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**Title Page**

**Title:** Basal and stress-induced salivary testosterone variation across the menstrual cycle and linkage to motivation and muscle power

**Header:** Testosterone variation, motivation and power

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**Abstract**

This study investigated salivary testosterone (sal-T) variation across the menstrual cycle in female athletes, at different competitive levels, and its association with motivation and neuromuscular power. Six elite and 16 non-elite female athletes were monitored on days 7 (D7), 14 (D14) and 21 (D21) across three menstrual cycles for basal sal-T concentrations and self-appraised motivation to train and compete. Two further measures were taken on D7, D14 and D21 across two menstrual cycles; (1) the sal-T response (delta change) to a physical stress test and (2) peak power (PP) response to a 6-sec cycle sprint following a post-activation potentiation (PAP) stimulus. Basal sal-T concentrations increased by  $17\pm 27\%$  from D7 to D14 before decreasing by  $-25\pm 43\%$  on D21 ( $p<0.05$ ), but this result was biased by elite females with higher sal-T ( $>102\%$ ) who showed larger menstrual changes. Motivation, sal-T reactivity to stress and the PP responses to a PAP stimulus also varied by testing day ( $p<0.05$ ), in parallel to basal sal-T and in favour of the elite group. Furthermore, stronger within-subject relationships ( $p<0.001$ ) between basal sal-T and motivation emerged in the elites ( $r = 0.70-0.75$ ) versus the non-elites ( $r = 0.41-0.50$ ). In conclusion, menstrual cycle changes in sal-T were more obvious in high-performing female athletes with higher sal-T concentrations. This was accompanied by greater training motivation, a more pronounced sal-T response to a physical stressor and greater neuromuscular power in the elite group. These results support observations that female athletes with higher T are more represented at elite levels of performance.

**Key words:** Anabolic; Androgens; Trainability; Recovery; Adaptation

## Introduction

There are reports of elite female athletes, particularly those involved in power-speed sports, demonstrating elevated free testosterone (T) levels compared to non-elite women.<sup>1,2</sup> This was first noted across a mixed-athlete cohort when the pooled salivary T (sal-T) profiles of elite (87 pg/ml) and non-elite (41 pg/ml) groups were evaluated.<sup>1</sup> Likewise, females participating in speed-based sports (e.g. sprinting) have presented much higher blood T concentrations than individuals from other (e.g. volleyball) sporting disciplines.<sup>3</sup> The mechanisms underlying this T variance and its influence on performance are not entirely clear; however, some have hypothesised that T supports motivated behaviours, such as aggressiveness, risk taking and self-efficacy.<sup>4-6</sup> Others have linked higher sal-T to increased voluntary training load choice among female athletes,<sup>7</sup> as a proxy for motivational drive.

The past decade has seen considerable interest in how T changes across female athletic competition, often with a focus on theoretical models of gaining and maintaining social status.<sup>8-10</sup> Acute physiological changes in T might also have functional relevance. For instance, a rise in female T prior to competition was linked to subsequent playing abilities<sup>11</sup> and competitive behaviours.<sup>4</sup> Across physical exercise or similar competitive “stressors”, both sexes display T increases<sup>11-14</sup> and the male T response shows some ability to predict subsequent performance,<sup>13</sup> particularly when the moderating effects of cortisol (C) are considered.<sup>12</sup> The induction of a T response using exercise is further interesting in that, when allowing for fatigue recovery, it may have a priming effect for further activity later in the same day,<sup>13,15</sup> and it associates with less circadian decline in T across the day.<sup>15</sup>

A specific example of short-term priming using an exercise stressor is post-activation potentiation (PAP). The PAP phenomenon refers to an excited neuromuscular state of skeletal

muscle following a prior stimulus.<sup>16</sup> The induction of a PAP response has been seen in both weight-bearing (e.g. sprint running) and non-weight-bearing (e.g. sprint cycling) examples in sport.<sup>16-18</sup> On a mechanistic level, an increase in T can modify intracellular calcium release within muscle cells,<sup>19</sup> as one possible contributor to PAP.<sup>16</sup> There is further speculation that T and C reactivity might work together to ensure that other potentiating mechanisms (e.g. myosin phosphorylation, motor unit recruitment) are activated through prior exercise.<sup>12</sup> It is also possible that performance during short-term intense exercise, which arguably requires a high level of motivation, is partly dependent upon T availability.

