ORIGINAL ARTICLE



Testosterone, Athletic Context, Oral Contraceptive Use, and Competitive Persistence in Women

Kathleen V. Casto¹ · Lindsie C. Arthur² · Dave K. Hamilton³ · David A. Edwards⁴

Received: 27 July 2021 / Revised: 1 October 2021 / Accepted: 4 October 2021 / Published online: 16 October 2021 © The Author(s), under exclusive licence to Springer Nature Switzerland AG 2021

Abstract

Objectives The purpose of this study is to provide a descriptive account of salivary testosterone levels in women in relation to being an athlete, sporting level, competitive context, and oral contraceptive (OC) use and, to explore the relationship between testosterone levels and performance in a task of competitive persistence.

Methods Saliva samples were collected from teams of women athletes at the recreational, collegiate varsity, and elite-international levels, and a university participant-pool sample of athletes and non-athletes (N = 253). Among the elite athletes, additional saliva samples were collected before and after on- and off-field training sessions and competition. University participants competed in a timed weight-holding competition in the laboratory.

Results Testosterone levels were highest in elite athletes compared to university students ($\eta^2 = .07$) and were elevated in the context of competitive training (+13–51%) and formal competition (69%) contexts. OC users had significantly lower testosterone levels than non-users ($\eta^2 = .14$). For university athletes, testosterone levels were positively correlated with performance in a task of competitive persistence ($R^2 = .23$). OC use was associated with lower competitive persistence (d = .42) – a relationship explained by OC users' lower testosterone levels relative to non-users (d = 1.32).

Conclusions Results suggest that salivary testosterone levels in women may depend on sport participation and OC use, are malleable to competitive contexts, and among athletes, are positively related to competitive task persistence. Given the testosterone suppressing effects of OC use, this study provides insight on psychophysiological risks of OC use that could be relevant to sport performance.

Keywords Testosterone \cdot Oral contraceptives \cdot Competition \cdot Competitive persistence \cdot Sport \cdot Women athletes

Kathleen V. Casto kcasto@ncf.edu

Extended author information available on the last page of the article

Introduction

Testosterone is a steroid hormone that plays a major role in masculinizing anatomy, physiology, and behavior in males during periods of development (for review, Arnold, 2009; Berenbaum & Beltz, 2011). In adulthood, both men and women produce this hormone, although circulating levels are substantially higher in men (e.g., Clark et al., 2019). There is a demonstrated sporting benefit, e.g., physical strength and power, to both testosterone-mediated developmental masculinization and the long-term exogenous supplementation of testosterone in men and women (for review, Handelsman et al., 2018; Hilton & Lundberg, 2020; Wood & Stanton, 2012). Testosterone is also thought to be positively associated with behaviors that benefit sport performance such as aggression and social-status-oriented motivation, although these relationships have proven complex (Carré & Archer, 2018; Casto & Mehta, 2019; Geniole et al., 2020).

The relationship between long-term testosterone exposure and sport performance prompts questions about how individual differences in endogenous levels of testosterone differ by sport type and sport level. From an evolutionary perspective, genetic signaling for higher testosterone levels could be an adaptation for the promotion of greater dominance, status motivation, and physical strength, stamina, and power necessary for resource acquisition in early-human social conflicts (e.g., Trumble et al., 2013; for review, Archer, 2006; Roney, 2016). These psychological and physical traits are also beneficial for success in modern sport competition, perhaps depending on the type of sport. Yet, little is understood about sport-type and sport-level differences in testosterone levels. Perhaps because theories about the evolutionary role of testosterone are almost exclusively stated in relevance to men (for exception, van Anders et al., 2011), women are understudied in this context (Casto & Prasad, 2017).

Although two studies have attempted to explore sport-based differences in testosterone levels with a dataset of nearly 700 men and women elite athletes (Healy et al., 2014; Sönksen et al., 2018), the blood levels of testosterone were biased due to the fact that samples were taken within two hours after the end of national and international competition (Ritzén et al., 2015). In a study of Swedish women, Eklund et al. (2017) found that Olympic-level athletes (N=106) did not differ from sedentary controls (N=117) in blood levels testosterone, although the athletes had significantly higher in levels of adrenally-sourced androgen precursors. When grouping these athletes based on the type of sport, whether power, endurance, or technical, neither testosterone nor adrenal androgens differed on average between the groups.

Testosterone levels in blood can be represented as the portion bound to blood proteins, the portion that is free or unbound, or the total concentration of both. Salivary levels of testosterone represent the amount of hormone that is free to pass into salivary gland fluid and thus, represents the relative proportion that is available to act on target tissues peripherally and centrally at the moment of sampling (Gröschl, 2017). Although the concentration of testosterone in saliva is substantially lower than what is measured in blood, blood and salivary levels are

positively correlated and are considered to be a valid marker of individual differences in testosterone (de Wit et al., 2018; but see Prasad et al., 2019). Therefore, differences in salivary testosterone among athletes by sporting level and in relation to sport motivation and performance could be particularly relevant to understanding testosterone's adaptive role in promoting competitive readiness. In a small sample case study of 22 women athletes, Cook et al. (2018) showed that the 6 elite athletes in the sample (who competed at the national level within their respective sports) had, on average, higher salivary testosterone levels, higher motivation to train, and greater cycle-ergometer performance than the 16 nonelite athletes (who competed at the club and recreational level within their respective sports). A larger study of women athletes who compete at different levels of sport and non-athletes would be necessary to substantiate this "sport-level effect" of salivary testosterone in women.

One factor that is particularly relevant to testosterone levels in women is the prescription use of oral contraceptives (OCs), the pill form of a broader class of hormonal-based contraceptive methods. Most OCs contain either a combination of synthetic estrogens and progestins, or just progestins; Consistent intake of these hormones prevents the occurrence of pregnancy via the disruption of the endogenous secretion of ovarian hormones, the suppression of ovulation, and/or other mechanisms that interfere with fertilization and implantation (Frye, 2006; Rivera et al., 1999). Whether measured in serum or saliva, testosterone levels have been shown to be significantly lower among OC users compared to non-users (e.g., Liening et al., 2010) and typically fall after commencing OC use (e.g., Graham et al., 2007). According to a large systematic review and meta-analysis, OC use decreases serum total and free testosterone by an average of 31% and 61%, respectively (Zimmerman et al., 2014). The antagonistic effect of OCs on testosterone levels is also observed in collegiate level and elite women athletes in the context of both training and competition (e.g., Edwards & O'Neal, 2009; Crewther et al., 2015). This raises the question: For women within the normal range of testosterone, does the suppression of testosterone secretion by OCs have a negative effect on athletic performance?

Prior research has addressed this question with various methods, yet a clear answer remains elusive. For example, in a study of elite field hockey athletes, Crewther et al. (2018) showed that despite the difference in testosterone levels, there was little to no overall difference between OC users and non-users in various metrics of performance including perceived exertion, video-coded positive and negative actions in competition, and both player and coach subjective ratings of performance. However, when measuring within-subjects change in physical performance from before to after 4 months of OC use among a sample of moderately active women, Casazza et al. (2002) showed an OC-related decrease in peak oxygen consumption (a measure of "aerobic exercise capacity") and power output in a cycle ergometer exercise test. Mackay et al. (2019) found that, in a small sample of healthy volunteers (presumably non-athletes), OC users compared to non-users had significantly slower recovery from exercise-induced muscle damage following a test of eccentric cycling (at 90% of their maximal power). But, the metrics for muscle damage were uncorrelated to salivary testosterone levels.

A recent systematic review of 42 studies (Elliott-Sale et al., 2020) reported that OC use appears to have a small negative effect on sport performance, a conclusion tempered by inconsistent findings among the different studies, perhaps owing to between-study differences in the ways in which performance was measured (e.g., absolute muscle strength, oxygen uptake levels during aerobic exercise). Psychological motivation, an important contributor to sport performance (Gillet et al., 2012), is rarely considered in studies of the relationship between OC use and athletic performance. Although OCs could directly impact physiological systems involved in physical tasks, their use could also affect sport performance through alterations in psychological and cognitive systems that affect mood, motivation, competitiveness, perceptions of stress, and feelings of fatigue (Del Río et al., 2018; Montoya & Bos, 2017). Perhaps it's no coincidence that these psychological experiences also appear to be positively influenced by or related to testosterone (e.g., Crewther et al., 2020; Losecaat Vermeer et al., 2020). Given the suppressive effect of OC use on testosterone levels, OC use could influence performance via its direct effects on testosterone, particularly in tasks that where optimum performance requires the combined contribution of physical and psychological elements.

The endocrine system aids individual survival (and broader evolutionary goals of the species) through its sensitivity and responsivity to the external world – to alert, prepare, and activate adaptive behaviors relative to survival and reproduction in coordination with other physiological systems. A complete understanding of the relationship between hormones and behavior should consider the role of environment and social context. Thus, analysis of testosterone levels measured in different training and competition contexts could highlight hormone-sensitive periods of psychological and physical arousal or challenge (Casto & Edwards, 2016a). Despite the sport-relevant physical benefits of long-term exposure to testosterone, little is known about how individual differences in endogenous levels of this hormone relate to competitive behavior, including sport-performance, particularly in women (Castanier et al., 2021; Casto & Prasad, 2017).

