS statistical package was used for calculation of independent *t*-tests (two-tailed), repeated measures ANCOVA, and within-subjects post-hoc contrasts for hormone levels across the different sampling times. Pearson zero-order correlations were used to relate cortisol and testosterone reactivity for the first and second performances. In all cases,  $p \le .05$  was required for statistical significance. Effect sizes, Cohen's d (*d*) and partial eta squared ( $\eta_p^2$ ), were calculated for *t*-tests tests and ANCOVAs respectively. In keeping with procedures in descriptive reports of hormone levels associated with athletic competition (e.g., Casto & Edwards, 2016; Edwards and Kurlander 2010), raw data are used for all figures and statistical analyses in this report.

As the sphericity assumption was not satisfied for any of our ANCOVA's (as judged by a statistically significant Mauchly's test of sphericity), the Greenhouse-Geiser test (robust to violation of sphericity) was used to test for within-subject effects.

# 7.3. Within-Person Calculation of Cortisol/Testosterone Coupling

Depending on team/sport, participants gave a total number of samples that ranged from 6 to 13, over a period of several days to a week. These included one or more neutral-day samples, game day samples obtained before and after each game/match, and for some participants a mid-warm-up (volleyball 2008) or after warm-up (soccer 2013) sample, and (for soccer 2013) a sample given thirty minutes after the end of competition. Within-person Pearson zero-order

correlation between cortisol and testosterone was calculated for each participant. A total of 33 participants showed a positive statistically significant ( $p \le .05$ ) association between cortisol and testosterone, with r-values ranging from .63 to .92. For purposes of further analysis, these individuals were considered to have C and T values that are "coupled." For the remaining individuals C and T levels were considered "uncoupled." It is important to note that hormone values from every saliva sample, even ones taken for games that were not used in any other analysis in this study (e.g., games 1 and 2 for the volleyball 2002 team), were used to calculate the correlation between C and T and subsequently whether a participant is coupled or not.

A second coupling variable (basal coupling) was calculated in the same way, except only before practice hormonal values/hormonal values on the day of consent (hormone values used to create baseline values) and hormonal values assayed before warmup on match day were used in creating it (3 to 8 samples total depending on the team). This was done in order to test whether coupling without the inclusion of after-competition values could predict hormone reactivity to competition.

### 8. Results

### 8.1. Effects of Athletic Competition on Salivary Levels of Cortisol and Testosterone

For each hormone and for each of two games/matches a mixed-design analysis of covariance (ANCOVA) was used to compare hormone levels for samples obtained at three different times (the within-subjects variable) for women who played in the competition and those who did not (the between-subjects variable) while controlling for OC use. In this analysis, hormone values were for samples obtained for neutral-day (baseline), before warm-up on the day of competition, and immediately after the end of the competition. With the exception of the tennis team, saliva samples for two previously chosen competitions were used. For convenience these are referenced as the "first" and "second" competitions in accordance with the order in which they were played.

## 8.2. Game 1

Mean C and T values for the first game/match are shown in Figure 1. Forty-seven women out of a total of 57 that played (82.46%) experienced an increase in C levels from neutral day baseline to after competition. Forty-eight women (84.21%) experienced an increase in T levels from neutral day baseline to after competition.

C levels changed significantly from baseline to after the end of competition ( $F(1.36, 122.01) = 5.07, p = .017, \eta p 2 = .05$ ). There was a significant overall between-subjects effect, as individuals that played had significantly higher mean C than those that did not play ( $F(1, 90) = 19.98, p < .001, \eta p 2 = .18$ ). There was a significant interaction between group (played vs did not play) and the change in C levels at different timepoints ( $F(1.36, 122.01) = 33.85, p < .001, \eta p 2 = .27$ ). There was no effect of OCs on C level changes in response to competition ( $F(1.36, 122.01) = .48, p = .549, \eta p 2 = .01$ ). There was a significant interaction between group and the change in C levels between before warm-up and after competition ( $F(1, 90) = 39.65, p < .001, \eta p 2 = .31$ ). Figure 1. shows that the change seems to largely stem from the increase in C experienced by the individuals that played.

