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PII: S1440-2440(17)30447-4
DOI: http://dx.doi.org/doi:10.1016/j.jsams.2017.05.021
Reference: JSAMS 1539

To appear in: *Journal of Science and Medicine in Sport*

Received date: 12-1-2017
Revised date: 31-3-2017
Accepted date: 24-5-2017

Please cite this article as: Crewther Blair T, Potts Neil, Kilduff Liam P, Drawer Scott, Cook Christian J.Can salivary testosterone and cortisol reactivity to a mid-week stress test discriminate a match outcome during international rugby union competition?.*Journal of Science and Medicine in Sport* http://dx.doi.org/10.1016/j.jsams.2017.05.021

Title: Can salivary testosterone and cortisol reactivity to a mid-week stress test discriminate a match outcome during international rugby union competition?

Short title: Stress-test predictions in rugby competition

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Abstract

Objectives: Evidence suggests that stress-induced changes in testosterone and cortisol are related to future competitive behaviours and team-sport outcomes. Therefore, we examined whether salivary testosterone and cortisol reactivity to a mid-week stress test can discriminate a match outcome in international rugby union competition.

Design: Single group, quasi-experimental design with repeated measures.

Method: Thirty-three male rugby players completed a standardised stress test three or four days before seven international matches. Stress testing involved seven minutes of shuttle runs (2 × 20 m), dispersed across one-minute stages with increasing speeds. Salivary testosterone and cortisol were measured in the morning, along with delta changes from morning to pre-test (Morn-PreΔ) and pre-test to post-test (Pre-PostΔ). Data were compared across wins (n = 3) and losses (n = 4).

Results: The Morn-PreΔ in cortisol increased before winning and decreased prior to losing ($p<0.001$), with a large effect size difference ($d = 1.6$, 90% CI 1.3-1.9). Testosterone decreased significantly across the same period, irrespective of the match outcome. The Morn-PreΔ in testosterone and cortisol, plus the Pre-PostΔ in testosterone, all predicted a match outcome ($p\leq0.01$). The final model showed good diagnostic accuracy (72%) with cortisol as the main contributor.

Conclusions: The salivary testosterone and cortisol responses to mid-week testing showed an ability to discriminate a rugby match outcome over a limited number of games. The Morn-PreΔ in cortisol was the strongest diagnostic biomarker. This model may provide a unique format to assess team readiness or recovery between competitions, especially with the emergence of rapid hormonal testing.

Key Words

Sport; Behaviour; Readiness; Training; Neuroendocrine

Introduction

Considerable research has examined the role of testosterone and cortisol in mediating athletic performance during competition,[1-4] with behavioural mechanisms often predominating. Evidence now suggests that acute hormonal changes can help explain future competitive behaviours. For instance, testosterone responsiveness to non-physical competition predicted motivation and willingness to engage in future events,[5, 6] as well as risk-taking behaviours.[7] The cortisol response to a stressor might also predict resilience to future stressors,[8] mental toughness in sport,[9] and adaptability to training stress.[10] Hence, stress-induced hormone reactivity might be informative in terms of better understanding a competitive outcome, particularly in those sports where the expression of dominant behaviours and resilience are key indicators of success.

Previous work has identified a link between the hormonal responses to training sessions and subsequent outcomes in team-sport competition. As an example, larger testosterone and/or lower cortisol responses to a mid-week training session was found three or four days before winning (vs. losing) in professional rugby union[11] and rugby league matches.[12] Elevated testosterone responses to a mid-week training session also corresponded to better match rankings in professional rugby union competition.[13] Similarly, among freshman college football players, the cortisol changes across a summer testing session contributed to a model that predicted their contribution to on-field performance evaluated post-season.[9] Therefore, monitoring the responses of these hormones across a mid-week stress test could have some value in projecting a better team outcome later in the week.

The above studies are limited by several methodological issues. First, the training sessions employed were not always uniform to enable effective weekly comparisons. Second, no morning measures were taken to better reference the stress-induced changes, which in turn might aid in the construction of more accurate explanatory models. Third, the data were not modelled to account for repeated testing on some athlete cohorts. Finally, these models have yet to be assessed in the international sporting arena to determine if the results derived from professional or recreational athletes are applicable at the

highest level of competitive sport. Addressing these issues would add new insight regarding stress physiology, with novel applications that go beyond traditional monitoring to include the early assessment of readiness to compete.