The present study provides a descriptive account of testosterone levels in women athletes and non-athletes across context, sporting level, and sport type and in relation to OC use and competitive performance. First, we present data on testosterone levels from samples of female athletes at the recreational (flag-football), collegiate varsity (cross-country running, soccer, and rifle), and international-elite level (field hockey) as well as a university participant-pool sample of athletes and non-athletes. We also compare, across athlete and non-athlete groups, the testosterone levels of OC users and non-users. Next, we offer a detailed look at a subset of elite athletes and how their testosterone levels vary in accordance with different aspects of their training and competition environment. Finally, using the larger participant pool data, we statistically assess the relationships between testosterone levels and performance in a task of competitive persistence according to athlete "identity" (designation as a club or varsity athlete as well as self-identification as an athlete) and OC use. This descriptive account provides much needed information to the debate on the meaning of individual differences in testosterone in women athletes and more broadly contributes to a greater understanding of women in sport and the hormonal underpinnings of athleticism.

Analysis 1: Baseline Testosterone Across Different Sports, Sport Level, and by OC Use

Analysis 1 Methods

Participants

This study synthesizes data from different samples of collegiate varsity, collegiate recreational, international-elite women athletes, and self-identified women athletes and non-athletes from a university participant pool (total N = 253). Sample size and characteristics for each subgroup are listed below. In all instances, research was approved by Emory University's Institutional Review Board and participants gave written informed consent prior to participation.

Collegiate Varsity Athlete Sample This sample includes 29 members of the 2010 and 2011 Emory University varsity (NCAA Division III) women's cross-country team, 25 members of the 2013 Emory University women's soccer team, 11 members of the from Texas Christian University All-women's Rifle team, and 4 women members of the West Point Military Academy Rifle team. Because the sample of cross-country athletes spanned two consecutive years, for members of the team that were on the team both years, only data for the 2011 racing season were included.

Recreational Athlete Sample This sample includes 34 recreational athletes who were undergraduate or graduate participants in an Emory University intramural all-women flag-football league playing for one of several different teams during the 2013 or 2014 intramural seasons.

International-Elite Athlete Sample This sample includes 22 members of the 2015–2016 United States Women's National Field Hockey Team. Data for these participants were collected during the year leading up to the 2016 Rio Olympic games.

University Participant Pool Sample This sample is a subset of 128 women participants from a larger sample of undergraduates (age range of 18–25) from Emory University recruited for a prior publication (Casto et al., 2020, Study1). Sex was identified by asking participants to select their biological sex. In a questionnaire format, participants also provided their age, height, and weight in addition to answering the following questions "Are you currently a varsity or club athlete?" and "Regardless of whether or not you play a club or varsity sport, do you identify as an athlete?" Thirty-two percent (N=41) of the sample reported being a club or varsity athlete. All club or varsity athletes also identified as an athlete, as did an additional 30 individuals (total N=71) so that 56% of the sample identified themselves as an athlete.

Oral Contraceptive Use

As part of the consent procedure, all athlete sample participants were asked to respond "yes" or "no" to the question "Are you currently using an oral contraceptive?" and to one other: "Are you currently using any injected, implanted, or patch-delivered hormone contraceptive?" For the university participant pool sample, each participant was asked to circle "yes" or "no" to four questions: "Are you currently using an oral contraceptive?"; "Are you currently using an injected or patch-delivered hormone-based contraceptive?"; "Are you currently using an intrauterine device (IUD)?"; and "Are you currently using a Nuvaring?" Women using non-oral hormonal methods of contraception (N=15) were excluded from analyses. Thus, the sample for analyses by OC use compared OC users (N=117) and non-users (N=121).

Saliva Sampling and Assays

Saliva Collection Procedure Participants in all cases were instructed to rinse their mouth with water prior to saliva collection. Following the rinse, participants were instructed to allow saliva to pool in the mouth and then gently push the pooled liquid into a small plastic vial until full (or nearly full). For all athlete groups, participants were provided with a piece of Trident, original flavor sugar-free gum to stimulate saliva production following the rinse and instructed to chew and swallow saliva for at least one minute prior to beginning the process of pooling the saliva into the plastic vial. The use of gum increases the speed of saliva production, minimizing the disruption to the athletes' routine and preparation in the competition environment. As reviewed in Casto and Edwards (2016a), this practice is common in studies of salivary hormone levels in athletes and has no apparent effect on testosterone levels, provided participants chew Trident, original flavor and chew and swallow saliva for at least one minute prior to spitting.

When and where the Sample Was Obtained Cross-country and rifle athlete saliva samples were obtained at the location of sanctioned NCAA intercollegiate matches/ competitions, prior to the beginning of the warm-up activities at approximately 9 AM. Baseline soccer and field-hockey athlete saliva samples were obtained off-field on a neutral non-competition day at approximately 2 PM. Recreational athlete saliva samples were obtained at the location of the intramural matches prior to the beginning of warm-up activities between 7 and 8 PM. The university participant pool saliva samples were obtained following a 15-min period after arriving to the lab and between 1 and 4 PM. Although a within-individual circadian decrease in salivary testosterone is well-documented (e.g., Dabbs Jr, 1990), between-individual and between-sample mean comparisons of testosterone levels taken at different times of day may not capture this effect. Nonetheless, we assessed absolute levels of testosterone in relation to time of day; results below.

For all the studies contributing data to this article, additional saliva samples were obtained for the purpose of assessing hormonal reactivity to competition; these results are detailed elsewhere (e.g., Casto & Edwards, 2016b; Casto et al., 2014,

2017, 2020; Edwards & Casto, 2019). For this analysis, we focus only on the most neutral, baseline sample that was obtained for each group of participants.

Storage Saliva samples collected from athlete groups were initially stored on ice before being transferred to a - 80 °C freezer. Saliva samples collected in the lab from the participant pool individuals were initially stored at -20 °C before being transferred to a - 80 °C freezer.

Assay Specifications Saliva samples for the cross-country and soccer athletes were assayed in duplicate for testosterone using competitive enzyme immunoassay kits from Salimetrics by the Biomarkers Core laboratory of the Yerkes Primate Center in Atlanta, Georgia. The average intr-aassay CV for samples ranged from 5.5–6.4% and the inter-assay CV was 8.1%. The rifle, field hockey, recreational athlete, and university participant pool saliva samples were assayed in duplicate by the Emory Clinical Translational Research Laboratory (Atlanta, GA) using competitive enzyme immunoassay kits from Salimetrics (State College, PA). The average intra-assay CVs ranged from 2.5–7%. The average inter-assay CVs ranged from 6 to 14%.

Analysis 1 Results

Mean testosterone levels for samples collected in the morning (cross-country and rifle; M=42.8, SD=17.0) were higher than samples collected in the afternoon (soccer, field hockey, university participant pool, M=34.4, SD=17.2), and evening (soccer, field hockey, university participant pool, M=35.1, SD=12.7), F(2, 252)=4.61, p=.011. However, this effect was small to medium, η^2 =.04.

Across all participants, salivary testosterone levels ranged from 5.0–81.1 pg/ml (M=35.9, SD=16.9). The individual spread and descriptive statistics for salivary testosterone levels by sport and sporting level are shown in Fig. 1. Differences in absolute level of testosterone across sport category should be interpreted with caution due to differences in sampling time of day. Matched on time of day and assay lab, field hockey athletes (Olympic level, M=41.7, SD=15.7) had significantly higher testosterone levels on average than the athlete (M = 30.1, SD = 16.1) and nonathlete (M = 29.7, SD = 15.6) university subject pool participants, F(2, 149) = 5.31, p = .006, $\eta^2 = .07$. Post hoc analysis using Tukey HSD comparisons showed that mean testosterone levels for the elite field hockey team was significantly higher than both the group of university subject pool athletes (p = .017; CI of diff = 1.7-21.5) and the group of university subject pool non-athletes (p = .005; CI of diff=3.1–20.9). Rifle shooters (M = 27.4, SD = 15.7) also had significantly lower testosterone (mean diff = -14.3, p = .046, CI of diff = -28.4--.2) than field hockey athletes, an effect that was observed despite the fact that rifle samples were collected in the morning, when testosterone levels are typically higher.

Finally, we conducted an analysis of covariance (ANCOVA), with time of day as a covariate, to compare testosterone levels by the broader categories of collegiate athlete (total N=110, including the varsity cross-country, soccer, rifle, and university participant pool athletes), recreational athlete (N=34, flag-football),

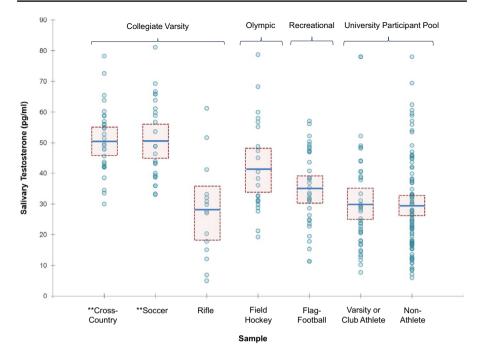


Fig. 1 Salivary testosterone levels at baseline in women across sample and sport categories. Circles represent individual participants and darker shading within circles indicates overlap. Horizontal bars and surrounding rectangles represent the sample mean for that group and the 95% confidence interval of the mean, respectively. **All testosterone levels were determined using Salimetrics enzyme immunoassay kits; however, these two samples were assayed at a different lab than the others. Thus, the levels could be systematically influenced by unaccounted for differences in laboratory procedures and category comparisons should be interpreted with caution

Olympic-level athlete (N=22, field hockey), and non-athlete (N=87, university participant pool non-athletes). The covariate, time of day, was not related to testosterone levels (p=.126). The overall effect for category was significant, F(3, 248)=6.38, p < .001, $\eta^2 = .07$. Post-hoc pairwise comparisons revealed that although none of the athlete groups, regardless of level, significantly differed in levels of testosterone, all athlete groups had significantly higher testosterone than the non-athlete sample (collegiate athletes: *mean diff*=8.2, p=.002, *CI of diff*=3.0–13.5; recreational athletes: *mean diff*=10.2, p=.026, *CI of diff*=1.2–19.2; Olympic-level athletes: *mean diff*=12.0, p=.002, *CI of diff*=4.3–19.6). Due to large sample size differences between groups, results should be interpreted with caution.