T levels changed significantly from baseline to after the end of competition ( $F(1.78, 159.78) = 4.93, p = .011, \eta p 2 = .05$ ). There was a significant overall between-subjects effect, as individuals that played had significantly higher mean T than those that did not play (F(1, 90) =

6.24, p = .014,  $\eta p 2 = .07$ ). There was a significant interaction between group (played vs did not play) and the change in T levels at different timepoints (F(1.78, 159.78) = 20.04, p < .001,  $\eta p 2 = .18$ ). There was no effect of OCs on T level changes in response to competition (F(1.78, 159.78) = 1.23, p = .291,  $\eta p 2 = .01$ ). There was a significant interaction between group and the change in T levels between before warm-up and after competition (F(1, 90) = 30.44, p < .001,  $\eta p 2 = .25$ ). Figure 1. shows that the change seems to largely stem from the increase in T experienced by the individuals that played.

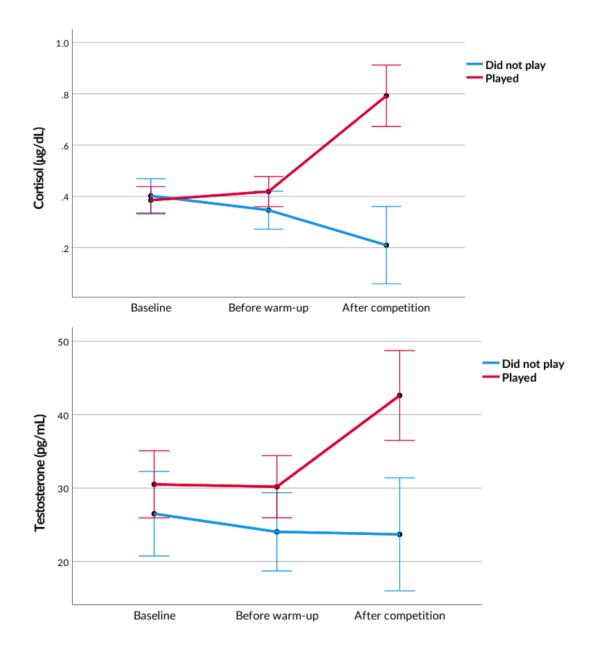


Figure 1: Mean C and T values for Game 1 at baseline, before warm-up, and after competition for players that played and those that did not (total N = 93). *Error bars* represent 95% confidence intervals

Mean C and T values for the second game/match are shown in Figure 2. Forty-two women out of a total of 57 that played (73.68%) experienced an increase in C levels from neutral day baseline to after competition. Forty-nine women (85.96%) experienced an increase in T levels.

Overall, C levels changed significantly from baseline to after the end of competition  $(F(1.61, 128.89) = 3.34, p = .049, \eta p 2 = .04)$ . There was a significant overall between-subjects effect, as individuals that played had significantly higher mean C than those that did not play  $(F(1, 80)=15.76, p < .001, \eta p 2 = .17)$ . In addition, there was a significant interaction between group (played vs. did not play) and the change in C levels at different timepoints ( $F(1.61, 128.89) = 18.50, p < .001, \eta p 2 = .19$ ). There was no effect of OC use on C level changes in response to competition ( $F(1.61, 128.89) = .24, p = .738, \eta p 2 = .00$ ). There was a significant interaction between group and the change in C levels between before warm-up and after competition ( $F(1, 80) = 22.24, p < .001, \eta p 2 = .22$ ). Figure 2. shows that the change seems to largely stem from the increase in C experienced by the individuals that played.

Overall, T levels changed significantly from baseline to after the end of competition  $(F(1.78, 142.51) = 3.51, p = .038, \eta p 2 = .04)$ . There was no significant overall between-subjects effect, with individuals that played having higher (but not significantly) mean T than those that did not play  $(F(1, 80) = 3.49, p = .066, \eta p 2 = .04)$ . In addition, there was a significant interaction between group (played vs did not play) and T levels at different timepoints  $(F(1.78, 142.51) = 21.47, p < .001, \eta p 2 = .21)$ . There was no effect of OC use on T level changes in response to competition  $(F(1.78, 142.51) = .61, p = .526, \eta p 2 = .01)$ . There was a significant interaction between group and the change in T levels between before warm-up and after competition  $(F(1, 78, 142.51) = .61, p = .526, \eta p 2 = .01)$ .