This study examined whether the stress-test responses of salivary testosterone and cortisol can be used to discriminate a match outcome during subsequent international rugby union competition. A squad of male rugby players completed a standardised physical test several days before seven international matches. After pooling data across wins and losses, we hypothesised that the players would exhibit a more positive stress-test response (i.e., larger testosterone increase, smaller cortisol increase) prior to winning, relative to losing. We also explored the possibility of combining hormonal measures (i.e., morning concentration, pre- and post-stress change) to construct a stronger explanatory model.

Methods

Forty professional male rugby union players were recruited for this study, as part of a training squad preparing for the 2010 International Autumn Series and the Six Nations Championship in 2011. Only those athletes playing at least one competitive match (n = 33) were included in the final analysis. The cohort had a mean (±SD) age, height, and body mass of 27.9 ± 3.2 years, 1.87 ± 0.09 m and 101.8 ± 13.3 kg, respectively. The participants were healthy and without any injuries or medical problems that would limit their ability to complete this study. The players did not report taking any medications, drugs or doping agents, and they were routinely tested in this capacity. Each player received a full explanation of the protocols and signed an informed consent form. The Ethics Committee at Swansea University provided ethical approval (Number 2010.001R).

A single group, quasi-experimental design with repeated measures was employed. A standardised stress test was completed either three or four days before seven international rugby union matches. The stressor involved a modified version of the Yo-Yo intermittent recovery test.[14] This exercise was chosen due to ease of implementation and likely compliance, whilst also being relatively stressful and with some specificity to the running demands of rugby union. Salivary testosterone and cortisol were

monitored in the morning, and in the afternoon before and after stress testing. Our analyses focused on three testosterone and cortisol measures; morning concentration, plus the delta change from morning to pre-test (Morn-PreΔ) and from pre-test to post-test (Pre-PostΔ). To assess the discriminative ability of these hormonal measures, data were pooled across those matches won and lost for comparisons. The match outcomes were further indexed by ratings of team performance and perceived exertion.

This study was conducted over a seven-week period spanning two competitive phases. In phase one, three matches were played across the Autumn International series (weeks 1, 2 and 3). In phase two, five matches were played during the Six Nations Championship, but only four were monitored (weeks 13, 14, 18 and 19). Five games were played at home and two away, producing three wins and four losses for this team. Their world ranking fluctuated between six and nine, whilst their opponents were rated between one and 12. The players entered a training camp environment before each match, at which time they trained four days a week (1-3 sessions per day).

Stress testing was completed three to four days before each match[11-13] and at least three days after a prior contest to ensure recovery of the physiological systems.[15-17] Testing was conducted indoors at a similar time of day (3:00 pm to 5:00 pm) to account for circadian variation.[3, 17] The test involved repeated shuttle runs (2 × 20 m) dispersed across seven one-minute stages; 1 (1 shuttle at 10 km/hr), 2 (1 shuttle at 11.5 km/hr), 3 (2 shuttles at 13 km/hr), 4 (3 shuttles at 13.5 km/hr), 5 (4 shuttles at 14 km/hr), 6 (8 shuttles at 14.5 km/hr) and 7 (8 shuttles at 15 km/hr). A sound cue from a CD player indicated the start and finish of each stage. As a sub-maximal and speed-controlled assessment, exercise reliability was not deemed an issue for elite athletes. To improve adherence, each stress test was used as a warm-up for other training sessions. A skill-based session was conducted earlier in the day, but we anticipated little to no influence as exercise intensity was very low (RPE<4) and the athletes had at least four hours to recover. This session was kept uniform across the experiment.

The players provided a saliva sample before breakfast (8:00 am) to assess morning hormones. This sample was taken around 30-45 minutes after waking. In the afternoon (>7 hours later), saliva samples were taken five minutes before and 10 minutes after stress testing to coincide with expected hormonal changes.[11] The time between samples was ~22 minutes. No food was taken 90 minutes beforehand. All samples were collected by passive drool and stored frozen at -80°C. After thawing and centrifugation, the samples were assayed in duplicate using commercial immunoassay kits (Salimetrics LLC, USA). The testosterone and cortisol plates had a lower range of 6.1 pg/ml and 0.12 ng/ml, respectively, with inter-assay coefficients of variation (CV) from 5.2% to 10.1%. To eliminate inter-assay variance, each athlete's samples were run within the same assay.