Combining across all groups of athletes, Fig. 2 shows the salivary testosterone levels of athletes and non-athletes by OC use. A 2×2 analysis of variance (ANOVA) revealed a significant Athlete by OC use interaction, F(3,238) = 5.84, p = .016, $\eta_p^2 = .02$. Although the main effect for OC use was significant and strong, F(3,238) = 37.02, p < .001, $\eta_p^2 = .14$, non-athletes who were also OC users had the lowest testosterone compared to athletes who did not use OCs (*mean*

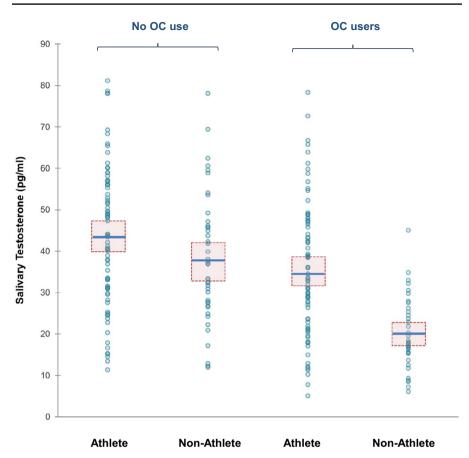


Fig. 2 Salivary testosterone levels for all athletes and non-athletes (from the participant pool) grouped by OC use. Athlete/non-users N=79; Non-athlete/non-users N=42; Athlete/OC users N=81; Non-athlete/OC users N=36. Circles represent individual participants and darker shading indicates overlap. Horizontal bars and surrounding rectangles represent the sample mean for that group and the 95% confidence interval of the mean, respectively

diff = -23.3, p < .001, CI of diff = -31.2 - .15.3), athletes who were OC users (mean diff = -17.9, p < .001, CI of diff = -26.9 - .90), and non-athletes who did not use OCs (mean diff = -15.5, p < .001, CI of diff = -23.4 - .7.6).

Analysis 2: Testosterone Levels in Elite Athletes Across Context and by OC Use

Analysis 2 Methods

With the sample of elite-international field hockey women athletes, additional saliva samples were collected to assess testosterone reactivity in the separate

contexts of training and competition. The contexts surveyed were two training sessions, which included an off-field pre-session mental preparation component and an on-field training component, and an international competition "test match," described below.

Training Day off-Field Preparation Period On each of the two consecutive days, athletes were asked to participate in an off-field preparation period lasting for approximately 30 mins total. This period included providing an initial saliva sample, followed by a 10 min "mental preparation" session and a second saliva sample. The mental preparation sessions consisted of either an exercise in guided mental imagery or an un-guided quiet meditation. On day 1, half the team completed the guided mental imagery task while the other half completed the un-guided quiet meditation. On day 2, the groups switched such that those who completed guided mental imagery on day 1 completed un-guided meditation on day 2, and vice versa. As described in Edwards and Casto (2019), a field-hockey-specific mental imagery script was developed with the purpose of provoking feelings of confidence, team unity, joy, and success through a detailed visual and first-person perspective of the field hockey environment during optimal performance. Athletes sat quietly in a room with their eyes closed while the experimenter read the script. For the un-guided quiet meditation, athletes were asked to clear their minds and rest quietly for same amount of time. The training day mental preparation sessions began at 1:30 PM.

Training Day on-Field Session Each training session consisted of the same onfield shoot-out simulation and target practice set-up. In field hockey, which team advances in tournament-style event for matches that end in a draw can be decided by "shoot-out" competition. In a shoot-out, individual members from each team take alternating one-on-one attempts to move the hockey ball past the goalkeeper of the opposing team and into the net. The "attacker", starting from the 25-yd line with the ball and "charging" towards the goal, has 8 s to attempt to score on the opponent goalkeeper who starts from the goal line. Both players can move freely within the space, but no contact fouls are permitted. To simulate this type of event for the purposes of practice, members of the team first warmed-up and completed target practice and then took turns making shoot-out attempts against the team's own two goal keepers. Successful attempts were noted for each player and the top performers were announced at the end of the training session for the purpose of stimulating intra-squad competition. Training day sessions began at 2:00 PM and lasted for approximately 45 min.

Competition The competition was an international "test match" against Japan. A test match is a competition that is officiated, recorded, and counts towards a player's international appearance totals ("number of caps"), but does not count towards the international team rankings or Olympic qualification. Nonetheless, the competition may affect coaches' decisions about who should make the Olympic squad and thus, is highly meaningful to the coaches and players. The competition consisted of two 35 min halves with a half-time break of 15-min. As agreed in advance by

the coaching staff for both teams, the end of match play (USA 2–0 over Japan) was immediately followed by a simulated shoot-out competition between the two teams. Twenty members of the team were present for the competition, five of which did not play in the match. Only five members of the 15 who played in the match also participated in the shoot-out portion of the competition. Saliva samples were collected about 1 h before the beginning of the warm-up period (5 PM), at half-time, and immediately following the completion of the match and shoot-out. The match began at 7 PM and ended around 8:30 PM.

Analysis 2 Results

Figure 3 shows testosterone levels of the elite athletes chronologically across various baseline, training, and competition states. Due to the small sample size, tests of statistical significance were not conducted; data are assessed descriptively. Although there were individual differences in the patterns of change, testosterone levels were typically elevated relative to baseline following the cessation of the two training sessions. On average, testosterone levels were equivalent at the beginning of both the training day sessions (40.9 and 42.3 pg/ml, respectively, 30 min prior to the field session). Following the mental preparation session, both the guided imagery (+46.3%, SD=46.2) and the un-guided meditation (+33.2%, SD=48.3) groups showed increased levels from before to after the session on day 1 and slightly decreased levels from before to after the session on day 2 (imagery, -18.8, SD = 13.4; no imagery, -8.1%, SD=23.1). Thus, regardless of the mental preparation condition, testosterone levels were higher following the training day preparation period on day 1 compared to day 2 (Fig. 3). Post-training session testosterone levels relative to pretraining baseline were also, on average, higher on day 1 (M = +51.1%, SD = 56.0) compared to day 2 (M = +13.2%, SD = 29.3).

For athletes who played in the international test match, testosterone levels at half-time were, on average, highly elevated relative to before-warm-up values (M = +69.3%, SD = 77.1) and remained at this level through to the end of the postmatch shoot-out. For the five athletes who did not play, testosterone levels (not shown in Fig. 3) remained at or near baseline throughout the competition event. Although OC users had lower testosterone levels at every time point than non-users, the pattern of change across periods of training and competition was remarkably similar on average for OC users and non-users (Fig. 3, bottom panel).

Analysis 3: Testosterone Levels in Relation to Competitive Persistence by Athlete Identity and Hormonal Contraceptive Use

Analysis 3 Methods

For the university participant pool sample, individual differences in performance were assessed in a task of competitive persistence. These data are a part of a larger

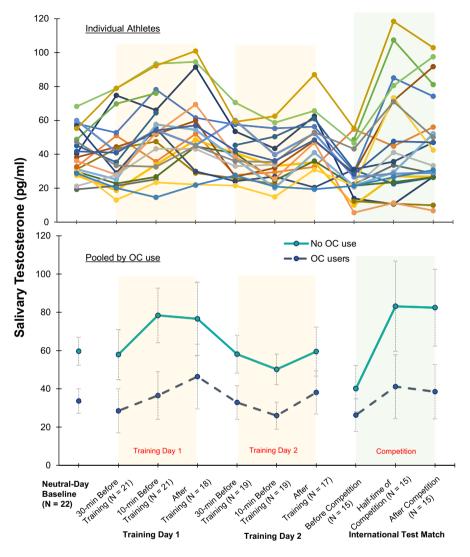


Fig. 3 Salivary testosterone levels for the elite-international field hockey athletes across baseline, training, and competition contexts by individual athlete (top panel) and pooled by oral contraceptive (OC) use (bottom panel). Points on the x-axis are arranged chronologically and sample sizes are different for each point. For each of the two consecutive training days, half the team completed a guided mental imagery task while the other half completed un-guided quiet meditation during the 30 min pre-training session period. Eleven of the 22 members of the team were using OCs. Data for the competition include only the 15 members of the team who played in the match (OC users N=9; non-users N=6)

study on testosterone reactivity and competitive persistence in men and women detailed in Casto et al. (2020).

Competitive Will Task Participants held a 1 lb. weight (a common dumbbell used for resistance training) at arm's length and shoulder height, with arm extended from the body at a 90° angle, for as long as they felt they could in competition against other participants according to the procedure in Casto et al. (2020). Participants' shoulder height was marked with a line on an index card taped to the wall and each participant was instructed to hold their arm at that height, dropping it once they "no longer wished to compete or could no longer physically keep their arm above the line, whichever came first."

