Title: A longitudinal investigation of bidirectional and time-dependent interrelationships between testosterone and training motivation in an elite rugby environment

Header: Testosterone and motivation interrelationships

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Abstract

In sport, testosterone has been positioned as a substrate for motivation with both directional and time dependencies. However, evidence is scarce when considering the complexities of competitive sport and no work has explicitly modeled these dependencies. To address these gaps, we investigated the bidirectional and time-dependent interrelationships between testosterone and training motivation in an elite rugby environment. Thirty-six male athletes were monitored across training weeks before and after eight international rugby matches. Pre-breakfast measures of salivary testosterone and training motivation (1-10 rating) were taken on training, competition, and recovery days (up to 40 tests). Using a continuous-time (CT) model, within-person estimates of autoregressive effects (persistence) and cross-lagged effects (relationships) were derived. A stronger, more persistent temporal association was identified for testosterone than for motivation. Cross-lagged effects verified that training motivation was positively related to testosterone at latter time points ($p < .001$). Discrete-time analyses revealed a non-linear association; increasing in strength from a zero-time lag to peak after 2.83 days (standardized effect = .25), before dissipation over longer lagged intervals. The testosterone relationship with ensuing training motivation was also positive, but non-significant. Match effects also appeared ($p <.001$) with a predicted decline in training motivation, but a rise in testosterone, at match onset. In summary, a positive association emerged between within-person fluctuations in self-appraised motivation to train and testosterone concentration in an elite rugby environment. The lagged, non-linear nature of this relationship and match predictions on both outcomes support, and extend, theoretical models linking testosterone and competitive behaviors.

Key words: Neuroendocrine; Training Stress; Competitiveness; Adaptation

1. Introduction

The steroid hormone testosterone plays a key role in regulating behaviors related to social motivation and dominance. This interplay is often conceptualized within the theoretical framework of the Challenge Hypothesis (Archer, 2006; Wingfield et al., 1990) and the Biosocial Model of Status (Mazur, 1985), where testosterone is thought to regulate behaviors that serve to gain and maintain social status in human or animal competition. Such a perspective is restrictive as testosterone can affect, both consciously and unconsciously, broad-spectrum motivations to act (Aarts & van Honk, 2009). Furthermore, descriptive and experimental studies indicate that many elements of social motivation (e.g., persistence, perceived physical dominance, status-seeking, competitive endurance, fear reduction) are related, positively, to individual changes or differences in testosterone (Casto et al., 2020; Enter et al., 2014; Hermans et al., 2006; Losecaat Vermeer et al., 2020; Welker & Carré, 2015; Welling et al., 2016), and often in the absence of overt human-to-human competition.

In recent years, testosterone has been positioned as a biological substrate for motivation in sport (Wood & Stanton, 2012), which could mediate training gains in muscle size and strength (Cook et al., 2013), competitive performance (Casto & Edwards, 2016), and post-competition recovery (Crewther & Cook, 2012). Direct evidence comes from studies on athletic populations as they train for, and compete in, different sports. Examples include positive associations between testosterone concentration or response and training motivation (Crewther et al., 2016), motivation to win (Salvador et al., 2003; Suay et al., 1999), competitiveness (Crewther & Cook, 2018), and voluntary selection of training workloads (Cook et al., 2013), as a proxy for motivation. Likewise, differences in athlete testosterone concentration or response were found to be positively related to social bonding, status and connectedness (Bateup et al., 2002; Edwards et al., 2006), as possible primers of motivation and interpersonal engagement in a team-sport environment.

Debate still exists regarding the cause-effect nature of the testosterone relationship with motivated behaviors. A high testosterone concentration may, for instance, indicate a predisposition for competitive drive or it could reflect a shift in status motivation to reinforce dominance (Chichinadze et al., 2012; Crewther & Cook, 2018). The notion of reciprocity is consistent with the Biosocial Model of Status (Mazur, 1985), whereby divergent testosterone responses to victories (i.e., rising) and defeats (i.e., falling) promote behaviors that serve to achieve and preserve social status, respectively. Whilst this model has theoretical appeal, not all research on human competition adhere to these outcome-specific hormone responses (Casto & Edwards, 2016). Competitive sport is a complex pursuit comprising of multiple activities (e.g., physical and skill training, competition, recovery days), with each arguably producing differential shifts in testosterone and motivation depending on factors like mood, anticipation of victory (Chichinadze et al., 2012), fatigue state (Schiphof-Godart et al., 2018), and rewards (Vallerand, 2012). It remains to be seen whether the theorized bidirectional testosterone and motivation relationship holds true under these conditions.