80) = 31.81, p < .001,  $\eta p2 = .28$ ). Figure 2. shows that the change seems to largely stem from the increase in T experienced by the individuals that played.

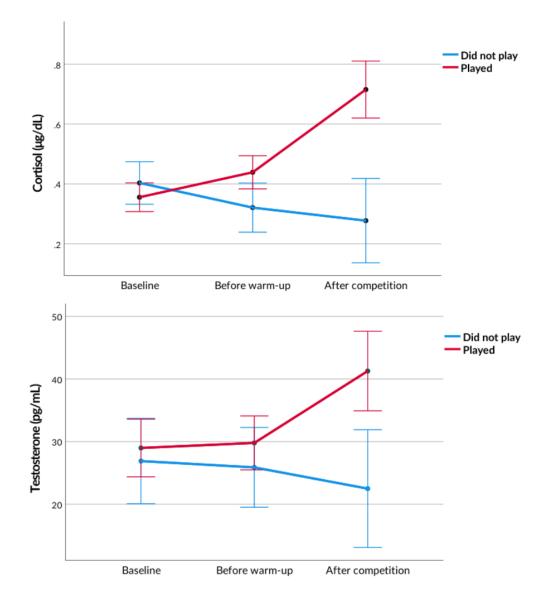


Figure 2: Mean C and T values for Game 2 at baseline, before warm-up, and after competition for players that played and those that did not (total N = 83). *Error bars* represent 95% confidence intervals

# 8.4. Relationship Between T and C Reactivity

Pearson zero-order correlations for all possible combinations of C and T reactivity across the two games/matches are presented in Table 1. Whether for C or T, for women who played in both competitions (total N = 47), hormone reactivity for the first competition is significantly correlated with hormone reactivity for the second. Second, for women who played in both competitions, whether for the first or second competitions, C reactivity is significantly correlated with T reactivity.

Reactivity conclations						
		T reactivity game 2	C reactivity game 1	C reactivity game 2		
T reactivity game 1	Pearson Correlation	.810**	.436**	0.257		
	Sig. (2-tailed)	0.000	0.002	0.081		
T reactivity game 2	Pearson Correlation		0.257	.407**		
	Sig. (2-tailed)		0.082	0.005		
C reactivity game 1	Pearson Correlation			.617**		
	Sig. (2-tailed)			0.000		

**Reactivity correlations** 

Table 1: C and T reactivity correlations (total N = 47), \*\*  $p \le .01$ 

## 8.5. Cortisol and Testosterone Coupling and Hormone Reactivity

Whether for the first or second competitions, salivary C and T levels increase dramatically in association with athletic competition for individuals who played, principally during the period from before-warm up to after competition. For purposes of further analysis, for individuals who played, hormone reactivity to competition was calculated as percent change in hormone level from neutral-day baseline to after the end of competition. This was done separately for the first and second competitions. Figures 3. and 4. show C and T reactivity for the first and second competitions, respectfully, for individuals who played grouped according to whether C and T were coupled or not. For the first and second competitions, C and T reactivity means were significantly higher for coupled participants than for uncoupled participants (see details below).

#### **8.6.** Competition Coupling – Game 1

For game 1, coupled individuals (M = 254.81, SD = 245.04) showed a significantly greater C percentage increase from baseline to the end of the first game (t (29.25) = 3.45, p = .002, d = 1.03) compared to the non-coupled ones (M = 70.97, SD = 105.64). Coupled individuals (M = 86.04, SD = 69.03) showed a significantly greater T percentage increase from baseline to the end of the first game (t (55) = 3.11, p = .003, d = .83) compared to the non-coupled ones (M = 36.20, SD = 52.12).