Overlaying these outcomes in adult females is the menstrual cycle. It has been debated by some<sup>20,21</sup> that the T variation across this cycle is small compared to inter-day and circadian changes and thus, can be largely ignored. Others however have reported larger T fluctuations at different stages of the menstrual cycle, particularly an elevation in T during the late follicular phase and at ovulation.<sup>22,23</sup> In healthy (non-athletic) women, this variation maps onto reported changes in competitive behaviours and preferences related to perceived social status, and perception and preference of facial features around the time of ovulation.<sup>24,25</sup> To our knowledge, there is no corresponding data on motivation to train or compete among female athletes. Addressing this issue would provide insight into the unique nature of the female athlete and the influence of normal T variation across the menstrual cycle.

The current study monitored female athletes participating at different competitive levels (elites, non-elites) on three different days (7, 14 and 21) across their menstrual cycle. The primary outcomes were changes in basal sal-T concentrations and motivational state, the sal-T response to a short physical stressor and cycling peak power (PP) following a PAP stimulus. The main aims were to determine if: (1) the basal concentrations of sal-T vary across the

menstrual cycle; (2) self-reported motivation to train and compete parallel the changes in basal T concentrations; (3) the sal-T response to a set physical stressor and cycling power after a PAP stimulus also differ across the menstrual cycle; (4) the magnitude of change in any outcome differs between elite and non-elite female athletes.

## **Materials and Methods**

### *Participants*

Twenty-two healthy female athletes were recruited with a mean ( $\pm$ SD) age, height, and body mass of  $21.0\pm 1.1$  years,  $1.69\pm 0.05$  m and  $66.9\pm 4.7$  kg, respectively. These athletes were currently competing in one or more of four sports (i.e. figure shaping  $n = 6$ , netball  $n = 8$ , soccer  $n = 5$ , triathlon  $n = 3$ ). On average, they reported training 2-5 days a week involving a mixture of general and sport-specific sessions, including some endurance components. Each athlete also reported a regular resistance-training history of at least two years before this study. Of the 22 athletes recruited, six were competing at a national level (i.e. elites), 13 at a club level and three at recreational events (i.e. non-elites). The participants were healthy and without any injuries or medical problems that would limit their ability to complete this study. They did not report taking any medications, drugs or doping agents, and all had been hormonal-based contraceptive free for a minimum of six months. The T values for each athlete were also within the normal physiological range for healthy women (40-300 pmol/L),<sup>26</sup> further suggesting that no anabolic doping agents were taken. Each female received a full explanation of the protocols and signed an informed consent form before study commencement. A university ethics committee provided ethical approval.

### *Study design*

A two-group, longitudinal study design involving a combination of observational, quasi-experimental and experimental testing was employed. The athletes self-collected saliva samples in the morning (within 15 min after rising and before breakfast) on days 7 (D7), 14 (D14) and 21 (D21) across three consecutive menstrual cycles to assess basal sal-T concentrations. Day 1 was the self-reported start of menses, recorded 30 minutes after awakening. A short motivational questionnaire was completed after saliva collection. On two occasions per menstrual cycle day (D7, D14 and D21), the athletes also performed a short physical stress test in the morning (0900 hours) to assess sal-T reactivity and a strength-based PAP stimulus in the afternoon (1500 hours) to examine changes in cycling PP. All menstrual cycle repeats were randomised. The study protocols and variables are outlined in Table 1. The subjects were familiarised with all procedures across two sessions and their 90% one repetition maximum (1RM) load for the PAP exercise (leg press) was calculated.

Insert Table 1 here.

### *Salivary hormones*

Each athlete was provided with labelled tubes with written instructions for the self-collection of saliva at home. The samples were collected using a passive drool method without stimulation and stored in a commercial freezer (-20°C) for no more than two days, before transference to a -80°C freezer for long-term storage. The stress-test samples were taken by an experienced technician in the laboratory. All samples were assayed in duplicate using a commercial immunoassay kit (Salimetrics LLC, USA), after an initial process of thawing and centrifugation (2000 g × 10 minutes). The sal-T plate had a lower detection range of 6.0 pg/ml with inter-assay coefficients of variation below 10%. To eliminate inter-assay variance, each athlete's samples were tested in the same plate. Salivary T correlates ( $r=0.71$ ,  $r^2=0.50$ ,