To our knowledge, no studies have modeled the time-dependency of any testosterone and motivation interactions. This is a fundamental gap in the literature, as fluctuations in testosterone and dominance behaviors or emotional state do not always covary on the same timescale (Crewther et al., 2016), especially around competition (Shearer et al., 2015; West et al., 2014), with added heterogeneity across individuals (Cook et al., 2018). Testosterone also affects the motivational circuitry via rapid and delayed pathways (Wood & Stanton, 2012), thereby mapping onto future behaviors and performance on timescales spanning several minutes to hours, or even days, later (Booth et al., 1989; Carré et al., 2013; Crewther & Cook, 2012; Mehta & Josephs, 2006; Zilioli & Watson, 2014). To capture these complexities, a more detailed analysis of time-lagged effects of testosterone and motivation on each other is

needed. Addressing these dependencies, both in direction and time, would provide a better etiological understanding of social neuroendocrinology in a sporting context.

A longitudinal, descriptive study was undertaken to investigate the bidirectional and time-dependent interrelationships between testosterone and training motivation in an elite rugby environment. If testosterone does promote competitive motivation, and vice versa, then such interplay could be exaggerated amongst those individuals who both enjoy competing and possess high motivation to win (i.e., elite athlete). Salivary testosterone and training motivation were measured repeatedly (up to 40 times) on training, competition, and recovery days. The primary aim was to test the within-person effect of testosterone/motivation on motivation/testosterone via time-lagged analyses. Evidence indicates that testosterone and motivational factors positively covary (Casto & Edwards, 2016; Cook et al., 2013; Wood & Stanton, 2012), so we hypothesized that the lagged and reciprocal association between testosterone and training motivation would be positive as well. We then explored how these relationships emerge over time by examining the direction and strength of each association at different time lag intervals. As a secondary aim, we examined the match effect on both variables, but no firm hypotheses were made in this regard.

To assess the time-lagged interplay between study variables, a continuous-time (CT) model was implemented. The CT approach uses differential equations to model data as continuous processes over time (Voelkle et al., 2018), which offers a more realistic framework for investigating hormone and behavior system dynamics. Continuous-time models afford other benefits by (1) permitting the analysis of data from longitudinal designs with differing time intervals between and within individuals, (2) accommodating sampling schedules with different start times and missing data, (3) allowing for effects or associations to be explored across time, and (4) facilitating cross-study comparisons (Driver et al., 2017; Hecht & Voelkle, 2019). Subsequently, CT models are well suited to a growing number of

studies utilizing experience sampling, ambulatory, and ecological momentary assessment approaches (de Haan-Rietdijk et al., 2017; Hecht & Voelkle, 2019; Voelkle et al., 2018).

Despite the potential benefits offered by CT modeling within neuroendocrine and behavioral research, some barriers still exist regarding their wider use and interpretation (e.g., understanding of differential calculus, concepts and terms related to time-series modeling, availability of software and code). To overcome these problems, we highlight some key CT concepts and terms, as part of our statistical procedures, before providing a brief working example using a simulated dataset. Next, we present step-by-step guidelines for applying a bivariate CT model to the actual and simulated rugby data. Annotated R code for conducting all analyses and plotting results are provided as a supplemental file, including a link to download the simulated dataset and tabled results for this working example. The CT models in this study were implemented using the ctsem (version 3.0.9) package (Driver et al., 2017) in the R (version 3.5.1) programming environment (R Core Team, 2020). This software package is freely available online (https://cran.r-project.org/web/packages/ctsem/index.html).

2. Materials and Methods

2.1. Participants

The study cohort consisted of 36 professional male rugby players with a mean age of 27.7 (*SD* = 3.3) years, height of 1.88 (*SD* = .09) m, and body mass of 102.3 (*SD* = 13.0) kg. These athletes formed part of a training squad preparing for an international rugby series in 2010 and an international tournament in 2011. Three matches were played on consecutive weekends (weeks 1, 2, 3) in the rugby series, whilst the tournament comprised of five matches with intermittent scheduling (weeks 13, 14, 16, 18, 19). These matches were played at home (n = 6) and away (n = 2) venues. Each athlete received a full medical screening upon squad selection, before entering a training-camp environment (<2 weeks). All training

activities, team meetings, meals, sleeping habits, and social events were strictly controlled in this environment. Each participant was briefed on the study aims, procedures, and benefits, before providing written informed consent. Ethical approval was given by the Swansea University Human Research Ethics Committee (Number 2010.001R).

