BRIEF REPORT

The utility of salivary testosterone and cortisol concentration measures for assessing the stress responses of junior athletes during a sporting competition

Blair T. Crewther¹ | Zbigniew Obmiński¹ | Joanna Orysiak¹ | Emad A. S. Al-Dujaili²¹Institute of Sport—National Research Institute, Warsaw, Poland²Centre for Cardiovascular Sciences, Edinburgh University, Edinburgh, UK**Correspondence**

Blair T. Crewther, Department of Endocrinology, Institute of Sport—National Research Institute, Warsaw, Poland.
Email: blair.crewther@gmail.com

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Background: This study examined the utility of salivary testosterone (T) and cortisol (C) concentration measures for assessing the acute stress responses of junior athletes during a sporting competition.

Methods: Paired capillary blood and saliva samples were taken from 71 junior athletes (45 males, 26 females) before and after a simulated Olympic weightlifting competition. The concentration measures of salivary T and C were referenced against blood total T (TT) and C (TC) concentrations. All hormones were tested by immunoassay.

Results: The competition promoted an increase in the blood TT concentrations of the male ($10.1\pm 18.7\%$) and female ($12.9\pm 19.9\%$) athletes ($P<.05$), with TC also rising in females ($37.5\pm 69\%$) and tending to increase in males ($23.2\pm 56.1\%$). No significant changes in salivary T or C were identified. Locally-weighted regression revealed that salivary T was strongly related to blood TT in males ($r^2=.84$), but not females ($r^2=-.15$), whereas salivary C was moderately related to blood TC in both sexes ($r^2=.43$, $r^2=.48$), respectively.

Conclusions: A short, high-intensity competition promoted a predictable stress-induced rise in blood TT and TC concentrations. We observed no hormonal stress responses in saliva under the current format, which could be explained by individual variability and blood-saliva differences in hormone kinetics. Still, the salivary T and C measures did show a moderate to strong ability to track individual blood TT (males only) and TC (both sexes) concentrations in a young athletic cohort across a broad physiological range. Recommendations for researchers and practitioners are presented.

KEYWORDS

androgen, binding proteins, glucocorticoid, stress, validation

1 | INTRODUCTION

Saliva is gaining broader acceptance as a biological fluid for monitoring steroid hormones in sports medicine, psychology, stress research and chronobiology,¹ especially the androgen testosterone (T) and the glucocorticoid cortisol (C). In comparison to other biological fluids (eg, urine, sweat, venous blood), saliva is relatively easy to collect, stress-free with high compliance among different populations, and it enables rapid and repeated sampling under conditions (eg, sports competition)

where the collection of other fluids are difficult or undesirable.^{1,2} Recent developments in enzyme-linked immunoassays are also contributing to greater use of salivary steroids among researchers, practitioners and clinicians alike, due to their simplicity, high sensitivity and low cross reactivity, as well as reliability.²

Under resting conditions, salivary T and C measures correlate with blood total T (TT) and C (TC) respectively in healthy and clinical populations, but more so blood-free T and C.³⁻⁷ Hence, saliva is thought to represent the biologically active hormone.^{1,2} Recent work suggests

that saliva is a separate matrix with its own binding protein properties, though salivary T ostensibly mirrors blood-free T.⁸ Still, the saliva-blood T relationships are stronger among males,^{3-5,9} because female salivary T is often assessed at the lower detection limits of many assays and are strongly influenced by binding protein activity.⁸ Cortisol is a much larger molecule and less affected by the same detection issues. It is also present at comparable levels in men and women^{7,10,11}; hence, C data from both sexes are often pooled for comparisons with moderate to strong relationships reported.^{7,10,11}

These comparisons have been extended to include an exercising format,¹²⁻¹⁹ as an ecologically valid model of "stress". In general, these studies support the use of salivary hormones to assess blood TT and TC activity, but with some important considerations. Most of these studies are limited by a small population (eg, 12 subjects or less) and no work has compared both sexes during athletic competition, as perhaps the most stressful form of exercise. Addressing these issues would ensure that salivary T and C can be validated across a large physiological range, which in turn will improve the strength of these findings and their applicability in other exercise, sport and health settings. Research on younger populations is also lacking,²⁰ who are arguably more susceptible to stress than adults and may benefit from a valid, non-invasive tool for monitoring their hormonal status.

Given the aforementioned issues and potential benefits for researchers, practitioners and clinicians, we examined the utility of salivary T and C as measures for assessing the acute stress responses of junior athletes (male and female) during a simulated weightlifting competition. These data were referenced against blood TT and TC. We hypothesized that: (i) athlete T and C concentrations in both fluids would rise under the stress of competition; (ii) males would exhibit stronger salivary T and blood TT relationships than females; and (iii) salivary C and blood TC would be similarly related in both sexes.