$p < 0.001$ ) with blood-free T measurements among women,<sup>27,28</sup> as we found during the pilot testing of a young athletic cohort ( $r = 0.69$ ,  $r^2 = 0.48$ ,  $p < 0.001$ ). These data verify the use of sal-T, although the measured values are not directly comparable to blood.<sup>28</sup>

### *Motivational testing*

Immediately after saliva collection, brief measurements of motivation to train and compete were recorded in a training diary. Motivation is a complex construct exhibiting elements of task, situational and environmental specificity.<sup>29</sup> To capture some of these elements, each athlete was instructed to rate their current motivational state on a Likert scale, anchored from 1 (I have no motivation to train / I have no desire to compete) up to 7 (I am extremely motivated to train / I feel extremely competitive). Single-item behavioural ratings are often used to assess athletic populations in sport,<sup>30-32</sup> due to ease of application and interpretation with low intrusiveness, all of which lead to high compliance among athletes.

### *Physical stress-testing and PAP protocols*

Physical stress testing began with a 5-minute warm-up on an air-braked cycle ergometer (Wattbike Pro, UK), maintaining a minimum of 50 W. This was followed by three minutes of increasing intensity (to 125-150 W) and then a maximum 10-second effort, followed by recovery pedalling to make a total of 10 minutes duration. Saliva was collected five minutes before and after the test for T determination. The PAP assessment involved a standard 5-minute warm-up comprising of steady state cycling (on the Wattbike) at no less than 50 W, followed by a progressive series of  $3 \times 6$  s sprint trials. The athletes pedalled slowly for a minute between each trial and three minutes after the last trial. Two minutes after warm-up completion,  $2 \times 6$  s maximum sprints were completed with a 1-minute recovery. As a baseline reference, peak power (PP) output was assessed using proprietary software. After a further 20-



minute recovery, the subjects completed a PAP stimulus comprising of  $3 \times 3$  repetitions at 90% 1RM on a 45° leg press machine or the control trial, consisting of slow pedalling at a minimum of 50 W for the same time period. Following 15 minutes of recovery, both groups repeated the  $2 \times 6$  s cycle test. The PP recorded was the best across the two trials.

All testing was standardised in this study. Verbal encouragement was given, by the same research investigator, to ensure maximal effort during stress testing, PAP induction and cycle ergometry. Room temperature was maintained between 20 and 24 °C across all sessions and the athletes wore similar items of clothing, socks, and shoes. Consumption of water (500 ml) was allowed during the study procedures, but food and sports drinks were not permitted until after the final saliva sample was collected. The athletes were also permitted to consume food and water between the morning and afternoon tests, but they were asked to cease eating one hour before the afternoon assessment to remove the effect of food intake. In the interim period, they were also asked not to engage in any additional physical activity.

### *Statistical analyses*

The study data were analysed with a generalized estimating equation using an exchangeable correlational structure.<sup>33</sup> Before analysis, the sal-T and PP results were log-transformed to approximate normality and reduce non-uniformity bias, but the raw results are shown to aid interpretation. Data were collapsed across all three menstrual cycles after initial testing revealed no significant effect (of each repeat) on any variable. To assess the impact of cycle day and competitive level, we assessed basal sal-T and each motivational measure with testing day (D7, D14, D21) and competitive status (Elite, Non-elite) as factors, plus their interaction. The same approach was used to examine stress-induced T reactivity, based on the delta changes (post – pre) in sal-T. Delta changes in PP were also computed and analysed with

treatment (Control, PAP), testing day and competitive status as factors, along with selected interactions. The change scores were compared to a zero baseline using paired T-tests. Where appropriate, post-hoc contrasts were conducted with the sequential Bonferroni test. Finally, we investigated the within-subject relationships ( $r$  values with 95% confidence intervals) between the sal-T, motivational and PP measures using the R package *rmcorr*.<sup>34</sup> All other data were analysed with IBM SPSS 24 with significance set at  $p < 0.05$ .