To incentivize the competition, participants were informed that a \$20 cash prize would be offered to the one participant who held her arm up the longest of all the other participants who took part in the study during that semester (data was collected across two semesters). Some participants competed against each other in dyads whereas others competed individually. For the participants who competed against a man. Performance time was unrelated to the sex of the other participant or whether the participant competed individually or in a dyad (Casto et al., 2020). Regardless of the presence or absence of an opponent, all participants were competing against each other for the same cash prize. Participants were not given any reference times for other participants' performances or any feedback about how well how their performance ranked overall. Prize winners were privately contacted at the end of the semester via email.

"Competitive Performance" in this task was operationalized as the time in seconds that a participant held up her arm before quitting the contest. Holding a weight for time and has been previously and independently validated as a measure of mental toughness, a psychological construct in which competitive persistence is a core feature (Crust & Clough, 2005). Further, performance in the competitive will task has been shown to be significantly and positively related to individual differences in traits having to do with competitiveness and dominance motivation as well as taskspecific desire to win and perceived effort (Casto et al., 2020). In our previous report on this task, physical qualities associated with strength – height, weight, and BMI – were unrelated to task performance and did not explain variance in the relationship between performance and psychological traits.

Analysis 3 Results

As detailed above, each participant in the university pool was asked to indicate whether or not they were a club or varsity athlete as well as, regardless of that formal designation, whether or not they self-identified as an athlete, described here as having an "athlete identity." Descriptive statistics and t-tests for the mean differences are shown in Table 1 by grouping category: athletes (club and varsity) compared to non-athletes; athlete identity compared to non-athlete identity; and OC users compared to non-users. Salivary testosterone levels were not significantly different between athletes and non-athletes whether by club/varsity designation or by self-identification. Testosterone levels were not related to individual differences in

Grouping Cat- egory	Club or Varsity Athlete (N=41)	Non-athlete $(N=87)$				
	Mean (SD)	Mean (SD)	t(df)	р	CI/D ^a	Hedges' g*
Testosterone (pg/ ml)	30.1 (16.1)	29.7 (15.6)	.12 (126)	.904	-5.56-6.28	.03
Performance (sec)	254.4 (93.6)	230.6 (73.1)	1.57 (126)	.120	-6.26-53.82	.30
	Athlete Identity (N=71)	Non-athlete Identity (N=57)				
Testosterone (pg/ ml)	29.7 (16.1)	30.1 (15.4)	16 (126)	.874	-6.00-5.11	.03
Performance (sec)	256.6 (88.8)	215.4 (62.6)	2.96(126)	.003	14.65–67.73	.52
	No OC use $(N=64)$	OC use $(N=54)$				
Testosterone (pg/ ml)	37.8 (16.1)	20.3 (8.4)	7.23 (116)	<.001	12.95–22.12	1.32
Performance (sec)	251.7 (62.6)	220.6 (83.6)	2.11 (116)	.037	1.91–60.14	.42

 Table 1
 Salivary testosterone levels and competitive will performance time by athlete designation (club or varsity), athlete self-identification, and OC use

 $CI-D^a = CI$ of difference. *Cohen's d was corrected for unbalanced groups using Hedges' g; values for Hedges' g are interpreted the same as for Cohen's d *Note*. There were 128 total participants. For analyses involving OC use, 10 participants are excluded from the analyses due to use of a hormonal contraceptive other than the pill

height, weight, or age (all correlations < .04). As with the larger sample included in Analysis 1, OC users had significantly lower testosterone than non-users (Table 1). There was no interaction between athlete designation and OC use in predicting testosterone levels; non-athlete OC users testosterone levels were, on average, nearly identical to athlete OC users.

Competitive will task performance was not significantly different for athletes and non-athletes by club/varsity designation. However, task performance was significantly better for self-identified athletes compared to non-athletes and for OC nonusers compared to OC users, and the effect sizes for both comparisons were small to medium (Table 1).

Overall, individual differences in testosterone levels were modestly correlated with task performance, r(128) = .21, p = .016. To test the moderating effects of being a club or varsity athlete, a hierarchical linear regression predicting performance time with testosterone, athlete designation (yes/no), and their interaction was conducted. The interaction between athlete designation and testosterone was significant, $R^2_{change} = .05$, $F_{change}(1124) = 7.52$, p = .007, b = -2.52, SE = .92, CI = -4.34--.70. As shown in Fig. 4 (left panel), higher testosterone predicted higher performance times among the women who were club or varsity athletes ($R^2 = .23$, b = 2.76, SE = .82, t = 3.38, p = .002, CI = 1.11-4.42), but not among the women who were non-athletes ($R^2 = .003$, b = .24, SE = .51, t = .48, p = .632, CI = -.76-1.25). The

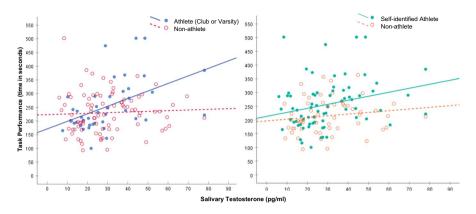


Fig. 4 Correlation between salivary testosterone and performance in the competitive will task by athlete designation and athlete self-identification. Left panel: Athlete (club or varsity, N=41) $R^2=.23$, Non-athlete (N=87) $R^2<.01$. Right panel: Self-Identified Athlete (N=71) $R^2=.07$, Non-athlete (N=57) $R^2=.02$

interaction of club or varsity athlete designation and testosterone remained significant after controlling for height, weight, and BMI. However, repeating this analysis with the athlete self-identification variable, the interaction was no longer significant, $R^2_{change} < .01$, F_{change} (1124)=.67, p=.415, b=-.71, SE=.87, CI=-2.43-1.01. Instead, there were significant main effects for athlete identification ($R^2=.07$, b=-45.61, SE=.13.51, t=-3.38, p=.001, CI=-72.35-18.87) and testosterone level ($R^2=.05$, b=1.07, SE=.43, t=2.50, p=.014, CI=.22-1.92) such that identifying as an athlete and higher testosterone levels independently predicted better task performance (Fig. 4, right panel).

To test the moderating effects of OC use on the relationship between testosterone and performance time, a hierarchical linear regression was conducted. The interaction between OC use and testosterone in predicting performance was not significant, $R^2_{change} = .01$, F_{change} (1114)=1.68, p = .198, b = -1.85, SE = 1.43, CI = -4.67-.98. Perhaps owing to multicollinearity between testosterone and OC use, the main effects of both OC use (t-test results in Table 1) and testosterone on task performance were no longer significant. To account for this shared variance, we regressed testosterone levels on performance, saved the unstandardized residuals, and then conducted a simple regression of OC use on the unstandardized residuals. OC use did not account for any additional variance in the relationship between testosterone levels and performance, $R^2 = .01$, F(1,117) = .685, p = .409, b = -12.02, SE = 14.52, $CI = -40.77 \cdot 16.73$. This suggests that the small effect of OC use on performance (R^2 = .04) is due to the large suppressing effect of OC use on testosterone levels (R^2 = .31). Although the mean unstandardized residual of testosterone on performance was negative among OC users (M = -7.3, SD = 84.5) and positive among non-users (M=4.8, SD=73.2), the difference between the groups was not significant (mean diff=12.0, p = .409, CI of diff=-16.7-40.8). Thus, there is some additional benefit of non-use above and beyond the effect of testosterone on performance, but this effect is trivial in light of large variability of the testosterone-performance relationship within OC users and non-users.

Discussion

Sport-Type and Sport-Level Differences in Basal Testosterone

Generally, we found that mean salivary testosterone levels by sample group did not systematically differ by sporting level. Specifically, increasing level from recreational, to collegiate, to elite-international was not associated with stepwise or distinct elevations in testosterone. However, elite-international level athletes had higher testosterone on average than both athlete and non-athlete university students. Combining all of the collegiate athlete groups together, and controlling for sampling time of day, all levels of athletes – recreational, collegiate, and elite-international – had higher salivary testosterone on average than the sample of non-athletes. These effects are consistent with prior reports that salivary testosterone levels are higher in elite women athletes compared to non-elite athletes (Cook et al., 2012, 2018; Crewther & Cook, 2018). This effect could be due to a selection bias: higher testosterone women are perhaps more likely to pursue and succeed in sport than women with lower levels of testosterone for reasons of interest, physical ability, or some combination of these factors. It is also possible that regular sport training and competing could elevate baseline testosterone levels.

On average, baseline testosterone levels for women rifle shooters were lower than corresponding levels for the collegiate varsity soccer and cross-country athletes as well as the elite field hockey players, an effect that emerged despite the fact that rifle athletes' testosterone values were determined from samples collected in the morning, when testosterone levels are typically higher (see Edwards & Casto, 2019 for analysis of testosterone reactivity to rifle competition in men and women athletes). The reason for the women rifle-shooters' relatively lower testosterone levels is not clear. Rifle is a sport in which there is no known sex-based advantage and is the only US National Collegiate Athletic Association (NCAA) sport that is not segregated by sex; men and women compete against each other for the same titles, rankings, and awards. Likewise, unlike other sports, aerobic capacity and explosive power are not necessary for skill-development and performance in this sport. Although rifle competitions can last several hours and require high mental and physical endurance, the primary physical task is to remain as still and psychologically calm as possible while aligning visual and body information with the precise timing of a trigger pull. In comparison, field-hockey, a sport requiring high strength and explosive power, might have a selection bias for higher testosterone women athletes. The small sample analysis in this study requires further research support and replication, yet raises questions about how testosterone levels relate to aspects of the sport type and the particular physical demands for success within each sport.