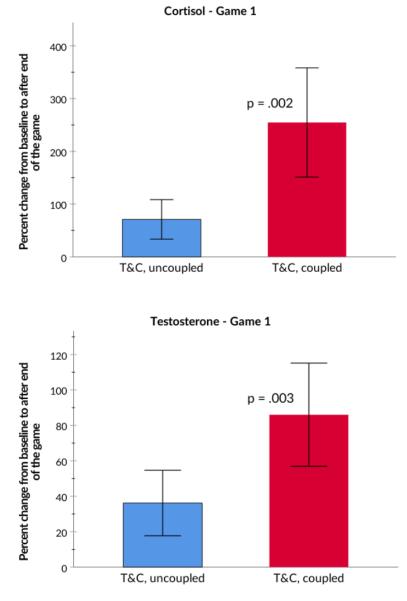


Figure 3: C and T reactivity from baseline to after competition for coupled and uncoupled individuals – Game 1 (total N = 57). *Error bars* represent 95% confidence intervals

# 8.7. Competition Coupling – Game 2

For game 2, coupled individuals (M = 200.14, SD = 201.02) showed a greater C percentage increase from baseline to the end of the second game (t (55) = 2.17, p = .035, d = .58) compared to the non-coupled ones (M = 99.52, SD = 150.06). Coupled individuals (M = 80.29,

SD = 71.44) showed a significantly greater T percentage increase from baseline to the end of the second game (t (55) = 2.79, p = .007, d = .74) compared to the non-coupled ones (M = 34.85, SD = 51.59).

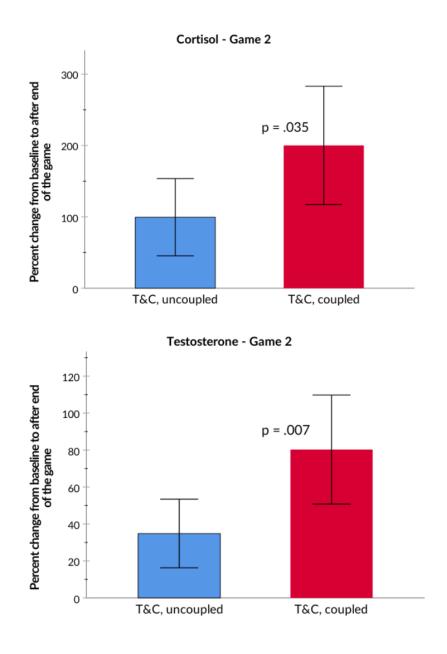


Figure 4: C and T reactivity from baseline to after competition for coupled and uncoupled individuals – Game 2 (total N = 57). *Error bars* represent 95% confidence intervals

#### 8.8. Differences in Basal C and T Between Coupled and Uncoupled Players

Coupled (M = .32, SD = .15) and uncoupled individuals (M = .43, SD = .22) have been found to significantly differ in levels of Basal C, with coupled individuals having lower basal C than the uncoupled ones (t (87.91) = 2.82, p = .006, d = .54). Coupled individuals (M = 25.61, SD = 16.08) did have, on average, lower basal T than uncoupled ones (M = 30.94, SD = 17.84), but no significant difference in basal T was observed between the groups (t (92) = 1.43, p = .156, d = .31)

# 8.9. Basal Coupling

Basal coupling, calculated by using only hormone values assayed from saliva samples obtained on neutral days (any hormone value used in creating neutral-day baseline values) and on before warm-up on the day of competition, does not predict hormone reactivity of C or T in response to competition. No significant difference in the percentage increase of either C or T between the basally coupled and uncoupled individuals was found in any period.

# 9. Discussion

## 9.1. Competitition-Induced Changes in Testosterone and Cortisol Levels

In acordance with previous studies (e.g., Bateup et al., 2002; Filaire et al., 1999; Hamilton et al., 2009; Jiménez et al., 2012), for the majority of intercollegiate women athletes included in this dataset that played their respective mathces, athletic competition is associated with a substantial increase in salivary levels of C and T occurring during the interval between the start of warm-up and the end of the competition. This is not surprising since the dataset compiled for this retrospective study included participant hormone values from many of these studies (e.g., Casto & Edwards, 2015; Edwards & Kurlander, 2010; Edwards, Wetzel, & Wyner, 2006). These increases are unique to women who play – mean after-competition hormone levels for teammates who did not play remained at or below before-warm-up levels. While the anticipation of the competition to come may be associated with an increase in hormone levels in some individuals in some sports (see Casto & Edwards, 2016 for a review), there was no clear evidence in this combined dataset data of such an effect., Whether for C or T, oral contraceptive use tested as a covariable had no influence on hormone reactivity to competition.