The players provided a rating of perceived exertion (RPE) on a Likert scale (from 0 = very low up to 10 = maximal effort), within five minutes of completing the stress test.[18, 19]

Twenty-two players (15 starters and 7 reserves) were selected for each match. Across the entire study, they played an average of 4.7 ± 2.1 games (range 1 to 7). All of these players provided a mid-week saliva sample in the morning and most (n = 17 to 22) completed the stress test corresponding to their weekly match selection. The match-day routine was consistent for all players and involved an early morning breakfast (8:00 to 9:00 am) before travelling to the contest venue. The team had a further meeting at the venue (<2 hours) and changed into their uniforms, followed by a standard warm-up on the pitch (<1 hour) and a final briefing (<15 minutes) before match kick-off.

In addition to the win-loss outcomes, player performance was appraised by three team coaches on a Likert scale (from 1 = extremely poor up to 10 = excellent) and aggregated to provide a coach rating of performance. These scores were based on key indicators such as handling errors, turnovers, line breaks, points scored, and penalties conceded. Performance ratings were also taken from the players themselves and they provided a RPE, using the above guidelines. These data were collected the next

morning to provide time to reflect upon the match just played. Given the importance of substitutions in rugby, we also monitored whether players were starters or non-starters and actual playing time.

Data were examined using a generalized estimation equation (GEE) with an auto-regressive correlational structure.[20] The GEE provides a semi-parametric approach to longitudinal data analysis using quasi-likelihood estimation, allowing for non-normal response variables, plus unbalanced and incomplete datasets.[21] Main effects were determined by testing of the Wald chi-square statistic. The match performance and stress-test hormonal measures (plus RPE) were compared across wins and losses. Effect sizes were also computed using Cohen's *d* with 90% confidence intervals (CI). Using a backwards stepwise approach, we employed a GEE logistic regression to identify the best stress-test predictors of match outcome. To avoid multicollinearity, morning testosterone and cortisol were excluded as predictors. A 10-fold cross-validation procedure was used to reduce model bias and model accuracy was evaluated using classification diagnostics. The significance level was set at $p \leq 0.05$. All analyses were performed with IBM SPSS Statistics 24 and Matlab R2016b.

Results

Relative to losing, winning was associated with higher performance scores from the coaches (5.58±1.59 vs. 6.02±0.94, p=0.005, d = 0.34, 90% CI 0.07 to 0.61) and players (5.33±1.49 vs. 6.14±1.48, p<0.001, d = 0.55, 90% CI 0.28 to 0.83), but with lower RPE (8.15±2.03 vs. 6.82±2.79, p<0.001, d = -0.54, 90% CI -0.82 to -0.27), respectively. These data were also modeled with player position, starting status, playing time, game venue, testing week, and opponent ranking entered separately as covariates. Covariate inclusion did not alter the initial differences identified (all p<0.01). Athlete playing time did not vary (p=0.415, d = 0.07, 90% CI -0.20 to 0.34) between wins (54.2±33.4 minutes) and losses (51.8±34.5 minutes), as this variable is more strictly controlled.

Morning testosterone and cortisol concentrations were lower before wins than losses (Table 1), but with small effect size differences (d = -0.24, 90% CI -0.51 to 0.03, d = -0.44, 90% CI -0.71 to -0.16).

A decline in the Morn-PreΔ in testosterone occurred before all matches won and lost (d = 0.17, 90% CI -0.12 to 0.46). No Pre-PostΔ in testosterone emerged and no win-loss differences (d = 0.18, 90% CI -0.11 to 0.46). The Morn-PreΔ in cortisol increased before winning and decreased prior to losing, with a large effect size difference (d = 1.6, 90% CI 1.27 to 1.93). We found no Pre-PostΔ in cortisol and no differences by match outcome (d = -0.30, 90% CI -0.59 to -0.01). The stress-test RPE scores were similar before all matches played (d = -0.23, 90% CI -0.52 to 0.05). To aid interpretation, the testosterone and cortisol values at each time point can be viewed in Figure 1A and 1B, respectively.

Insert Table 1 and Figure 1 (A and B) here.

The stress-test data were also adjusted for morning hormones, playing position, starting status, testing days before competition, testing week, and opponent ranking. The initial findings were stable with three exceptions. A non-significant win-loss difference in morning cortisol emerged when testing day (p=0.250, d = -0.21, 90% CI -0.48 to 0.06) and week (p=0.055, d = -0.34, 90% CI -0.62 to -0.07) were modeled, whilst the inclusion of player position resulted in lower RPE scores (p=0.039, d = -0.30, 90% CI -0.59 to -0.02) before wins (3.94±1.20) than losses (4.26±0.92).