Context Effects on Testosterone Levels

The current study tracked testosterone levels of elite women field hockey athletes over the course of training and competition in the year leading up to their participation in the Olympic games. Testosterone levels were higher relative to baseline following a pre-training mental preparation session on the first of two days, regardless of whether the preparation involved sport-achievement-themed guided imagery or un-guided meditation. On the second of two consecutive days, testosterone levels fell slightly over the course of these pre-training interventions. In women athletes, the warm-up period leading up to competition is marked by a rise in testosterone that temporally "anticipates" the formal competition to come (e.g., Casto & Edwards, 2016b; Edwards & Kurlander, 2010). In contrast to the sport-specific physical activities typical of a warm-up period, the two mental preparation periods in the current study involved physical relaxation and, for one of the periods, an attempt to "psychologically" activate the athlete by imagining great success in a field hockey contest. While some athletes showed large increases in testosterone during imagery compared to their response to un-guided meditation, this effect was specific to the first day of testing – athletes who completed the imagery task on day 2 did not show greater post-task testosterone levels compared to their day 1 un-guided meditation.

In male athletes, watching video clips, e.g., of sport training or aggressive content, can significantly elevate testosterone levels and these elevations positively predict subsequent performance in a strength-based task (3 rep max squat, Cook & Crewther, 2012; but see Crewther et al., 2016). Carré and Putnam (2010) showed that male ice-hockey athletes showed large increases in testosterone when watching a video of their own previous victory, but not when watching their own prior defeat or a neutral content video. A more recent study of male combat athletes (e.g., mix martial artists) showed that testosterone increased when watching a video of a teammate fighting in a formal contest, but only if he felt that being a member of the team was central to his own identity (Casto et al., 2021). Thus, psychological stimuli that motivate or arouse feelings of social dominance and success can, in the absence of both physical exertion and active physical engagement in a competition, elevate testosterone levels in men (for study in men and women sports fans, see Burk et al., 2019). Whether or not this can be consistently demonstrated for women athletes remains to be determined.

The two training sessions studied here involved a field hockey shoot-out contest. Training sessions were associated with increases in testosterone with the effect more evident on the first day than the second. The impact of a single training session on testosterone levels in elite women field hockey players has been previously shown to depend on whether the training session was "heavy" or "light", with only the relatively heavier training showing elevated testosterone (Crewther et al., 2015). These findings are consistent with meta-analytic results in men to the effect that moderate to intense physical activity is consistently associated with increases in salivary and blood levels of testosterone (D'Andrea et al., 2020). The most substantial increase in testosterone levels for the field hockey players occurred in association with an international test match - mean testosterone level at half-time was approximately 70% above the pre-competition baseline (1 h before the match), and the increase at halftime was sustained through to the end of the contest. The team studied led at halftime and went on to hold that lead through to the end of match play; prior research consistent with this effect has shown competition-associated elevations in testosterone even for matches that resulted in a defeat (e.g., Casto & Edwards, 2016a).

Athletic competition involves both physical activity and psychological striving for social status over others. As reviewed in Casto and Edwards (2016a) and Edwards and Casto (2019), competition in the absence of physical activity can also significantly elevate testosterone – the combined effects of physiological and psychological arousal presumably account for the substantial elevations of this hormone associated with athletic competition. We (Edwards & Casto, 2019) have previously postulated that there are three factors that influence testosterone reactivity in the context of social competition: 1) the individual must be psychologically engaged in the competition, 2) the outcome of the competition should be personally significant, and 3) the individual has to feel s/he has some agency with respect to the outcome. For athletes competing in a formal contest of their sport, all three of these criteria are typically met.

Individual Differences in Basal Testosterone Levels and Task Performance

Data from a laboratory study of competitive persistence (Casto et al., 2020) were retrospectively analyzed to further address the relationship between testosterone levels and performance in this task. Higher testosterone levels in women predicted better task performance, i.e., greater weight-holding persistence, but only among a subset of the participant sample that consisted of club and varsity athletes. The effect was not clearly present among non-athletes.

Although discomfort in this task arises from the fatigue and pain in the shoulder and arm associated with keeping a weight-bearing arm elevated, there are surely psychological elements at play that determine an individual's willingness to endure. Performance in the task has been positively correlated with trait competitiveness and dominance motivation as well as task-specific desire to win, while being unrelated to body weight and height (Casto et al., 2020). Essentially the same task was employed by Crust and Clough (2005) to provide criterion validity for a measure of "mental toughness," a concept from sport psychology having to do with competitive confidence, resilience, persistence, effective emotional control, and coping with adversity – qualities considered to be among the most important psychological predictors of success in sport (Crust, 2007; Liew et al., 2019). Testosterone-related individual differences in mental toughness could, at least in part, account for individual performance differences in this competitive task.

Findings from this study are consistent with prior research linking testosterone to competitive persistence (e.g., Welker & Carré, 2015), motivation, and effortbased task performance (for review, Losecaat Vermeer et al., 2016). For example, Mehta et al. (2009) showed that men and women with relatively higher testosterone performed better in an analytical reasoning test, but this effect emerged only when competing individually as opposed to collaboratively with another individual. Similarly, men given a single dose of exogenous testosterone showed increased competitive effort (reaction times) than men given a placebo, but only when the context provided an opportunity to improve social status (Losecaat Vermeer et al., 2020). Relevant to sport performance, one recent study of professional male rugby players showed a positive relationship between levels of training motivation and subsequent testosterone concentration approximately three days later over many successive weeks of sampling (Crewther et al., 2020). Future research on the relationship between testosterone levels and sport performance should consider the role of testosterone in promoting psychological motivation to compete and persist through the discomfort inherent in sport-associated exertion.

OC Use Effects on Testosterone and Performance

Consistent with other reports (e.g., Edwards and O'Neal; Crewther et al., 2015) salivary testosterone levels were significantly lower for OC users than non-users. Non-athletes who were also OC users showed the lowest testosterone levels overall (relative to non-athletes who were not using OCs as well as athlete users and non-users). Among the elite field hockey athletes, OC users had lower testosterone than non-users, but users and non-users showed remarkably similar context-dependent shifts in testosterone level (Fig. 3). These findings are consistent with earlier reports that OC users and non-users show similar increases in testosterone in connection with athletic competition (Edwards & O'Neal, 2009). Other research has shown that, when pooled across different training and competition contexts, the average testosterone response may be attenuated in OC users (Crewther et al., 2015).

Results from the current study inform, albeit tentatively, an understanding of OC use and competitive performance. Data from a laboratory study of competitive persistence (Casto et al., 2020) were retrospectively analyzed to explore the three-way relationship between OC use, testosterone levels, and performance. In general, and as shown in the previous analysis with this data, the performance of non-users was superior to that of OC users. Because of the known causal effect of OC use on the suppression of testosterone (e.g., Zimmerman et al., 2014), the present study tested if OC use predicted performance above and beyond the effects of testosterone on performance. Results from this new analysis showed that it did not, i.e., OC use did not significantly predict additional variance in performance after accounting for individual differences in testosterone levels.

Prior studies on the relationship between OC use and sport performance primarily rely on physiological tests associated with athletic ability such as muscle strength or oxygen uptake capacity (Elliott-Sale et al., 2020). As discussed above, success in sport also depends on mental toughness and motivation. There are reports that hormone contraceptive users display increased emotional and stress reactivity to aversive stimuli and are more likely to experience symptoms associated with anxiety and other mood disorders when compared to non-users (for review, Anderl et al., 2020; Del Río et al., 2018; Montoya & Bos, 2017; but see Robakis et al., 2019). Other studies have shown that OC users make disadvantageous competitive bidding decisions compared to non-users (Pearson & Schipper, 2013), are less likely to selfselect into a competitive than non-competitive tournament scheme (Buser, 2012), engage in lower self-reported competitive behavior directed towards other women (Cobey et al., 2013), and show reduced persistence in achievement-oriented cognitive tasks (Bradshaw et al., 2020; Griksiene et al., 2018). Thus, OCs could influence sport performance by altering physiological and/or psychological/cognitive systems

71

associated with competitive persistence. However, it is important to note that individual differences in endogenous testosterone levels and OC use do not appear to have large impacts on physiological variables associated with sport performance (e.g., Elliott-Sale et al., 2020), nor are the results from this study likely to extend to all sport-related actions (e.g., free-throw shooting or penalty kick accuracy). Results from this study suggest a slight overall benefit of higher testosterone among athletes in a task of competitive weight-holding persistence and additionally, that lower performance in this task among OC users is explained by their systematically lower testosterone.

Among a sample of 430 national and elite internationally-competing women athletes across 24 different sports, 50% were contemporaneously using some form of hormonal contraceptive (primarily the OC pill) and nearly 70% reported using one of these contraceptive methods at some point during the course of their competitive career (Martin et al., 2018). The ability to control the hormonal milieu and prevent ill-timed menstruation is seen as a sport-performance benefit by many women athletes (Schaumberg et al., 2018). Although the overall effect of OCs on performance may depend on individual differences in hormonal sensitivity and success in sport is influenced by a complex array of factors, women should be made aware of the potential risks of OC use regarding psychological, behavioral, and athletic outcomes. Continued research on this matter is clearly warranted.