## 9.2. Why do Cortisol and Testosterone Increase in Association With Athletic Competition?

Although "psychological" effects of competition on steroid concentrations (Booth et al., 1989; Oliveira & Oliveira, 2014; Salvador, 2005; Salvador & Costa, 2009) cannot be discounted, players and non-players differed with respect to the amount of physical exertion interspersed between the warm-up and the end of competition. There is a plethora of evidence that blood levels of T and C increase during aerobic exercise, generally in proportion to the duration/intensity of the exercise (Copeland et al., 2002; Enea et al., 2011; Farrell et al., 1983; Wilkerson et al., 1980) and the type of exercise (see Gatti & De Palo, 2010 for review). Intense exercise is a potent stressor which activates the HPA axis, resulting in the release of CRH by the PVN and the subsequent release of ACTH by the pituitary gland, which then stimulates the adrenal cortex to increase cortisol and testosterone production and secretion (Petrides et al., 1994).

One reason why C and T increase in parallel during competition may be because at least some of the increase in T derives from the conversion of increased levels of DHEA and DHEA-S whose adrenal secretion is stimulated by the same ACTH that promotes the secretion of cortisol (Stewart, 2003). Physical exertion may decrease the rate at which hormones are cleared from plasma (Enea et al., 2011), and exercise can reduce plasma volume by causing a shift of fluid out of intravascular space, effectively increasing the plasma levels of hormones (Kargotich et al., 1997) which is perhaps then reflected in increased salivary levels of these same hormones. Particularly with respect to testosterone, the source (ovarian or adrenal), and proximate cause(s) (secretion, decreased metabolic clearance, hemoconcentration) of increased salivary levels associated with athletic competition remain to be determined.

The results of the analysis of T and C reactivity differences between coupled and uncoupled individuals are, at least superficially, similar to the findings that coupled individuals have higher T reactivity during a musical performance in which there was no physical exertion (Turan et al., 2022).

While physical exertion alone can increase T and C concentrations, post-competition lactate, a blood metric of physical exertion, was found to be negatively correlated with competition-induced increases in T and not related to the number of minutes played in a match as well as other proxies of physical exertion (e.g., subjective perceived level of exertion), which implies that there are competition-related effects on T not directly related to physical exertion (Aguilar et al., 2013; Casto & Edwards, 2016). Person factors such as competitiveness, the subjective pleasure of competing, personal skill, and/or athletic ability likely influence in which way levels of C and T change during an athletic contest (Casto & Edwards, 2016). Important context factors such as whether a game/match is played at home or away, match outcome (wining/losing), the closeness of competition, and the current competitive hierarchy between teams might also affect hormonal response to competition (Casto & Edwards, 2016).

#### 9.3. Individual Differences in Hormone Reactivity and Cortisol/Testosterone Coupling

For women who played in two competitions, when after-competition hormone level is figured as a percent of neutral-day baseline, the most reactive individuals for the first competition tend to be the most reactive for the second. These results are in accordance with earlier studies (Edwards & Kurlander, 2010; Edwards & Casto, 2013) as would be expected since they include many of the same participants, and are consonant with the idea that women may have a "signature" change in hormone levels that is conserved from one competition to the next.

For both the first and second competitions, cortisol and testosterone reactivity were significantly correlated -- women who showed the most reactivity to one hormone tended to be the most reactive with respect to the other. This suggests a coordinated dual-hormone response to the physical and/or psychological stress of athletic competition.

There were 33 women for whom the zero-order correlation between C and T levels was statistically significant and, by this criterion, C and T levels were considered coupled. Considering only those individuals who actually played, whether for C or T, individuals whose C and T levels were coupled, showed significantly more hormone reactivity to competition than individuals in which these two hormones were not significantly coupled. Enhanced T reactivity has been reported for coupled individuals in the context of a music performance recital (Turan et al., 2022). The present study is the first to report enhanced T and C reactivity for women in which C and T are coupled in the context of intercollegiate athletic competitions. The positive connection between cortisol/testosterone coupling and hormone reactivity to competition is consonant with the idea of a coordinated, dual hormone response to the stress of athletic

competition. The extent to which cortisol/testosterone coupling is predictive of psychological coping style and/or performance in this setting as well as hormone reactivity to other stressors remains to be determined.

