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A longitudinal analysis of salivary testosterone concentrations and competitiveness in elite and non-elite women athletes

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ABSTRACT

Background: There is evidence linking women's testosterone (T) to competitive behaviours in sport and exercise. To advance this work, we examined the longitudinal relationships between salivary T (sal-T) and competitiveness in athletic women who differ in training status.

Methods: Elite (n = 9) and non-elite (n = 21) women athletes were monitored on days 6–8 (follicular phase), 13–15 (ovulatory phase) and 20–22 (Luteal phase) of a menstrual cycle with two repeats. Salivary T levels were assessed before breakfast, followed by two questions (each rated on a 1–7 scale) on competitive desire and training motivation. Using a linear mixed model, we evaluated the menstrual phase and training status effects on each variable, before assessing the within-subject effects of sal-T on competitiveness.

Results: Salivary T concentrations were higher at ovulation (effect size [ES] difference = 0.2–1.4), relative to the follicular and luteal phases, with a more marked response among elite women ($p < .01$). The competitiveness ratings showed similar menstrual-phase variation (ES difference = 0.6–1.0 at ovulation). A positive effect of sal-T on competitiveness emerged in both groups ($p < .001$), but with different slope patterns ($p < .015$). Specifically, the elite sal-T relationships with desire to compete (standardized β = 1.147, SE = 0.132) and training motivation (β = 1.195, SE = 0.124) were stronger compared with non-elite women (β = 0.631, SE = 0.114; β = 0.778, SE = 0.114), respectively.

Conclusions: Morning sal-T concentrations, competitive desire and training motivation all peaked around ovulation in women athletes. Notably, sal-T availability and its relationship with competitiveness was stronger among high-performing athletes. Our findings confirm menstrual fluctuations in T and competitiveness among naturally-cycling women, with population context as a moderating factor.

1. Introduction

In sport and exercise, there is growing interest in the androgenic and arousal roles of testosterone (T) as a social hormone, particularly regarding competitive behaviours among women [1,2]. Evidence shows that changes or differences in women's T levels are positively related to self-efficacy, pre-event focus, motivation for action, team bonding, and self-chosen workloads in athletic and non-athletic domains [1,3–5]. Consequently, the T contribution to female performance and training adaptation could be mediated by physical outputs (e.g., more work performed, greater load selection, exercise adherence) linked to the expression of such behaviours, some of which can occur somewhat outside of conscious awareness [5].

Studies have also implicated T in other dimensions of competitiveness, such as intrasexual competition [6–9]. Typically, this work is framed around the menstrual cycle focusing on distinct phases associated with relatively high T (i.e., ovulatory) and low T (i.e., luteal) concentrations [9], or correspondingly high-fertile and low-fertile periods [7,8], along with the exogenous manipulation of T subsequent to oral contraceptive (OC) use [7]. However, within-subject testing under natural conditions provides more compelling evidence [6]. Adopting the latter approach would provide unique insight into competitive behaviours in sport and the temporal nuances of reproductive endocrinology, which could then guide exercise planning and strategy application to maximise athlete performance.

Testosterone is highly responsive to training and competition, which can lead to chronic adaptations to differentiate elite from non-elite performers [10]. For instance, a positive association was reported

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between sal-T and pre-training motivation in professional male athletes [11]. Conversely, non-elite men showed no such linkage [11]. Elite women were also found to possess higher (more than double) sal-T levels than non-elites [12] and they can exhibit greater relative exercise-induced increases in T versus untrained women [13], thereby potentially supporting those behaviours necessary to maintain elite-level performance. To date, no research has examined the within-subject effect of sal-T on competitiveness in women athletes who differ in training status (i.e., elites, non-elites), as another crucial step towards explicating the behavioural role of T in sport.

A longitudinal study was undertaken to investigate the sal-T linkage to competitiveness in elite and non-elite women athletes. Testing was performed across three menstrual phases (i.e., follicular, ovulatory, luteal) to profile the variation in sal-T levels and two sport-related competitiveness measures: desire to compete and training motivation. Follow-up testing was conducted to assess the within-subject relationships between these variables. Three hypotheses were formulated; first, sal-T and competitiveness would be elevated at ovulation, relative to the follicular and luteal phases; second, these changes would more marked in elite women; third, the elite group would exhibit stronger sal-T relationships with competitiveness.

2. Materials and methods

2.1. Subjects

Thirty-six women athletes from different sports (i.e., triathlon $n = 3$, body shaping $n = 6$, swimming $n = 6$, rugby union $n = 6$, netball $n = 9$, football $n = 6$) were recruited under ethical approval. Six women reported taking some form of hormone-based OC, so they were excluded from data analyses. The remaining women ($n = 30$) had not taken any OCs in the last six months and they reported normal menstrual cycles (range: 24 to 33 days). This cohort was separated into elites ($n = 9$), who were national- or international-level competitors, and non-elites ($n = 21$) who were competitive at a club or recreational level [11,14]. All athletes were questioned about the use of performance-enhancing agents (e.g., anabolic steroids), but none were reported, and the elite group were routinely tested in this capacity. Written informed consent was provided before study commencement.