Evolutionary Perspectives

Results from the current study highlight the sensitivity of testosterone to contextual aspects of the sporting environment. Elite level women athlete's testosterone levels rose and fell – in some cases, drastically so – over course of mental and physical training and competition periods, a finding that is consistent with earlier reports of women and men athletes competing in a variety of sports (Casto & Edwards, 2016a). The presumption is that these fluctuations are adaptive in that they coordinate physiological and behavioral changes that increase the probability of meeting the goals of the individual in that context. With the goal of status or resource acquisition, the beneficial result of a testosterone increase would be enhanced competitive motivation, task persistence, or aggressiveness – psychological states that elevate the likelihood of behaviors that aid in defeating an opponent and demonstrating superior social and physical dominance (e.g., Archer, 2006, Carré & Archer, 2018; Losecaat Vermeer et al., 2016, 2020).

Higher testosterone levels among athletes compared to non-athletes, whether through genetic predisposition or increased environmental exposure to competition and physical training, could represent individual differences sport readiness – a baseline state of higher competitive motivation or willingness to compete. However, in line with life-history theory, predominant evolutionary perspectives position testosterone as a mediator of the tradeoff between mating effort and parental investment, strictly in males (Gray et al., 2020; Roney, 2016; Muller, 2017; Wingfield et al., 2001). Chronic elevation of testosterone is viewed as costly for individual survival by being metabolically expensive, immunosuppressing, and promoting

behaviors that risk physical injury. Thus, testosterone should only increase in contexts in which the reproductive benefits of testosterone-mediated behavior outweigh the costs of risk to survival. And as a result, situations that lead to higher circulating testosterone include male-male competition in the presence of potential mates or, presumably, greater access to hypothetical mates.

Yet, formal sport is perhaps an altogether different kind of human endeavor than mate-seeking and aggression in the context of competition for mates. Despite the lack of direct reproductive relevance inherent in life-history tradeoff theories about testosterone, organized sport is perhaps an ideal setting for studying testosteronebehavior relationships. Sport can be viewed as a coalitional contest for dominance, akin to tribal warfare for resources common in early human societies and non-human primates (Crofoot & Wrangham, 2010; Flinn et al., 2012). Team sports in particular require commitment to social group formation and maintenance, collective goal-set-ting and action, resource distribution, and strong in-group/out-group categorization processes – factors beneficial to social group functioning more broadly and likely selected for in men and women. As such, engaging in an athletic competition could be included in theoretical frameworks as a major input or "eliciting condition" for testosterone release in men and women (Roney, 2016; Rosvall et al., 2020).

Limitations

The research presented here has several limitations. In analysis 1, methodological variation between groups (e.g., time of day) limited our ability to draw conclusions about baseline testosterone levels across different sports and sport level. Additionally, the relatively small sample sizes for each of the sport teams limits generalizability. For the case study of testosterone change across context in the elite women field hockey athletes, sample sizes were low and varied across the different activities based on athlete availability/presence. For this reason, data are only presented descriptively. Further research would be necessary to confirm some of our more observational/correlational/and cross-sectional results.

Another limitation is the unaccounted-for variation in menstrual cycle phase within naturally cycling participants. In women, testosterone has a slight-to-moderate mid-cycle peak associated with the fertile window in some individuals (e.g., Bui et al. 2013; Rothman et al., 2011) and this mid-cycle peak could affect testosterone reactivity to stress (e.g., Cook et al., 2021). While cycle phase of the participants at the time of sampling is presumably random and thus, balanced across groups, we have no way of confirming that this was the case. Testing menstrual cycle phase effects requires a larger sample size as well as more technical methods (use of lute-inizing hormone test strips) and longitudinal tracking for validly determining cycle phase (Blake et al., 2016). In analysis 3 of the present study, participants were asked to indicate the first day of their last menstrual period. However, due to their overall low confidence ratings and the low validity of forward-only counting methods, these data were not included in analyses.

A final limitation to consider is the dearth of information regarding the type of OCs used by the participants in this research. Not all progestins and/or synthetic

estrogen formulas found in OCs suppress testosterone (Louw-du Toit et al., 2017; Sitruk-Ware & Nath, 2013). Future research could collect and report details regarding the specific hormone formula, schedule of use, and whether the participant is currently on the active or inactive (placebo) pill day. Exploration into the effect of other hormonal contraceptives (e.g., hormonal IUD, NuvaRing) would further our understanding of how exogenous hormones interact with athletic performance and testosterone levels in people who menstruate.

Conclusion

The purpose of this study was to assess factors related to individual differences in salivary testosterone levels in women and to explore how those levels relate to being an athlete, engaging in various athletic contexts, competitive performance, and OC use. Results suggest that salivary testosterone levels in women athletes are typically higher than in non-athletes and, particularly among athletes, are meaningful for competitive performance. But, levels of this hormone are also malleable and adaptable to the context – among elite athletes tested, testosterone levels increased in the context of competitive training and formal competition. Individual differences in testosterone among a larger university sample were associated with greater performance in a task of competitive persistence among those who identified as an athlete. Although OC use did not appear to impact or suppress context dependent shifts in testosterone, chronic suppression of testosterone via OC use was associated with lower competitive persistence. This study adds to a broader understanding of the role of testosterone in competitive behavior and sporting ability among women and, considers the adaptive value of these mechanisms from an evolutionary perspective. Further, this research provides information that could be helpful for women athletes in weighing the potential risks and benefits of OC use.

Data Availability The dataset for analysis #3 in the current study is available on the open science framework (https://osf.io/6r7da/). The datasets of teams of athletes for analysis #1 and #2 are available from the corresponding author on reasonable request, but are not publicly available to protect the privacy of the athletes whose data might be identifiable.

Declarations

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

- Anderl, C., Li, G., & Chen, F. S. (2020). Oral contraceptive use in adolescence predicts lasting vulnerability to depression in adulthood. *Journal of Child Psychology and Psychiatry*, 61(2), 148–156. https://doi.org/10.1111/jcpp.13115
- Archer, J. (2006). Testosterone and human aggression: An evaluation of the challenge hypothesis. Neuroscience & Biobehavioral Reviews, 30(3), 319–345. https://doi.org/10.1016/j.neubiorev.2004.12.007

- Arnold, A. P. (2009). The organizational–activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. *Hormones and Behavior*, 55, 570–578. https://doi. org/10.1016/j.yhbeh.2009.03.011
- Berenbaum, S. A., & Beltz, A. M. (2011). Sexual differentiation of human behavior: Effects of prenatal and pubertal organizational hormones. *Frontiers in Neuroendocrinology*, 32(2), 183–200. https:// doi.org/10.1016/j.yfrne.2011.03.001
- Blake, K., Dixson, B., O'Dean, S., & Denson, T. (2016). Standardized protocols for characterizing women's fertility: A data-driven approach. *Hormones and Behavior*, 81, 74–83. https://doi.org/10.1016/j. yhbeh.2016.03.004
- Bradshaw, H. K., Mengelkoch, S., & Hill, S. E. (2020). Hormonal contraceptive use predicts decreased perseverance and therefore performance on some simple and challenging cognitive tasks. *Hormones* and Behavior, 119. https://doi.org/10.1016/j.yhbeh.2019.104652
- Burk, C. L., Mayer, A., & Wiese, B. S. (2019). Nail-biters and thrashing wins: Testosterone responses of football fans during World Cup matches. *Physiology & Behavior*, 209, 112596. https://doi.org/10. 1016/j.physbeh.2019.112596
- Buser, T. (2012). The impact of the menstrual cycle and hormonal contraceptives on competitiveness. Journal of Economic Behavior & Organization, 83(1), 1–10. https://doi.org/10.1016/j.jebo.2011.06. 006
- Carré, J. M., & Archer, J. (2018). Testosterone and human behavior: The role of individual and contextual variables. *Current Opinion in Psychology*, 19, 149–153. https://doi.org/10.1016/j.copsyc.2017.03. 021
- Carré, J. M., & Putnam, S. K. (2010). Watching a previous victory produces an increase in testosterone among elite hockey players. *Psychoneuroendocrinology*, 35(3), 475–479. https://doi.org/10.1016/j. psyneuen.2009.09.011
- Casazza, G. A., Suh, S. H., Miller, B. F., Navazio, F. M., & Brooks, G. A. (2002). Effects of oral contraceptives on peak exercise capacity. *Journal of Applied Physiology*, 93(5), 1698–1702. https://doi. org/10.1152/japplphysiol.00622.2002
- Castanier, C., Bougault, V., Teulier, C., Jaffré, C., Schiano-Lomoriello, S., et al. (2021). The specificities of elite female athletes: A multidisciplinary approach. *Life*, 11, 622. https://doi.org/10.3390/life1 1070622
- Casto, K. V., & Edwards, D. A. (2016a). Testosterone, cortisol, and human competition. *Hormones and Behavior*, 82, 21–37. https://doi.org/10.1016/j.yhbeh.2016.04.004
- Casto, K. V., & Edwards, D. A. (2016b). Before, during, and after: How phases of competition differentially affect testosterone, cortisol, and estradiol levels in women athletes. *Adaptive Human Behavior* and Physiology, 2, 11–25. https://doi.org/10.1007/s40750-015-0028-2
- Casto, K. V., & Mehta, P. H. (2019). Competition, dominance, and social hierarchy. In L. Welling & T. Schackelford (Eds.), *The Oxford handbook on evolutionary psychology and behavioral endocrinol*ogy. Oxford University Press.
- Casto, K. V., & Prasad, S. (2017). Recommendations for the study of women in hormones and competition research. *Hormones and Behavior*, 92, 190–194. https://doi.org/10.1016/j.yhbeh.2017.05.009
- Casto, K. V., Elliot, C. M., & Edwards, D. A. (2014). Intercollegiate cross-country competition: Effects of warm-up and racing on salivary levels of cortisol and testosterone. *International Journal of Exercise Science*, 7, 318–328 https://digitalcommons.wku.edu/ijes/vol7/iss4/8
- Casto, K. V., Rivell, A., & Edwards, D. A. (2017). Testosterone and cortisol response to recreational sport competition in women and perceived personal success. *Hormones and Behavior*, 92, 29–36. https://doi.org/10.1016/j.yhbeh.2017.05.006
- Casto, K.V., Edwards, D.A., Akinola, M., Davis, C., & Mehta, P.H. (2020). Testosterone reactivity to competition and competitive endurance in men and women. In the special issue celebrating the 30th anniversary of the challenge hypothesis. *Hormones and Behavior*, 123, 104665. https://doi.org/10. 1016/j.yhbeh.2019.104665.
- Casto, K. V., Root, Z. L., Geniole, S. N., Carré, J. M., & Bruner, M. W. (2021). Exploratory analysis of the relationship between social identification and testosterone reactivity to vicarious combat. *Human Nature*, 32, 509–527. https://doi.org/10.1007/s12110-021-09407-7
- Clark, R. V., Wald, J. A., Swerdloff, R. S., Wang, C., Wu, F. C., Bowers, L. D., & Matsumoto, A. M. (2019). Large divergence in testosterone concentrations between men and women: Frame of reference for elite athletes in sex-specific competition in sports, a narrative review. *Clinical Endocrinol*ogy, 90(1), 15–22. https://doi.org/10.1111/cen.13840