Regression analyses identified three significant measures of winning and losing (Table 2); Morn-PreΔ in testosterone, Pre-PostΔ in testosterone, and Morn-PreΔ in cortisol. The final constructed model had a classification accuracy of 72.2% with the cortisol measure being the strongest contributor (~80%).

Insert Table 2 here.

Discussion

To our knowledge, this is the first study to employ a hormonal stress-test model to discriminate a rugby match outcome at the highest level of competitive sport. Winning was accompanied by higher appraisals of team performance and lower physical exertion versus losing. Partly supporting our initial

hypotheses, a large mid-week difference in the Morn-PreΔ in cortisol was observed before matches that were won (increasing) and lost (decreasing). The acute reactive measures of testosterone (Morn-PreΔ, Pre-PostΔ) and cortisol (Morn-PreΔ) also showed good diagnostic capabilities.

The main finding was a large mid-week rise in cortisol (Morn-PreΔ) prior to winning, whereas cortisol decreased before losing. The cortisol increase before exercise is not unexpected, as it prepares an individual for an impending challenge.[1, 22] Higher morning or pre-competition cortisol concentrations have preceded better outcomes in sport (e.g. winning, higher rankings, more weight lifted),[3, 4, 23] but little research has described this linkage on the same timescale and across multiple contests. A study on rugby players did report a negative correlation between the cortisol changes (%) across a mid-week training session and pre-game testosterone,[11] which in turn might influence performance. However, correlations by match outcome were not reported, nor did they factor in repeated measures on each player. The cortisol awakening response is another consideration, as it peaks at 30 to 45 minutes after waking,[24] coinciding with the morning samples. Our pre-test samples were taken more than seven hours later, so this response is unlikely to affect our results. The salivary cortisol values lie within the physiological range for male rugby players during training[11, 15 25] and competition.[17, 26]

The mid-week testosterone patterns were similar before winning and losing, either decreasing (Morn-PreΔ) or showing no change (Pre-PreΔ). Studies reporting a link between testosterone reactivity and competitive performance did employ longer exercising protocols (i.e., 20-60 minutes);[11-13] thus, a more sustained exercise stimulus might be needed to realise this association. Notably, the same work was conducted on professional club teams, so differences in team culture, management strategies, and game demands between the club and international environments could be equally important in explaining the poor discriminative ability of the testosterone measures. As such, one team may benefit more from monitoring testosterone and another from cortisol testing. Other psychological and environmental factors can also influence testosterone dynamics before a rugby competition (e.g.

motivation, knowledge of starting status, match importance)[17, 26, 27] and conceivably our mid-week results. Like cortisol, the salivary testosterone values are consistent with rugby literature.[2, 17, 25, 26]

Although no significant differences in testosterone were identified by rugby match outcome, we were able to classify a win or loss with 72% accuracy by combining the reactive measures of both testosterone and cortisol. This model could be used to make informed training and management decisions to increase the likelihood of team success, especially with the emergence of rapid hormonal testing. For example, comparing a stress-test result with reference data might help establish a readiness or recovery score to guide the training week, possibly including preconditioning strategies (e.g. videos, coach feedback, priming exercise) to optimise performance.[28] There are still some considerations, as these projections were achieved over a limited number of games. Furthermore, this model was validated using the same dataset, but this was partly offset by a more conservative estimate (i.e., 80.7% accuracy without validation). Given the current design, we are also unable to determine if the observed mid-week patterns are transient in nature or a temporal response to accumulating inputs.

The Morn-PreΔ in cortisol was the strongest predictive biomarker under the current format. As a glucocorticoid, cortisol exerts actions that can modulate an organism's response to a subsequent stressor.[22] Along these lines, a larger cortisol response under challenge was associated with greater resilience to future stressors in real life[8] and competitive weightlifting (unpublished data). Cortisol is connected to other psychological features (e.g. appraisal, anxiety, mood, coping, dominance) that are conceptually relevant to resilience and vulnerability to stress.[1, 29] As such, the observed mid-week rise in cortisol before winning could indicate a stronger capacity to prepare for, and cope with, other sporting stressors within a short timeframe. Other cortisol effects require consideration within the sport of rugby, as post-match adrenal activity is imbedded in the immune response, tissue damage and inflammation,[15, 16] thereby potentially influencing player recovery and ensuing performance.