It is important to remember that C/T coupling was determined by consideration of all available saliva samples. If however, coupling is determined by correlations calculated after the exclusion of after-competition hormone values, there is no effect of coupling on reactivity for either C or T. Put a bit differently, coupling-related enancement of hormone reactivity in the present study depends on the assignment of individuals as being either coupled or uncoupled using values for saliva samples that define the magnitude of competion-related increases in C and T. Even allowing for the problematic validity of C/T correlations based on hormone values for, in many cases, only three samples, the absence of a coupling effect here suggests that basal coupling and competition-related coupling are dissociable with respect to how each is related to hormone reactivity. It may be that participating in an athletic competition *induces* coupling to varying degrees in different participants and that neutral-day values in the dataset used for this retrospective study are relevant only because they provide an opportunity for the collection of saliva samples that can be used to make calculations of coupling whose real basis lies in the relationship between changing levels of C and T during the period of actual play. In this regard, it is worth noting that for women who played, competition-related changes in C and T are positively and significantly correlated. There are too few saliva samples in the dataset used for this study to convincingly dissociate basal coupling from performance-based coupling – what should be a major goal in future research on coupling and hormone reactivity in athletic and other performance settings.

#### 9.4. Possible Benefits of Hormonal Coupling

That hormone reactivity is higher for coupled individuals than uncoupled individuals prompts questions about the ways and extent to which enhanced C and T reactivity might be beneficial with respect to physiology, psychology, and performance in athletic as well as other kinds of circumstances.

Of the potential beneficial psychological effects associated with increased levels of T, two that stand out due to their possible implications in athletic contests are enhanced competitivness and persistence (Crewther & Cook, 2018; Hahn et al., 2016; Kutlikova et al., 2021, Welker & Carré, 2014). While studies have rarely looked at competitivness in women and its association with T, one study (Hahn et al., 2016) found that women reported stronger feelings of competitivness towards other women during test sessions where T was high compared to test sessions when T was low. Another (Crewther & Cook, 2018) reported a significant relationship between competitivness, menstrual cycle, and free salivary T in female athletes with elite athletes showing a stronger relationship between T and competitivness.

# 9.5. Sources and Mechanisms of T and C Coupling

There are three sources which might be responsible for the increase in levels of C and T in response to athletic competition, those being the adrenal cortex (both T and C), the ovary (T), and peripheral tissue (conversion of T precursors into T). Decreased clearance, hemoconcentration, and sympathetic activation of the HPA and HPG axes are some of the proximate mechanisms that might be involved (Casto & Edwards, 2016; Edwards & Casto, 2013). Since most of the T in women comes from the adrenal cortex (75 % total) and C is

exclusively secreted by the adrenal cortex, it is plausible that adrenal activation in response to factors associated with athletic competition is responsible for increased secretion and consequent co-fluctuation of C and T (Edwards & Turan, 2020). Alternatively, it is possible that the psychological and physiological elements of competition impact the ovaries and adrenal gland in a similar manner, producing the coupling effect seen in this study, as there is evidence to suggest that there is also coupling of T and estradiol (presumably of ovarian origin) seen in the context of athletic competition (Edwards & Turan, 2020).

It is possible that the HPA and HPG axes are not solely responsible for increasing levels of C and T that occur in connection with athletic competition (Casto & Edwards, 2016). It may be that sympathetic activation of the HPA and/or HPG axis is involved in the rapid competitionrelated increase in T and C levels. The sympathetic nervous system can activate the adrenal medulla to secrete catecholamines, which then stimulate the release of T precursors and C from the adrenal cortex, which then get converted to T in peripheral tissue (Ehrhart-Bornstein et al., 1994). Additionally, it is possible that the ovaries are activated via direct projections from the central nervous system (Gerendai et al., 2002) and that they contribute to the increase in T levels in the context of athletic competition, although there is little evidence for this.