## Results

### *Demographic profiles of the elite and non-elite female athletes*

At study commencement, the elite and non-elite females were of similar age ( $21.0 \pm 1.1$  years;  $21.1 \pm 1.2$  years), height ( $1.70 \pm 0.06$  metres;  $1.69 \pm 0.05$  metres) and weight ( $67.5 \pm 6.5$  kg;  $66.8 \pm 4.3$  kg), respectively (all  $p > 0.515$ ). Athlete strength levels did vary, with 90% 1RM leg press loads ranging from 220 kg to 410 kg. Individual 90% 1RM was not related ( $p = 0.489$ ) to basal T during the first week of testing. Menstrual cycle length was normal across all athletes (24 to 33 days) with a mean of  $28.7 \pm 1.9$  days. The length of each menstrual cycle was not related to basal sal-T on any testing day Spearman  $r = -0.32$  to  $0.16$ ,  $p > 0.201$ ).

### *Basal sal-T concentrations*

A main effect of testing day ( $p < 0.001$ ) and competitive status ( $p < 0.001$ ) emerged when examining basal sal-T concentrations. The testing day  $\times$  competitive status interaction was also significant ( $p < 0.001$ ). Post hoc testing (Figure 1) identified a significant rise in sal-T ( $42 \pm 27\%$ ) among the elites from D7 to D14, before falling at D21 by  $-26 \pm 30\%$  and  $-48 \pm 38\%$  from these respective days ( $p < 0.001$ ). In the non-elite group, sal-T increased slightly by  $9 \pm 22\%$  from D7 to D14 ( $p = 0.022$ ), before declining by  $-14 \pm 29\%$  at D21 ( $p < 0.01$ ). The elites had higher sal-T concentrations than the non-elites at all time points ( $p < 0.01$ ).

Insert Figure 1.

### *Motivational state*

In relation to training motivation, we found an effect of testing day and a day  $\times$  status interaction ( $p < 0.001$ ). Both athlete groups displayed higher ( $p < 0.01$ ) motivation to train on D14 versus D7 and D21 (Figure 2). The elites on D14 also reported higher motivation than the non-elites at all time points ( $p < 0.05$ ), whilst the non-elites on D14 had higher scores than the elites on D21 ( $p < 0.01$ ). A similar menstrual pattern was observed for motivation to compete ( $p < 0.001$ ); increasing in all athletes from D7 ( $3.8 \pm 1.0$ ) to D14 ( $5.0 \pm 1.3$ ), before decreasing below both days at D21 ( $3.3 \pm 0.9$ ) ( $p < 0.05$ ). Overall, the elites reported higher ( $p = 0.016$ ) motivation to compete ( $4.4 \pm 1.6$ ) than their non-elite counterparts ( $3.7 \pm 0.9$ ).

Insert Figure 2 here.

### *Sal-T responses to the physical stress test*

Preliminary analysis of pre-test sal-T revealed similar trends to the basal results; sal-T was higher on D14 ( $35.8 \pm 23.9$  pg/ml) versus D7 ( $26.3 \pm 13.4$  pg/ml) and D21 ( $20.5 \pm 6.7$  pg/ml), with a further difference between the elites ( $36.9 \pm 24.1$  pg/ml) and non-elites ( $18.2 \pm 9.9$  pg/ml) ( $p < 0.05$ ). Stress testing promoted a positive change in sal-T on D7, D14 and D21 in the elite ( $8 \pm 6\%$ ,  $16 \pm 6\%$ ,  $6 \pm 5\%$ ) and non-elite ( $6 \pm 4\%$ ,  $7 \pm 6\%$ ,  $5 \pm 5\%$ ) groups, respectively (Figure 3).

When comparing the T responses, a main effect for testing day and competitive status emerged, along with a significant interaction (all  $p < 0.03$ ). Contrasting testing revealed that the elite sal-T response on D14 exceeded all other days in both groups ( $p < 0.01$ ).

Insert Figure 3 here.

### *Cycling PP responses to the PAP stimulus*

Power testing at baseline mirrored the sal-T results; PP on D14 ( $383\pm 166$  W) exceeded the D7 ( $371\pm 169$  W) and D21 ( $369\pm 161$  W) results with elites also producing more PP ( $432\pm 280$  W) than non-elites ( $317\pm 170$  W) (all  $p<0.014$ ). In all PAP trials, positive changes in PP (from 3-7%) occurred (Figure 4,  $p<0.01$ ), whereas the control treatments had no significant effect. When comparing the PP responses, we identified a main effect by treatment, testing day and competitive status ( $p<0.003$ ). The first interaction of interest (treatment  $\times$  testing day) was also significant. Further testing showed that all PAP results were significantly different from control data, with the largest increase in PP on D14 ( $p<0.001$ ).