This work must be interpreted in light of some limitations. First, we had no control over team selections and training each week, though we anticipated that the physical activities would be consistent for all players within the training camp environment. Second, the pooling of data does not tell us about the manner in which the games were won or lost (e.g. a referring error, team strategies), nor the influence of environmental (e.g. pitch conditions, weather), and psychological factors (e.g. prior success against each opponent, match expectations). Third, we did not examine more complex positional × hormonal interactions or dual interactions the testosterone and cortisol measures. Some of these issues can be addressed within the professional club rugby environment, where seasonal games can reach 25 or more. It would also provide scope to include other routine measures (e.g. heart rate, speed and power, sleep hours) to develop a more global index of team readiness and recovery.

Conclusion

The salivary testosterone and cortisol responses to mid-week stress testing were associated with the outcomes of international rugby union matches over a limited number of games. The Morn-PreΔ in cortisol provided the strongest biomarker for discriminating wins from losses. For researchers and practitioners, being able to access this information via rapid hormonal testing would provide a unique format to assess team readiness or recovery status between contests.

Practical Implications

- Monitoring mid-week changes in salivary testosterone and cortisol concentrations before and after a physical test could indicate if a team is ready to compete several days later, particularly if reference data is available to signify an optimal or sub-optimal profile for performing.
- Cortisol provided the strongest diagnostic biomarker in an international rugby union environment, but it would be useful to assess testosterone and cortisol in tandem to account for possible environmental and cultural factors between different rugby teams.
- A short (<10 minute), intermittent running test provided an effective stressor for assessing these hormonal changes and could be implemented as a standalone test or as part of a wider testing

battery. Other exercises (e.g. tackle and scrum drills) could be used to improve sport or athlete specificity, once standardised for this role.

Acknowledgements

This project was supported by grants from the UK Engineering and Physical Sciences Research Council and the UK Sports Council, as part of the Elite Sport Performance Research in Training with Pervasive Sensing Programme [EP/H009744/1], and the Scottish Rugby Union.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Figure legend

Figure 1. Estimated marginal means (±SD) for salivary testosterone (1A) and cortisol (1B) concentrations at each time point.

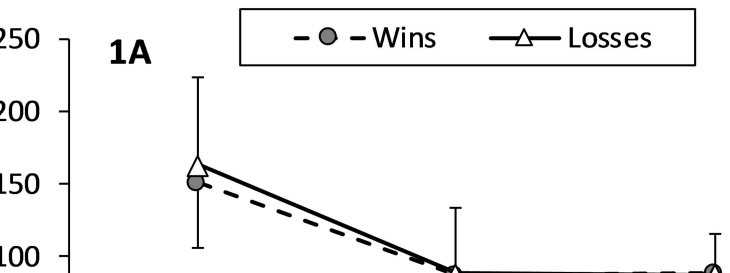
Table 1. Estimated marginal means (±SD) for the stress-test hormonal and perceived exertion measures before International rugby union matches won and lost.

Variable	Sample	Wins	Losses	*p value*
Testosterone (pg/ml)	Morning	150±44.7	162±56.0	0.010
	Morn-PreΔ	-57.7±45.1	-65.7±48.5	0.241
	p value	<0.001	<0.001	
	Pre-PostΔ	2.7±29.9	-2.4±28.1	0.215
	p value	0.498	0.431	
Cortisol (ng/ml)	Morning	2.97±1.50	3.61±1.47	0.014
	Morn-PreΔ	1.30±2.53	-1.93±1.32	<0.001
	p value	<0.001	<0.001	
	Pre-PostΔ	-0.54±2.26	-0.05±0.53	0.119
	p value	0.071	0.422	
RPE (score)	Post	3.94±1.54	4.26±1.22	0.058

Key: RPE = rating of perceived exertion.

Table 2. Parameter estimates predicting a match outcome from the stress-test hormonal measures.

Variable	B	SE	*p value*
Intercept	1.146	0.3990	0.004
Testosterone Morn-PreΔ	0.017	0.0055	0.001
Testosterone Pre-PostΔ	0.025	0.0098	0.010
Cortisol Morn-PreΔ	0.896	0.1752	<0.001


1A
Wins
Losses
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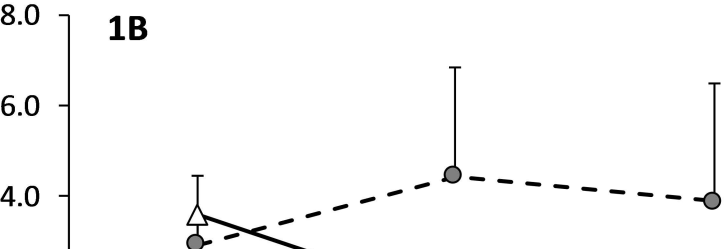
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