#### 9.6. Possible Differences Between Coupled and Uncoupled Individuals

While the mechanisms behind competition-related increases in C and T are likely the same for coupled and uncoupled individuals, there might be a relatively persistent difference between these individuals in how they respond to competition, both physiologically and psychologically.

Coupled individuals were found to have lower basal C (by about 30%) than uncoupled ones (competition coupling). T was not significantly different between the groups, even though it was smaller in coupled individuals by about 17%, but this analysis is most likely compromised because of different kits used to assay testosterone for the soccer team and all the others. This difference in basal C might be relevant for higher T reactivity, as it has been found that lower levels of basal C predict higher T reactivity in the TSST in men (Bedgood et al., 2014). More importantly, higher T reactivity in women from before the start until after the end of an athletic competition is predicted by lower levels of basal C (Edwards & Casto, 2015). It might be that there is a psychological/neurological difference between individuals that have lower levels of basal C and those that have higher levels of basal C that is driving higher T reactivity. There is evidence that suggests that an increase in T can be caused by stimuli that are purely psychological in nature, as watching "motivational" clips is known to increase T levels, with one report even showing that such an increase in T is closely followed by an improvement in physical performance (Cook & Crewther, 2012). This effect could be even stronger in the context of athletic competition, due to multiple factors, most of which are the same person and context factors used in explaining C and T fluctuations in response to athletic competition.

Coupled individuals might possess certain psychological characteristics (e.g., high levels of confidence, power motivation etc.) and the neuronal underpinnings of these characteristics might influence how competition-induced stressors are processed by the brain and how this processing subsequently influences the HPA axis, HPG axis, and/or the sympathetic nervous systems. As the adrenal cortex is solely responsible for the production/secretion of cortisol and is highly involved in the direct and indirect production/secretion of testosterone, stronger activation of the adrenal cortex in coupled individuals in response to competition-related stressors might be the most parsimonious explanation for the vast difference in hormone reactivity seen between coupled and uncoupled individuals. Furthermore, there is evidence that DHEA and cortisol are diurnally coupled within adolescents (Marceau et al., 2013). If the adrenal cortex is the element most responsible for the competition-induced increase in C and T levels, higher competition coupling is, by the very nature of things, a byproduct of an enhanced adrenal response to the challenges of competition rather than its cause.

# **9.7.** Limitations

Data for this project was obtained from past studies, all of which were designed to document the hormonal correlates of athletic competition. None of the studies were planned for testing the effects of C and T coupling on competition-related hormone reactivity. None of the studies collected data about the menstrual cycle phases of the participants because the small sample sizes in those studies would have prevented meaningful analysis of the relationship between different phases of the menstrual cycle and hormone changes associated with competition. Whether or not menstrual cycle phase affects hormone coupling in women remains an open question to be answered by a larger study intended for this purpose. Depending on team/sport, hormone assays were conducted using kits from different suppliers. The studies used in this dataset were conducted over a period of almost 20 years – the sensitivity of hormone assays have improved over this time. For these reasons, it would not have been possible to make any meaningful inference from team/sport differences in hormone levels, and no attempt was made to do so.

# **9.8. Future Directions**

While T and C have been researched in various contexts, most studies include assays for only one of these hormones. That levels of C and T both change in an apparently coordinated manner in relationship to athletic competition and other stressors (see Turan et al., 2015; Turan et al., 2022) emphasizes the need to include assays for both hormones in studies of the endocrine correlates of human competition. As for T and C coupling, I propose a stronger emphasis on administration studies that have the potential to fully examine the possible benefits of increased hormone reactivity for athletic as well as other competitive contexts. Studies looking at intraindividual differences in proxy measures of athletic ability might be particularly useful (e.g., reaction time and its association with T and C). By doing so, we may better understand the causal impact these hormones have on performance and each other. Finally, I once more note the need for studies with sufficient number of saliva samples to dissociate basal coupling from competition-related coupling. I hope that, eventually, this line of research might even lead to novel and safe ways of enhancing human performance in a wide range of contexts and situations.

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