Insert Figure 4 here.

The second interaction of interest (treatment  $\times$  competitive status) was marginally significant ( $p=0.051$ ). Contrast analyses (Figure 5) revealed no changes in PP under the control condition in both athlete groups ( $0\pm 3\%$ ,  $p>0.648$ ). Conversely, the PAP treatment elicited a positive PP response for the elite ( $7\pm 4\%$ ) and non-elite ( $4\pm 4\%$ ) athletes ( $p<0.001$ ). The observed changes in PP (both groups) differed from the control treatments ( $p<0.001$ ), and the elite group were more responsive to the PAP protocols than the non-elites ( $p=0.015$ ).

Insert Figure 5 here.

#### *Relationships between sal-T, motivation and cycling PP*

Basal sal-T was positively related ( $p<0.001$ ) to both measures of motivation among elite and non-elite women, but these associations were stronger in the former group (Table 2).

Additional relationships ( $p<0.014$ ) were identified in the non-elite group, linking pre-test sal-T and the sal-T changes to the corresponding measures of cycling PP, but these were weak

associations. It is also important to note that, apart from basal sal-T and motivation, the paired variables were all collected at different times of the day (see Table 1).

Insert Table 2 here.

## **Discussion**

This study investigated the physiological variance in sal-T concentrations, both basal and in response to a set physical stressor, at three different time points across the female menstrual cycle. The effectiveness of a standard PAP stimulus on PP during cycle testing was also examined. Overall, we found that most parameters (i.e. basal sal-T, motivation, sal-T responses to stress, PAP-induced PP) peaked on D14 of the menstrual cycle compared to D7 and D21, though many of our findings were influenced by competitive status in sport.

Across all athletes, basal sal-T concentrations rose from D7 to D14 of the menstrual cycle before declining on D21. This change was biased by elite females who not only possessed higher (>102%) waking and early morning sal-T than non-elite women, as others have reported,<sup>1</sup> but also showed greater T variation. These differences must be interpreted carefully, due to large sal-T variation within the elite group (Figure 1) that could reflect other genetic, behavioural or training factors.<sup>1,2,32</sup> The two groups did not differ in age, height and weight, nor did they present any irregularities in menstrual cycle duration. Whilst studies support menstrual rises in T, particularly near late follicular and early ovulation,<sup>22,23,35</sup> there is contention as the interpretation of this. Some have reported little or no T variation that is meaningless against a background of daily change.<sup>20,21</sup> Others have introduced the concept of a “critical difference”,<sup>36</sup> whereby a sal-T difference must exceed daily variation and other sources of error to claim a biological effect. While this concept is somewhat contentious,

given that daily T changes in themselves may be of some biological value,<sup>7,32,37</sup> it is noteworthy that the sal-T variation exhibited by the elite women across the menstrual cycle exceeded the critical difference of 90% suggested for healthy young men.<sup>36</sup>

Two simple questions relating to one's motivation to train and compete were taken. Both were consistently higher in the elite group with elevated sal-T and both increased towards D14 (from D7) before decreasing on D21. Coinciding with our results, women often exhibit more interest in keeping resources, a higher libido, more energy and competitiveness, as they progress towards ovulation (days 13-15).<sup>25,38</sup> Conversely, female competitiveness can decline during the follicular (days 6-12) and luteal (days 16-23) phases,<sup>39</sup> but seldom do we see corresponding changes in aggressiveness. More typically, it is the behaviour of assertiveness, the quality of being self-assured and confident without resorting to more aggressive stances. On an individual level, the fluctuations in basal sal-T were also positively related to both motivational outputs, but with elite women presenting stronger associations, as seen in a recent study on male athletes.<sup>32</sup> Speculatively, this linkage might reflect the expression of more male-typical competitive behaviours among elite (vs. non-elite) women,<sup>40</sup> which might then influence T release to reinforce such behaviours and their competitive status. This is still a difficult area to interpret as T may be permissive to certain behaviours, but they are also highly individual with some situational and environmental dependency.