2 | MATERIALS AND METHODS

2.1 | Participants

Seventy-one junior athletes were recruited, comprising of 45 males (age 18.1±1.2 years, height 174.3±8.5 m, weight 79.6±18.0 kg) and 26 females (age 17.6±1.2 years, height 162.2±7.6 m, weight 63.8±13.6 kg) with a background in Olympic weightlifting. The participants did not report any injuries, medical or health conditions that would limit their ability to complete the competition protocols. The athletes were also questioned about drugs, medicines and doping usage, but none were reported. Participant and parental consent were provided before the study commenced. This work was performed in accordance with the Helsinki Declaration and was approved by an Institutional Ethics Committee.

2.2 | Testing procedures

The young athletes were monitored across a simulated Olympic-style weightlifting competition. This involved strength testing across

two exercises (ie, snatch, clean and jerk) using single-repetition trials at self-selected loads, as per a normal weightlifting competition. Exercise intensity was deemed to be very high, as the loads chosen were typically 94% of the personal best lifts for each athlete, whereas the volume of exercise was relatively low (ie, 6 trials in total). The exercises performed are very reliable in trained weightlifters with low (<2.8%) coefficients of variation (CV).²¹ Two paired capillary blood and saliva samples were taken across the weightlifting competition; before warming up for the first exercise trial (ie, pre-competition) and then after the last trial (ie, post-competition), equating to a 30-minute sampling period. Athlete testing was conducted over four consecutive days in smaller manageable groups, but each group was assessed at a similar time each day (between 10 AM and 1 PM) to account for diurnal variation.^{2,4,22}

2.3 | Hormonal testing

Capillary blood provides a valid, non-invasive alternative to venous blood for assessing TT and TC.^{17,23,24} Blood samples (~300 µL) were taken from the fingertip and the serum portion separated by centrifugation. Saliva (~500 µL) was collected by passive drool without stimulation.⁸ To prevent saliva contamination, the participants were instructed to not brush their teeth, consume any food or drink fluids (except water) for 2 hours before testing.²⁵ All samples were stored at -80°C and assayed within a month. All hormone values in blood and saliva were determined using commercial immunoassay kits (DRG, Marburg, Germany). The lower detection limit for the blood TT and TC kits were 0.69 and 55 nmol/L, respectively. The salivary T and C kit detection limits were 35 pmol/L and 0.55 nmol/L, respectively. The inter-assay CV's did not exceed 10%, but the samples from each athlete were tested within the same plate to eliminate this variability.

2.4 | Statistical analyses

To assess the acute hormonal responses to competition, change scores were calculated (post-pre expressed as a % change) for each variable and compared to a zero baseline using the Wilcoxon signed-rank test. Sex differences in hormone reactivity were assessed by comparing the change scores with the Quade test, once controlling for pre-competition hormones. Effect sizes (ES) were calculated using Cohen's *d*. Associations between the saliva and blood hormone measures were tested with locally-weighted regression,²⁶ after pooling the pre- and post-competition data to produce a broader physiological range. A low order (1st degree) polynomial fit was applied with bisquare weightings to adjust for outliers. The models are presented with 90% prediction intervals. The significance level was set at $P \leq 0.05$.

3 | RESULTS

The blood TT concentrations of males and females increased across the competition ($P < .009$, $ES = 0.8-0.9$, Table 1), but the TT changes in each group were no different ($P = .834$, $ES = 0.1$). A rise in blood TC

TABLE 1 Blood and salivary hormone responses of junior male and female athletes across a simulated weightlifting competition (means $\pm$ SD)

Measure	Sex	Pre	Post	% change
Blood TT (nmol/L)	Males	19.9 $\pm$ 12.1	22.1 $\pm$ 15.0	10.1 $\pm$ 18.7*
	Females	1.67 $\pm$ 0.49	1.88 $\pm$ 0.58	12.9 $\pm$ 19.9*
Salivary T (pmol/L)	Males	319 $\pm$ 352	325 $\pm$ 370	0.5 $\pm$ 35.6
	Females	67.4 $\pm$ 42.0	55.0 $\pm$ 34.9	7.3 $\pm$ 88.4
Blood TC (nmol/L)	Males	434 $\pm$ 140	493 $\pm$ 181	23.2 $\pm$ 56.1
	Females	381 $\pm$ 94.2	497 $\pm$ 199	37.5 $\pm$ 69.0*
Salivary C (nmol/L)	Males	21.1 $\pm$ 6.5	20.2 $\pm$ 9.0	-1.5 $\pm$ 38.3
	Females	20.4 $\pm$ 3.9	23.4 $\pm$ 9.9	14.4 $\pm$ 42.4

Key: T=testosterone, C=cortisol, TT=total testosterone, TC=total cortisol.

*Significant change from baseline $P < .05$.

also emerged in females ($P=.016$, $ES=0.8$), whilst in males TC tended to increase ($P=.067$, $ES=0.6$). The blood TC responses of each group did not differ ($P=.967$, $ES=0.2$). No changes in salivary T and C were seen in males ($P>.144$, $ES=0.0-0.1$) or females ($P>.234$, $ES=0.1-0.4$), nor did we find any sex differences in salivary hormone reactivity ($P>.07$, $ES=0.1-0.4$). The amount of T and C measured in saliva, when expressed relative to blood TT and TC concentrations, was 2%-4% for males and 3%-5% for females. These percentages are consistent with the literature.^{3-5,7,10}

Regression analyses revealed a strong positive association between individual salivary T and blood TT concentrations in males (Figure 1A). For females, the individual variation in salivary T was a poor predictor of blood TT (Figure 1B). The negative r^2 outcome indicates that the

model is worse than simply fitting a horizontal line. When comparing individual salivary C and blood TC concentrations, positive relationships of moderate strength were found in the male (Figure 2A) and female (Figure 2B) cohorts tested.