- Cobey, K. D., Klipping, C., & Buunk, A. P. (2013). Hormonal contraceptive use lowers female intrasexual competition in pair-bonded women. *Evolution and Human Behavior*, 34(4), 294–298. https:// doi.org/10.1016/j.evolhumbehav.2013.04.003
- Cook, C. J., & Crewther, B. T. (2012). Changes in salivary testosterone concentrations and subsequent voluntary squat performance following the presentation of short video clips. *Hormones and Behavior*, 61(1), 17–22. https://doi.org/10.1016/j.yhbeh.2011.09.006
- Cook, C. J., Crewther, B. T., & Smith, A. A. (2012). Comparison of baseline free testosterone and cortisol concentrations between elite and non-elite female athletes. *American Journal of Human Biology*, 24(6), 856–858. https://doi.org/10.1002/ajhb.22302
- Cook, C. J., Kilduff, L. P., & Crewther, B. T. (2018). Basal and stress-induced salivary testosterone variation across the menstrual cycle and linkage to motivation and muscle power. *Scandinavian Journal of Medicine & Science in Sports*, 28(4), 1345–1353. https://doi.org/10.1111/sms.13041
- Cook, C. J., Fourie, P., & Crewther, B. (2021). Menstrual variation in the acute testosterone and cortisol response to laboratory stressors correlate with baseline testosterone fluctuations at a within- and between-person level. *Stress*, 1–10. https://doi.org/10.1080/10253890.2020.1860937
- Crewther, B. T., & Cook, C. J. (2018). A longitudinal analysis of salivary testosterone concentrations and competitiveness in elite and non-elite women athletes. *Physiology & Behavior*, 188, 157–161. https://doi.org/10.1016/j.physbeh.2018.02.012
- Crewther, B. T., Hamilton, D., Casto, K. V., Kilduff, L. P., & Cook, C. J. (2015). Effects of oral contraceptive use on the salivary testosterone and cortisol responses to training sessions and competition in elite women athletes. *Physiology & Behavior*, 147, 84–90. https://doi.org/10.1016/j.physbeh. 2015.04.017
- Crewther, B. T., Hamilton, D., Kilduff, L. P., Drawer, S., & Cook, C. J. (2018). The effect of oral contraceptive use on salivary testosterone concentrations and athlete performance during international field hockey matches. *Journal of Science and Medicine in Sport*, 21(5), 453–456. https://doi.org/10. 1016/j.jsams.2017.09.017
- Crewther, B. T., Hecht, M., Potts, N., Kilduff, L. P., Drawer, S., Marshall, E., & Cook, C. J. (2020). A longitudinal investigation of bidirectional and time-dependent interrelationships between testosterone and training motivation in an elite rugby environment. *Hormones and Behavior*, 126, 104866. https://doi.org/10.1016/j.yhbeh.2020.104866
- Crofoot, M. C., & Wrangham, R. W. (2010). Intergroup aggression in primates and humans: The case for a unified theory, In mind the gap (pp. 171–195). Springer.
- Crust, L. (2007). Mental toughness in sport: A review. International Journal of Sport and Exercise Psychology, 5(3), 270–290. https://doi.org/10.1080/1612197X.2007.9671836
- Crust, L., & Clough, P. J. (2005). Relationship between mental toughness and physical endurance. Perceptual and Motor Skills, 100(1), 192–194. https://doi.org/10.2466/pms.100.1.192-194
- D'Andrea, S., Spaggiari, G., Barbonetti, A., & Santi, D. (2020). Endogenous transient doping: Physical exercise acutely increases testosterone levels—Results from a meta-analysis. *Journal of Endocrinological Investigation*, 1–23. https://doi.org/10.1007/s40618-020-01251-3
- Dabbs Jr., J. M. (1990). Salivary testosterone measurements: Reliability across hours, days, and weeks. *Physiology & Behavior*, 48(1), 83–86. https://doi.org/10.1016/0031-9384(90)90265-6
- de Wit, A. E., Bosker, F. J., Giltay, E. J., de Kloet, C. S., Roelofs, K., van Pelt, J., et al. (2018). Testosterone in human studies: Modest associations between plasma and salivary measurements. *Andrologia*, 50(1), e12779. https://doi.org/10.1111/and.12779
- Del Río, J. P., Alliende, M. I., Molina, N., Serrano, F. G., Molina, S., & Vigil, P. (2018). Steroid Hormones and their action in Women's brains: The importance of hormonal balance. *Frontiers in Public Health*, 6, 141. https://doi.org/10.3389/fpubh.2018.00141
- Edwards, D. A., & Casto, K. V. (2019). The social neuroendocrinology of athletic competition. In P. Mehta & O. Schultheiss (Eds.), *The international handbook of social neuroendocrinology*. Routledge/Psychology Press.
- Edwards, D. A., & Kurlander, L. S. (2010). Women's intercollegiate volleyball and tennis: Effects of warm-up, competition, and practice on saliva levels of cortisol and testosterone. *Hormones and Behavior*, 58(4), 606–613. https://doi.org/10.1016/j.yhbeh.2010.06.015
- Edwards, D. A., & O'Neal, J. L. (2009). Oral contraceptives decrease saliva testosterone but do not affect the rise in testosterone associated with athletic competition. *Hormones and Behavior*, 56(2), 195– 198. https://doi.org/10.1016/j.yhbeh.2009.01.008