The physical stressor promoted a consistent rise (5-16%) in sal-T, with the largest change among elite females at ovulation (D14). Like basal sal-T, heterogeneity in the T responses to stress were also evident (Figure 3). Testosterone responsiveness to a physical stressor has been well documented in both males<sup>12-14</sup> and females.<sup>11,14</sup> In males, it is somewhat predictive of subsequent performance,<sup>12,13,15</sup> but it has not been demonstrated if T reactivity can predict female performance. The sal-T measures around the morning stress test (non-elites only) were

positively related to baseline PP and/or the PP changes in the afternoon. While it is tempting to link performance directly to T, there are major caveats on doing so. First, these were weak associations and we did not assess if sal-T remained elevated before PAP testing some six hours later. In males C is another, but not the only, essential confounder with moderation effects demonstrated<sup>12</sup> and, in some cases, C may be a better predictor of performance than T.<sup>41-44</sup> Cortisol was not measured in this study due to experimental costs. In addition, both estradiol and progesterone will certainly have changed across the menstrual cycle,<sup>21-23,39</sup> and estrogens will likely mirror T with similar behavioural effects.<sup>25,39</sup>

Substantial interpretation of our results cannot be reliably made without a more comprehensive study on a larger sample of athletes. Nevertheless, it does appear that elite female athletes exhibit more marked changes in sal-T and its responsiveness to a physical stressor across the menstrual cycle. The same group was also more motivated to train, perhaps ensuring greater voluntary exertion and consistency in their efforts during exercise testing. While our numbers justify caution, it may be an observation that needs careful consideration in future studies, as ignoring it could produce difficulty in replication and considerably increased variability. Similarly, at the high end of speculation a more “favourable” response to a physical stressor may suggest that the late follicular to ovulatory period of the menstrual cycle is ideally primed for intense power – heavy load-type training. In fact, there is evidence that maximum force development is enhanced when strength training is conducted in the follicular phase, relative to luteal-phase training.<sup>35</sup>

Lastly, it is interesting to note that the PAP-induced changes in cycling PP varied across the menstrual cycle (highest on D14), in parallel to the sal-T and motivational measures. This is perhaps not surprising given its similarity to the set physical stressor, but nonetheless is

noteworthy in terms of acute PP enhancement from the same exercise stimulus. Testosterone might contribute to PAP via several neuromuscular mechanisms (e.g. intracellular calcium movement, corticospinal activity)<sup>19,45</sup> or as a motivational substrate for exercise<sup>6,7,32</sup> and this may, in part, account for our group trends or within-subject relationships. The finding of a larger PAP response among the elites is not unexpected, since the induction and utilisation of PAP tends to increase with a higher level of physical training.<sup>16</sup> Arguably, PAP could be viewed as a competitive task with a physical outcome and, if so, would suggest that there may be some menstrual cycle-related advantages to athletic competition. However, despite our results, an acute increase in cycling PP may have little transference to those complex physical movements that determine most competitive outcomes.

The current findings do need to be balanced against the complexity of undertaking this work on athletes, where issues pertaining to sample size, unbalanced groups and environmental confounds (i.e. training stressors, competition) exist. Some of these issues were partly offset by the repeated measures design, whereby up to 54 (elites) and 144 (non-elites) data points were collected per variable, and data collapsing for analysis. In addition, D7, D14 and D21 are somewhat arbitrary divisions and care must be taken to assign them as truly representative of follicular, ovulatory and luteal phases. Still, given the cycle length (~28 days) each day will likely have fallen within quite different hormonal phases and close to these natural divisions. Previous studies also indicate that hormone reactivity is influenced by situational and cultural factors<sup>44,46</sup> that are difficult to control in sport. Despite these limits, there are some important outcomes pertaining to how we view and understand female athletes and the advantages of understanding T variation across the menstrual cycle. One key feature is the presence of large sal-T differences across female populations and how they may affect both variability and repeatability of a related measure (e.g. muscle power, motivation).



## Perspective

In summary, the sal-T concentrations of athletic females varied across the menstrual cycle and most markedly in athletes performing at higher levels of competition, who in themselves displayed higher sal-T levels irrespective of menstrual cycle timing. Salivary T responsiveness to a physical stressor also showed menstrual cycle changes, as did the increase in PP following a PAP stimulus. It must be cautioned, again, that previous studies have suggested strong complexities in these types of hormonal responses that may limit them to the athletes, environment and testing situation under which they are obtained. In other words, they may simply be informative case studies. These intricacies provide progress to explain the conflicting reports of T involvement, not only in competitive performance in sport, but also in more general behavioural studies. A key aspect of this is awareness of large differences or changes in sal-T across female populations and how they may affect both variability and repeatability of associated measures (e.g. motivation to train, power output).