4 | DISCUSSION

This is the first study to compare the acute stress responses of T and C (measured in blood and saliva) among junior athletes in a weightlifting competition. The simulated event promoted increases in blood TT and TC concentrations in both sexes, whereas salivary T and C showed no such change. A strong relationship was also demonstrated between

FIGURE 1 Locally-weighted regression predicting blood total testosterone (TT) concentrations from salivary testosterone (T) concentrations in junior male (A) and female (B) athletes

FIGURE 2 Locally-weighted regression predicting blood total cortisol (TC) concentrations from salivary cortisol (C) concentrations in junior male (A) and female (B) athletes

the salivary T and blood TT measures, but limited to the males tested, whilst salivary C and blood TC were moderately related in both sexes.

The rise in blood TT (10%-13%) and TC (23%-37%) concentrations is not surprising given the inherent stressors of athletic competition. The lack of a hormonal stress response in saliva was unexpected, particularly for C, as exercise-induced increases in both fluids are common.^{12-17,19} Heterogeneity in salivary T and C reactivity is one consideration,^{18,19,27} as evidenced by the large SD's relative to the group changes. Alternatively, physical exercise might artificially elevate blood hormones due to a hemoconcentration effect,^{13,28} but not observable in other biological fluids. Differences in the timing and magnitude of the peak hormonal increases in blood and saliva offers a more plausible explanation,^{14,16,18} but not quantifiable under the current format (ie, paired samples). As such, we suggest that studies may benefit by offsetting the post-stress saliva sample to capture the expected hormonal changes in blood (eg, 10-15 minutes later).^{12,14}

Our data confirms that males exhibit stronger salivary T and blood TT relationships than females,^{3-5,9} despite the similar group responses in both fluids. This is explainable by the fact that women's T values are much lower than men, as we identified in their relative proportion of T in blood (8%) and saliva (19%), and often measured at the lower detection limits for most immunoassay kits.² Having less T available than males, coupled with a higher amount of sex-hormone binding protein,²⁹ means that a greater proportion of blood T is also "bound" in females and not freely able to enter saliva. The conversion of T to other steroids in peripheral tissue (eg, salivary glands),³⁰ plus T binding to salivary proteins,⁸ might also have a greater net effect on T measured in female saliva and subsequent blood TT relationships. Thus, whilst salivary T provides a valid measure for assessing individual blood TT concentrations among adult and younger men, extreme care should be taken when employing salivary T measures in female populations under resting and dynamic conditions.

The moderate salivary C and blood TC relationships are consistent with exercise research,^{12,14-20,22,31} but with the added novelty of a competitive stressor and concurrent testing of younger men and women. The sex-related differences noted for salivary T are not evident for C, due to its larger molecular size and similar concentrations among male and female populations. The fact that C is largely (>90%) bound to corticosteroid-binding globulin (CBG) in blood²⁹ can still influence interpretation. For example, different relationships emerged in adults at the CBG saturation threshold (~500 nmol/L), possibly due to a greater relative rise in blood-free C above this point.^{11,12,31} We retested our data with an exponential model to examine this inflection point (as 34% of the TC values were >500 nmol/L), but found no improvements on the linear models applied. This suggests that a younger athletic cohort might possess a different saturation threshold and that the results for adults may not necessarily apply to this population. Nevertheless, salivary C appears to be a valid biomarker for tracking individual TC concentrations in both sexes.

We do acknowledge some of the shortcomings of this work. The saliva results were referenced against total hormones in blood, though it does provide a reservoir for the free hormone. The unbalanced group design is another limitation, but unavoidable given

the smaller pool of female athletes available. From an analytical viewpoint, commercial immunoassays developed for saliva (eg, IBL, Salimetrics, DRG) also tend to inflate T and C values,^{32,33} with the operational range of different assay kits adding to these complexities. However, conversion guidelines are now available, at least for salivary C, when common assays (eg, IBL, DRG, Salimetrics, DSL, DELFIA)³³ are used by researchers, practitioners or clinicians. Alternatively, one could focus on percent changes or differences in salivary T and C, once these measures have been validated in the population and situation of interest.

In conclusion, the blood TT and TC concentrations of young weightlifters showed a predictable rise across a short, high-intensity competition. No hormonal changes in saliva (paired samples) emerged under these stressful conditions, which could be due to individual variability and blood-saliva differences in hormone kinetics. Despite this, the salivary measures showed a moderate to strong ability to track individual blood TT (males only) and TC (both sexes) concentrations in a young cohort across a broad physiological range.

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