



# Women's intercollegiate volleyball and tennis: Effects of warm-up, competition, and practice on saliva levels of cortisol and testosterone

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## ABSTRACT

In virtually all sports, participants “warm-up” prior to formal competition. Women athletes from a highly ranked varsity college volleyball team and, in a second study, a highly ranked varsity college tennis team gave saliva samples before warm-up, at mid-warm-up (volleyball) or after warm-up (tennis), and immediately after intercollegiate competition. For volleyball and tennis, warm-up was associated with a substantial elevation in saliva levels of testosterone which was carried over through the period of actual competition. Cortisol levels were relatively unchanged during warm-up, but typically rose during competition. Thus, as women prepare for athletic competition by warming up, testosterone levels rise in apparent anticipation of the coming contest and then remain high through the period of play. In volleyball and tennis, after-practice testosterone level was significantly higher than before-practice level, and practice session increases in testosterone (but not cortisol) were positively correlated with increases in testosterone during intercollegiate competition. When practice and competitive play share as yet undetermined key elements, individual differences in this endocrine response to “competition” appear stable across practice and intercollegiate competition.

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## Introduction

In women's team athletics, competition is usually associated with an increase in salivary testosterone level (e.g., Bateup et al., 2002; Edwards et al., 2006, 2007) and, depending on the sport, an increase in salivary cortisol (e.g., Edwards et al., 2006, 2007; Filaire et al., 1996, 1999; Haneishi et al., 2007). Similar changes have been noted for individual (Booth et al., 1989) and team (Edwards et al., 2006) athletic competitions in men. In men and women these changes presumably parallel changes in serum levels of testosterone and cortisol.

In women's team athletics, testosterone (T) and cortisol (C) levels also appear to increase in anticipation of competition. In one study with college rugby players (Bateup et al., 2002), T and C levels measured in saliva were significantly higher 15 min before competition than 24 h earlier. In another study with women soccer players (Oliveira et al., 2009), saliva T and C levels measured 30 min before competition were significantly higher than levels of these same hormones measured on an earlier neutral day. Anticipatory increases in T and C have been noted for male tennis players (Booth et al., 1989) and judo competitors (Salvador et al., 2003; Suay et al., 1999) as well.

Increases in hormone levels that anticipate competition are typically determined from comparison of samples taken a few minutes before the start of competition with samples obtained either

the day before competition or some other “neutral” day of non-competition. Thus, it is not possible to know from these studies precisely when the anticipatory increases in T and/or C begin.

In virtually all sports, participants “warm-up” prior to formal competition. Warm-up routines may include running, stretching, light calisthenics, and sport-specific skill-sharpening drills. All of this is intended to decrease the potential for injury and, physiologically and psychologically, prepare participants for the coming competition. Surprisingly, there are no published studies specifically connecting this period to the changes in hormone levels that appear to anticipate actual athletic competition.

On the premise that a full understanding of the effects of any hormone on physiology and behavior is informed by an appreciation of the settings and circumstances that affect its secretion, we here report the results of two studies designed to determine the effects of warm-up and competition on saliva levels of cortisol and testosterone. The first was conducted with members of a highly ranked women's intercollegiate volleyball team who gave saliva samples immediately before a structured warm-up prior to an important intercollegiate match, again at mid-warm-up, and immediately after the finish of competition. The second study was conducted with members of a highly ranked women's intercollegiate tennis team who gave saliva samples immediately before and after warm-up for their doubles matches against another school, after the completion of the doubles matches, and then again after their singles matches that followed. For purposes of comparison, each study also included analysis of C and T levels for samples obtained immediately before and after practice sessions.

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## Methods

### Participants

Participants for the first study were the 15 active members of the 2008 Emory University varsity women's intercollegiate (Division III) volleyball team. A 16th volleyball participant suffered a season-ending injury prior to the start of the study and, although she was at courtside and gave saliva samples, her values were not figured in the analyses for this report. The study was conducted in late October. At the time, Emory was ranked second nationally and the team would finish the season as the National Collegiate Athletic Association (NCAA) champion.

Participants for the second study were the 13 members of the 2009 Emory University varsity women's intercollegiate (Division III) tennis team. The study was conducted in early April when Emory was ranked fourth in the country, and the team would finish third in the NCAA championship tournament in May.

Each study was approved by Emory's Institutional Review Board. Potential participants were informed about the purpose of the study as well as the method and frequency of saliva sampling, and each member of the team gave written informed consent prior to participation. As part of the consent procedure women were specifically asked to respond "yes" or "no" to two questions: "Are you currently using oral contraceptives?" and "Are you currently using any injected, implanted, or patch-delivered hormone contraceptive?"