2.2. Study procedures

A longitudinal, descriptive design was employed to address the study aims. A 5-day testing schedule was planned in the morning across each week of competition (see Figure 1), where participants rated their motivation to train and provided a saliva sample for testosterone determination. Tests 1 and 2 coincided with normal training days (-4 and -3 days before competition), test 3 was a light training day (-1 day), test 4 was the morning of competition, and test 5 was the post-match recovery day (+1 day). Subsequently, up to 40 test sessions (5 weekly tests × 8 matches) were planned across this study. Training generally began within 2 hours of the morning assessment, apart from competition and recovery days. The competitions all started in the afternoon (from 2:30 pm to 5:15 pm local time) some 7 hours, on average, after the morning assessment. Each match was played over two periods of 40 minutes, separated by a 15-minute interval, although playing time can be extended due to foul play or injuries. The next-day morning assessment was completed around 14-16 hours later. We did not anticipate any measurement bias from matches played on consecutive weekends, as hormone concentration and subjective state in professional rugby teams return to pre-match values within three days (Cunniffe et al., 2010; Shearer et al., 2015; West et al., 2014).

Insert Figure 1 here.

Studies conducted on athletic populations under real sporting conditions have greater ecological validity than laboratory-based research (Atkinson & Nevill, 2001). There is, however, some inherent trade-off due to several uncontrollable factors in elite sport (e.g., athlete injuries, illness, training plans and any alterations thereof), leading to some missed tests in this study, and other logistical constraints (e.g., storage facilities for samples during international travel) that prevented saliva collection on some days. Financial constraints further limited our assay of samples taken around each rugby match (-1 day, morning of match, +1 day) to those athletes (n = 22) selected to play each week. Overall, the mean number of tests completed by each participant was 28.1 with a *SD* of 11.4 (minimum = 7, maximum = 40), producing a total of 643 observations for testosterone and 916 for training motivation. The R package ctsem uses full information maximum likelihood estimation to handle missing data (Driver et al., 2017).

2.3. Salivary testosterone assessment

Saliva sampling began before breakfast (8-9 am) on all test occasions, which accounted for circadian variation in testosterone concentration (Beaven et al., 2010). Sample collection before breakfast also eliminated the possibility of sample contamination, due to food or fluid intake. Briefly, a small (~1 mL) sample was provided by passive drool without artificial stimulation before storage according to published guidelines (Toone et al., 2013). After thawing and centrifugation, each sample was assayed in duplicate for testosterone concentration using an enzyme-linked immunoassay kit (Salimetrics LLC, USA). The assay kit had a sensitivity limit of 1 pg/mL with a calibrator range of 6.1 to 600 pg/mL. The inter-assay coefficients of variation (CV) on low and high controls were all <11% and the CVs on sample duplicates within an assay were, on average, <4%. To eliminate inter-assay bias, each athlete's samples were assayed in the same plate.

2.4. Training motivation assessment

Our assessment of training motivation formed part of a larger, but still brief, inventory to assess athlete wellbeing, readiness, and recovery on an almost daily basis. Immediately after saliva collection, each athlete appraised their motivation to train on a 10-point Likert scale (from extremely poor = 1 up to excellent = 10). To assess the transient and situational nature of motivation (Vallerand, 2012), a response timeframe was created by asking participants to rate their perception at that moment in time. Data were collected verbally by a team trainer after showing each participant a laminated card with all ratings and explanations. This measure was based on the Utrecht Work Engagement Scale for the subscale assessment of vigor (see items 1 and 8) (Schaufeli et al., 2006), and subsequently adapted for sports training and competition settings (Guillén & Martínez-Alvarado, 2014). Pilot testing on a sub-group of 18 athletes (k means = 5) revealed acceptable test-retest reliability (intraclass correlation coefficient [ICC] = .60) and internal consistency (Cronbach's alpha = .84).

2.5. Statistical analyses

2.5.1. The discrete-time (DT) framework

In a DT framework, the measured variables may be assumed to represent a cyclical or *stationary process* that continuously fluctuate around a mean value, as part of a larger dynamical system. Here we considered morning fluctuations in salivary testosterone and self-appraised motivation and the process mean is the concentration or score to which each variable is regulated towards[1]. Temporal relationships within and between variables are described by *autoregressive effects* and *cross-lagged effects*. An *autoregressive effect* is the

[1] The process mean can obtain a value of zero when standardization techniques are applied.

regression coefficient when the state of a variable is regressed on its former state. Different concepts exist regarding interpretation of the autoregressive effect. In line with recent work (Hardt et al., 2019), we interpret the autoregressive coefficient as representing the *persistence* of a process with higher values indicating that the testosterone concentration at one time point is more predictive for the testosterone concentration at the next time point. Conversely, a low autoregressive coefficient indicates weaker persistence in the sense of low predictiveness of testosterone concentration across successive time points.