2.2. Study protocols

The elite and non-elite groups were monitored across three distinct phases of a single menstrual cycle with two further cycle repeats. Menstrual phase assignment was consistent with previous work [8,15–17]. Based on a 28-day cycle, testing occurred in discrete windows from days 6 to 8 (follicular phase), 13 to 15 (ovulatory phase), and 20 to 22 (luteal phase), following the onset of previous menses. This window method provided objective cut-off points to assess general shifts in sal-T and behaviour across the menstrual cycle, but the primary analyses to predict competitiveness from sal-T included all results, as continuous data. To ensure that separate phases were captured, a minimum period of seven days between collection points was specified. Testing was performed in the morning (at home) to improve study compliance and the ecological validity of the results obtained. Saliva samples were self-collected for T determination, followed by a brief competitiveness questionnaire. The time of testing did vary (range: 6:30 AM to 8:00 AM), but each athlete administered her own tests at the same time of day, relative to waking (within 30 min).

All athletes maintained their normal training and competition schedules, but no physical activity was performed at least 12 h before data collection. The elite group trained up to five days a week using multiple forms of exercise (e.g., sport-specific fitness, strength conditioning, skill development) in a planned and periodised manner. The non-elite women trained no more than three days a week, comprising of sport-specific sessions and general conditioning. The athletes were monitored during the pre-season and/or in-season phases for their respective sports, so participation in some form of competitive encounter is likely. However, training sessions are often designed to mimic competition, and can promote similar hormonal responses and exertion ratings among female athletes [18], so athletic competition could be viewed as another stress stimulus in sport, particularly when it only comprises of 5–15% of all activities performed during a typical in-season training block [11,18,19]. The early morning testing sessions provided partial control for these effects.

Due to logistical constraints (e.g., travel away from home), subject non-compliance and some drop outs across the monitoring period, results from 40 testing sessions were missing (270 were originally planned), representing a data loss of 15%. Despite this, the number of completed tests per athlete (mean 7.7 ± 2.2) was deemed sufficient to address the study hypotheses. A linear mixed model was employed to examine the study results; a flexible statistical approach suitable for unbalanced, longitudinal studies with randomly or non-randomly missing data [20].

2.3. Salivary testosterone assessment

Prior to sample collection, the participants were asked to refrain from brushing their teeth, consume any food or drinking caffeine, and not to smoke [21]. A small (~1 mL) saliva sample was collected by passive drool into pre-labelled sterile containers [6,11,22] and subsequently stored in a commercial freezer (< 3 days) before transfer to a −80 °C freezer for long-term storage [22]. After thawing and centrifugation, the samples were analysed in duplicate using commercial enzyme-linked immunoassay kits (Salimetrics LLC, USA) in accordance with the manufacturers' guidelines. Each kit had a calibrator range of 6.1 pg/mL to 600 pg/mL. The inter-assay coefficients of variation (CV) were < 10%. To eliminate inter-assay variability, the samples for each participant were assayed within the same plate.

2.4. Assessment of competitiveness

Two simple competitiveness questions were completed immediately after saliva collection: desire to compete (from 1 = I have no desire to compete up to 7 = I feel extremely competitive) and training motivation (from 1 = I have no motivation to train up to 7 = I am extremely motivated to train). To assess the transient nature of these outcomes, each athlete was instructed to rate their feelings at that moment in time. Single-item psychological ratings are common in sport [11,23,24], as they enable rapid (and reliable) data to be collected with low intrusiveness, low costs, and are relatively easy to interpret [25]. In addition, the at-home testing procedures is likely to overcome any perceptual or self-reporting issues associated with social context [25], as well as T changes arising from olfactory cues among women [8].

2.5. Statistical analyses

To evaluate the impact of menstrual variability and population context on each variable, a two-factor (Menstrual phase, Training status) mixed analysis of variance was employed. Where appropriate, post-hoc assessments were performed using the Tukey test. Cohen's effect size (ES) statistics were also calculated with a 95% confidence interval (CI), as follows; < 0.2 = trivial, 0.2 to < 0.5 = small, 0.5 to < 0.8 = medium, ≥ 0.8 = large. The within-subject effect of sal-T on competitiveness was tested with a random intercept-linear mixed regression model. In separate analyses, competitive desire and training motivation were entered as the dependent variable with sal-T (standardized), training status (0 = elite, 1 = non-elite), and their interaction, entered simultaneously as predictors. The sal-T data were log transformed before testing to satisfy model assumptions, but the raw values are presented to aid interpretation. All model assumptions for the residuals (i.e., linearity, independence, normality, equal variance)