- Eklund, E., Berglund, B., Labrie, F., Carlström, K., Ekström, L., & Hirschberg, A. L. (2017). Serum androgen profile and physical performance in women Olympic athletes. *British Journal of Sports Medicine*, 51(17), 1301–1308. https://doi.org/10.1136/bjsports-2017-097582
- Elliott-Sale, K. J., McNulty, K. L., Ansdell, P., Goodall, S., Hicks, K. M., Thomas, K., Swinton, P. A., & Dolan, E. (2020). The effects of oral contraceptive on exercise performance in women: A systematic review and meta-analysis. *Sports Medicine*, 50, 1785–1812. https://doi.org/10.1007/ s40279-020-01317-5
- Flinn, M. V., Ponzi, D., & Muehlenbein, M. P. (2012). Hormonal mechanisms for regulation of aggression in human coalitions. *Human Nature*, 23(1), 68–88. https://doi.org/10.1007/s12110-012-9135-y
- Frye, C. A. (2006). An overview of oral contraceptives: Mechanism of action and clinical use. *Neurology*, 66(66 suppl 3), S29–S36. https://doi.org/10.1212/WNL.66.66_suppl_3.S29
- Geniole, S. N., Bird, B. M., McVittie, J. S., Purcell, R. B., Archer, J., & Carré, J. M. (2020). Is testosterone linked to human aggression? A meta-analytic examination of the relationship between baseline, dynamic, and manipulated testosterone on human aggression. *Hormones and Behavior*, 123. https:// doi.org/10.1016/j.yhbeh.2019.104644
- Gillet, N., Berjot, S., Vallerand, R. J., Amoura, S., & Rosnet, E. (2012). Examining the motivation-performance relationship in competitive sport: A cluster-analytic approach. *International Journal of Sport Psychology*, 43(2), 79.
- Graham, C. A., Bancroft, J., Doll, H. A., Greco, T., & Tanner, A. (2007). Does oral contraceptive-induced reduction in free testosterone adversely affect the sexuality or mood of women? *Psychoneuroendocrinology*, 32(3), 246–255 https://pubmed.ncbi.nlm.nih.gov/17586130/
- Gray, P. B., Straftis, A. A., Bird, B. M., McHale, T. S., & Zilioli, S. (2020). Human reproductive behavior, life history, and the challenge hypothesis: A 30-year review, retrospective and future directions. *Hormones and Behavior, 123*. https://doi.org/10.1016/j.yhbeh.2019.04.017
- Griksiene, R., Monciunskaite, R., Arnatkeviciute, A., & Ruksenas, O. (2018). Does the use of hormonal contraceptives affect the mental rotation performance? *Hormones and Behavior*, 100, 29–38. https:// doi.org/10.1016/j.yhbeh.2018.03.004
- Gröschl, M. (2017). Saliva: A reliable sample matrix in bioanalytics. *Bioanalysis*, 9(8), 655–668. https:// doi.org/10.4155/bio-2017-0010
- Handelsman, D. J., Hirschberg, A. L., & Bermon, S. (2018). Circulating testosterone as the hormonal basis of sex differences in athletic performance. *Endocrine Reviews*, 39(5), 803–829. https://doi.org/ 10.1210/er.2018-00020
- Healy, M. L., Gibney, J., Pentecost, C., Wheeler, M. J., & Sonksen, P. H. (2014). Endocrine profiles in 693 elite athletes in the post competition setting. *Clinical Endocrinology*, 81, 294–305. https://doi. org/10.1111/cen.12445
- Hilton, E. N., & Lundberg, T. R. (2020). Transgender women in the female category of sport: Perspectives on testosterone suppression and performance advantage. *Sports Medicine*, 1–16. https://doi. org/10.1007/s40279-020-01389-3
- Liening, S. H., Stanton, S. J., Saini, E. K., & Schultheiss, O. C. (2010). Salivary testosterone, cortisol, and progesterone: Two-week stability, interhormone correlations, and effects of time of day, menstrual cycle, and oral contraceptive use on steroid hormone levels. *Physiology and Behavior*, 99, 8–16. https://doi.org/10.1016/j.physbeh.2009.10.001
- Liew, G. C., Kuan, G., Chin, N. S., & Hashim, H. A. (2019). Mental toughness in sport. German Journal of Exercise and Sport Research, 49(4), 381–394. https://doi.org/10.1007/s12662-019-00603-3
- Louw-du Toit, R., Perkins, M. S., Hapgood, J. P., & Africander, D. (2017). Comparing the androgenic and estrogenic properties of progestins used in contraception and hormone therapy. *Biochemical* and Biophysical Research Communications, 491(1), 140–146. https://doi.org/10.1016/j.bbrc.2017. 07.063
- Mackay, K., González, C., Zbinden-Foncea, H., & Peñailillo, L. (2019). Effects of oral contraceptive use on female sexual salivary hormones and indirect markers of muscle damage following eccentric cycling in women. *European Journal of Applied Physiology*, 119(11), 2733–2744. https://doi.org/ 10.1007/s00421-019-04254-y
- Martin, D., Sale, C., Cooper, S. B., & Elliott-Sale, K. J. (2018). Period prevalence and perceived side effects of hormonal contraceptive use and the menstrual cycle in elite athletes. *International Journal* of Sports Physiology and Performance, 13(7), 926–932. https://doi.org/10.1123/ijspp.2017-0330
- Mehta, P. H., Wuehrmann, E. V., & Josephs, R. A. (2009). When are low testosterone levels advantageous? The moderating role of individual versus intergroup competition. *Hormones and Behavior*, 56(1), 158–162. https://doi.org/10.1016/j.yhbeh.2009.04.001

- Montoya, E. R., & Bos, P. A. (2017). How oral contraceptives impact social-emotional behavior and brain function. Trends in Cognitive Sciences, 21(2), 125–136. https://doi.org/10.1016/j.tics.2016.11.005
- Muller, M. N. (2017). Testosterone and reproductive effort in male primates. *Hormones and Behavior*, 91, 36–51. https://doi.org/10.1016/j.yhbeh.2016.09.001
- Pearson, M., & Schipper, B. C. (2013). Menstrual cycle and competitive bidding. Games and Economic Behavior, 78, 1–20. https://doi.org/10.1016/j.geb.2012.10.008
- Prasad, S., Lassetter, B., Welker, K. M., & Mehta, P. H. (2019). Unstable correspondence between salivary testosterone measured with enzyme immunoassays and tandem mass spectrometry. *Psychoneuroendocrinology*, 109, 104373. https://doi.org/10.1016/j.psyneuen.2019.104373
- Ritzén, M., Ljungqvist, A., Budgett, R., Garnier, P. Y., Bermon, S., Lindén-Hirschberg, A., et al. (2015). The regulations about eligibility for women with hyperandrogenism to compete in women's category are well founded. A rebuttal to the conclusions by Healy et al. *Clinical Endocrinology*, 82(2), 307–308. https://doi.org/10.1111/cen.12531
- Rivera, R., Yacobson, I., & Grimes, D. (1999). The mechanism of action of hormonal contraceptives and intrauterine contraceptive devices. *American Journal of Obstetrics and Gynecology*, 181(5), 1263– 1269. https://doi.org/10.1016/s0002-9378(99)70120-1
- Robakis, T., Williams, K. E., Nutkiewicz, L., & Rasgon, N. L. (2019). Hormonal contraceptives and mood: Review of the literature and implications for future research. *Current Psychiatry Reports*, 21(7), 1–9. https://doi.org/10.1007/s11920-019-1034-z
- Roney, J. R. (2016). Theoretical frameworks for human behavioral endocrinology. *Hormones and Behavior*, 84, 97–110.
- Rosvall, K. A., Bentz, A. B., & George, E. M. (2020). How research on female vertebrates contributes to an expanded challenge hypothesis. *Hormones and Behavior*, 123, 104565. https://doi.org/10.1016/j. yhbeh.2019.104565
- Rothman, M. S., Carlson, N. E., Xu, M., Wang, C., Swerdloff, R., Lee, P., et al. (2011). Reexamination of testosterone, dihydrotestosterone, estradiol and estrone levels across the menstrual cycle and in postmenopausal women measured by liquid chromatography-tandem mass spectrometry. *Steroids*, 76(1–2), 177–182. https://doi.org/10.1016/j.steroids.2010.10.010
- Schaumberg, M. A., Emmerton, L. M., Jenkins, D. G., Burton, N. W., de Jonge, X. A. J., & Skinner, T. L. (2018). Use of oral contraceptives to manipulate menstruation in young, physically active women. *International Journal of Sports Physiology and Performance*, 13(1), 82–87. https://doi.org/10.1123/ ijspp.2016-0689
- Sitruk-Ware, R., & Nath, A. (2013). Characteristics and metabolic effects of estrogen and progestins contained in oral contraceptive pills. *Best Practice & Research Clinical Endocrinology & Metabolism*, 27(1), 13–24. https://doi.org/10.1016/j.beem.2012.09.004
- Sönksen, P. H., Holt, R. I., Böhning, W., Guha, N., Cowan, D. A., Bartlett, C., & Böhning, D. (2018). Why do endocrine profiles in elite athletes differ between sports? *Clinical Diabetes and Endocrinology*, 4(1), 1–16. https://doi.org/10.1186/s40842-017-0050-3
- Trumble, B. C., Cummings, D. K., O'Connor, K. A., Holman, D. J., Smith, E. A., Kaplan, H. S., & Gurven, M. D. (2013). Age-independent increases in male salivary testosterone during horticultural activity among Tsimane forager-farmers. *Evolution and Human Behavior*, 34(5), 350–357. https:// doi.org/10.1016/j.evolhumbehay.2013.06.002
- Van Anders, S. M., Goldey, K. L., & Kuo, P. X. (2011). The steroid/peptide theory of social bonds: Integrating testosterone and peptide responses for classifying social behavioral contexts. *Psychoneuroendocrinology*, 36(9), 1265–1275. https://doi.org/10.1016/j.psyneuen.2011.06.001
- Vermeer, A. L., Riečanský, I., & Eisenegger, C. (2016). Competition, testosterone, and adult neurobehavioral plasticity. *Progress in Brain Research*, 229, 213–238. https://doi.org/10.1016/bs.pbr.2016.05. 004
- Vermeer, A. L., Krol, I., Gausterer, C., Wagner, B., Eisenegger, C., & Lamm, C. (2020). Exogenous testosterone increases status-seeking motivation in men with unstable low social status. *Psychoneuroendocrinology*, 113. https://doi.org/10.1016/j.psyneuen.2019.104552
- Welker, K. M., & Carré, J. M. (2015). Individual differences in testosterone predict persistence in men. European Journal of Personality, 29, 83–89. https://doi.org/10.1002/per.1958
- Wingfield, J. C., Lynn, S. E., & Soma, K. K. (2001). Avoiding the 'costs' of testosterone: Ecological bases of hormone-behavior interactions. *Brain, Behavior and Evolution*, 57(5), 239–251. https://doi. org/10.1159/000047243
- Wood, R. I., & Stanton, S. J. (2012). Testosterone and sport: Current perspectives. Hormones and Behavior, 61(1), 147–155. https://doi.org/10.1016/j.yhbeh.2011.09.010

Zimmerman, Y., Eijkemans, M. J. C., Coelingh Bennink, H. J. T., Blankenstein, M. A., & Fauser, B. C. J. M. (2014). The effect of combined oral contraception on testosterone levels in healthy women: A systematic review and meta-analysis. *Human Reproduction Update*, 20(1), 76–105. https://doi.org/10.1093/humupd/dmt038

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Kathleen V. Casto¹ · Lindsie C. Arthur² · Dave K. Hamilton³ · David A. Edwards⁴

- ¹ Social Sciences Division, New College of Florida, Bay Shore Road, Sarasota, FL 34243, USA
- ² Melbourne School of Psychological Sciences, University of Melbourne, Parkville, Victoria 3010, Australia
- ³ USA Field Hockey, Colorado Springs, CO, USA
- ⁴ Department of Psychology, Emory University, 36 Eagle Row, Atlanta, GA 30322, USA