### *When saliva samples were obtained for volleyball players and tennis players*

For the volleyball players, saliva samples were obtained from participants for an intercollegiate match played at home. The match (a 3–0 victory) began at 7:10 PM, and was against a Division III opponent, at the time ranked first in the country. Each participant gave three match-connected saliva samples. The first (baseline) was given while the team dressed for the match 30–40 min before the start of warm-up. Participants gave their second (mid-warm-up) sample 30 min after the start of a 60 min warm-up period. Following NCAA regulations, the home team has exclusive use of the court for the last 5 min of the warm-up period, and actual play begins 1 min after that—not enough time at the end of warm-up for collection of saliva samples. But, 30 min after the start of warm-up (i.e., mid-warm-up) the home team leaves the court to allow the visiting team full court access—this transition afforded an opportunity for a 5 min collection period at mid-warm-up that would not substantially interfere with the usual pre-match warm-up protocol for the players being sampled. Participants gave their third sample immediately after the completion of the match about 80 min after the start of play. We also obtained saliva samples before and after two consecutive practice sessions 2 weeks earlier in the season, with each session beginning around 3:30 PM and ending around 5:25 PM.

Tennis team participants gave saliva samples for a single intercollegiate match played outdoors at home. They gave their first (baseline) sample between 3:30 and 3:45 PM, immediately prior to warm-up for a match against the number-one ranked team (Division III) in the country. Participants gave a second sample at the end of the warm-up (5:05 PM) just prior to the start of doubles competition. As per Intercollegiate Tennis Association regulations, three doubles teams from the two colleges played a single set each, and then six players from each team played against each other in a best-of-three set singles competition. Each player gave a third sample immediately after the completion of her doubles set. The fourth sample was obtained immediately after the completion of each player's singles competition. Doubles matches began at 5:15 PM and were completed by 6:50 PM; the last singles match was completed at 8:57 PM. For the two teams, the outcome of the match was decided by the combined

results of the doubles and singles competitions. Emory won 1 of the 3 doubles matches and 3 of the 6 singles matches to lose the overall competition by a score of 4–5. Participants also gave saliva samples before and after a practice session held 1 week later. The practice session began at 4:30 PM and ended at 6 PM.

Whether for volleyball or tennis, the athletes gathered together with their coaches at the finish of the competition for a brief (5 min) talk, after which they dispersed for different destinations and purposes. Thus, it was not possible to obtain saliva samples for a sufficiently extended time (assuming a delay of 20–30 min before changes in hormone levels in blood are reflected in changes in hormone levels in saliva) after competition to allow meaningful analysis of the effects of competition outcome on levels of salivary cortisol and testosterone.

### *Saliva samples and hormone assay*

For each sample, participants were provided a piece of sugar-free gum (Trident®, original flavor) to stimulate saliva production and a 20 ml plastic vial which they were asked to fill to a 5 ml line marked on the side. Participants chewed for a timed 2 min period before starting to fill the vial. Including the pre-delivery chewing period, collection of the samples typically took 4–7 min. The mid-warm-up collection period for the volleyball players was capped at 5 min so as to not substantially interfere with the usual pre-match warm-up routine.

Samples were stored at  $-26^{\circ}\text{C}$  within 15 min after collection and the frozen samples were later sent to the Biomarkers Core Laboratory of the Yerkes National Primate Research Center of Emory University in Atlanta, Georgia for hormone assay. Salivary cortisol was assayed in duplicate using the Diagnostic Systems Laboratories (Beckman Coulter, Webster TX) enzyme immunoassay kit DSL10-67100, with a range of analytical sensitivity of .025–10  $\mu\text{g}/\text{dl}$  for test volumes of 25  $\mu\text{l}$ . All inter-assay coefficients of variation for the various assays were <5%; all intra-assay coefficients of variation were <10%. Salivary testosterone was assayed in triplicate using a modification (Granger et al., 1999) of the Diagnostic Systems Laboratories (Beckman Coulter, Webster TX) radioimmunoassay kit DSL-4100, with a range of analytical sensitivity of 2–500  $\text{pg}/\text{ml}$  for test volumes of 200  $\mu\text{l}$ . All intra-assay coefficients of variation for the various assays were <5%; all inter-assay coefficients of variations were <20%.

### *Statistical analyses*

The SPSS statistical package for personal computer was used for the calculation of independent and correlated *t*-tests (two-tailed) and Pearson product-moment correlations. In all cases,  $p < .05$  was required for statistical significance.

## Results

### *Hormone levels and hormone contraception*

Baseline (before-practice and before-warm-up on the day of competition) cortisol (C) and testosterone (T) means are shown in Table 1. Hormone values for participants were within the normal range for college-age women (e.g., Dabbs et al., 1995; Gozansky et al., 2005; Kirschbaum and Hellhammer, 1994). For volleyball and tennis, women using oral contraceptives had a slightly lower baseline (before-match-warm-up) testosterone mean than women not using oral contraceptives, but the difference between users and non-users was not statistically significant for study participants in either sport. Whether for volleyball or tennis, mean salivary C for women using oral contraceptives was not significantly different from that for non-users.

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