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**Table 1.** Overview of the study protocols and measured variables.

Repeat	Time of day	Menstrual cycle day	Measured
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testing		Day 7 (D7)	Day 14 (D14)	Day 21 (D21)	variables
1 <sup>st</sup> cycle*	Waking <sup>1</sup>	Morning	Morning	Morning	Sal-T, motivation
	0900 hours	Stress test	Stress test	Stress test	Pre and post Sal-T
	1500 hours	Cycle test PAP / control Cycle retest	Cycle test PAP / control Cycle retest	Cycle test PAP / control Cycle retest	Pre-treatment PP Post-treatment PP
2 <sup>nd</sup> cycle*	See above	Repeat as above	Repeat as above	Repeat as above	See above
3 <sup>rd</sup> cycle*	Waking <sup>1</sup>	Morning	Morning	Morning	Sal-T, motivation

Key: PAP = post-activation potentiation, PP = peak power, sal-T = salivary testosterone.

\*Menstrual cycle repeats were randomised, <sup>1</sup>Testing within 15 min of rising.

**Table 2.** Within-subject relationships between the salivary testosterone (sal-T) measures and the outcomes of motivation and cycling peak power (PP). Correlations are presented with 95% confidence intervals in brackets.

Measure	Response variable	Elite women	Non-elite women
Basal sal-T	Motivation to train	0.75 (0.60, 0.86)**	0.50 (0.35, 0.62)**
	Motivation to compete	0.70 (0.52, 0.82)**	0.41 (0.25, 0.54)**
Pre-test sal-T	Pre-treatment PP	0.23 (-0.15, 0.55)	0.27 (0.06, 0.47)*
	PP change	0.10 (-0.28, 0.45)	0.04 (-0.18, 0.26)
Sal-T change	Pre-treatment PP	0.16 (-0.22, 0.50)	0.30 (0.08, 0.49)*
	PP change	0.11 (0.27, 0.46)	0.31 (0.09, 0.49)*

Significant correlations \*p<0.05, \*\*p<0.001

## Figure Legends

**Figure 1.** Basal salivary testosterone concentrations at days 7, 14 and 21 of the menstrual cycle in elite and non-elite women athletes. Group means ( $\pm$ SD) are presented with individual data at each time point. \*Significant from elites on D7 and D21, and non-elites on D7, D14 and D21, #Significant from elites on D21 and non-elites on D7, D14 and D21, †Significant from non-elites on D7, D14 and D21, <sup>o</sup>Significant from non-elites on D7 and D21 all  $p < 0.05$ .

**Figure 2.** Motivation to train at days 7, 14 and 21 of the menstrual cycle in elite and non-elite women athletes. Group means ( $\pm$ SD) are presented. \*Significant from elites on D7 and D21, and non-elites on D7, D14 and D21, #Significant from elites on D21 and non-elites on D7 and D21 all  $p < 0.05$ .

**Figure 3.** Stress-induced changes in salivary testosterone concentrations at days 7, 14 and 21 of the menstrual cycle in elite and non-elite women athletes. Group means ( $\pm$ SD) are presented with individual data at each time point. \*Significant change from baseline, #Significant change from elites on D7 and D21, and non-elites on D7, D14 and D21 all  $p < 0.05$ .

**Figure 4.** Changes in cycling peak power (PP) under the control and post-activation potentiation (PAP) treatments on days 7, 14 and 21 of the menstrual cycle. Group means ( $\pm$ SD) are presented. \*Significant change from baseline, †Significant change from control data on D7, D14 and D21, #Significant change from PAP data on D7 and D14 all  $p < 0.05$ .

**Figure 5.** Changes in cycling peak power (PP) across the control and post-activation potentiation (PAP) treatments in the elite and non-elite groups. Group means ( $\pm$ SD) are presented. \*Significant change from baseline, †Significant change from elite and non-elite control data, #Significant from non-elite PAP data all  $p < 0.05$ .