When characterizing a dynamic system by two variables (i.e., bivariate CT model), the state of one variable can be also explained by the former state of the other variable. These coefficients, termed the *cross-lagged effects*, describe relationships between both variables at different time-lagged interval lengths. A larger cross-lagged estimate equates to a stronger bivariate temporal association and vice versa. For ease of interpretation, the cross-lagged estimates can be standardized for expression as within-person SDs. Another important consideration is that the dependence of cross-lagged effects on time interval lengths are, typically, non-linear in nature with a *peak lagged effect* that represents the maximal temporal association between two processes (see Figure 3B as an illustrative example). In other words, these relationships coexist at multiple time intervals for which they simply differ in strength. The *autoregressive matrix* ($\mathbf{A}_{\Delta t}$) combines the above elements, with autoregressive effects (e.g., testosterone persistence across successive time points, motivation persistence across successive time points) on the main diagonal and cross-lagged effects (e.g., testosterone relationship with motivation at the subsequent time point, motivation relationship with testosterone at the subsequent time point) on the off-diagonals.

2.5.2. Extending the DT framework to a CT framework

In the DT framework described above, model parameters (e.g., the autoregressive matrix $\mathbf{A}_{\Delta t}$) usually depend on the length of the time interval between time points (Δt). A more general way of describing the dynamics is to assume a continuous process, which can be described with a stochastic differential equation (Voelkle et al., 2012), in terms of how the position of the system at any given time (*t*) relates to the rate of change in each process (i.e., $\frac{dy(t)}{dt}$). The mathematical solution allows system dynamics to be calculated for any time interval of interest, both observed and unobserved (i.e., between observations), by drawing on the *drift matrix* **A** and the *diffusion matrix* **Q**, the former including auto- and cross-effects and the latter representing random process error. As an example, a CT model might identify a bivariate association between two processes using an assessment design with daily measurements over a 1-month period, but a researcher might then convert the CT estimates to DT parameters for an interval of 6 hours to better explore this interplay and identify temporal mechanisms. The CT to DT relationship can, as one example, be described by the equation, $\mathbf{A}_{\Delta t} = e^{\mathbf{A}\Delta t}$, where *e* is the matrix exponential function and Δt is the time interval.

There are important differences in nomenclature when parameters are discussed in either a CT or DT framework. For instance, the terms autoregressive effects and cross-lagged effects refer to a DT domain, whereas the terms auto-effects and cross-effects are the corresponding parameters when discussed within a CT framework (see Hecht & Voelkle 2019, Table 1 for an overview of DT and CT terms).

2.5.3. A working example of a bivariate CT model

Here we provide a brief working example to assist readers with the conversion of the key CT parameters into DT parameters and their subsequent interpretation. The drift matrix **A** relating the testosterone and training motivation processes in a simulated dataset (see supplemental file) is given by:

$$\mathbf{A} = \begin{matrix} \\ \text{Testosterone} \\ \text{Motivation} \end{matrix} \begin{matrix} \begin{matrix} \text{Testosterone} & \text{Motivation} \end{matrix} \\ \begin{bmatrix} -.305 & .440 \\ .354 & -.992 \end{bmatrix} \end{matrix} \quad (1)$$

The CT model results (see supplement Table S1) show that the auto-effects (elements on the main diagonal above – highlighted in red) for both processes and the cross-effects (off-diagonal elements above) between processes are all significantly ($p < .01$) different from zero. As detailed below, the CT parameters were converted into DT parameters for three different time interval lengths. Equations 2, 3, and 4 show the CT to DT calculations for the time periods of $\Delta t = 1$, $\Delta t = 4$, and $\Delta t = 7$ days, respectively. The DT autoregressive parameters indicate a decline in the persistence of each process as the time interval shifts from 1 day up to 7 days. This pattern is expected, because values of a variable lose their association with later values as the amount of time between measurements increase. The DT cross-lagged effects signify an initial improvement in the strength of the interrelationships between both processes, when modeled at time interval lengths from 1 day up to 4 days, before decreasing in strength at the longest interval of 7 days.

$$\mathbf{A}_{\Delta t=1} = e^{\begin{bmatrix} -.305 & .440 \\ .354 & -.992 \end{bmatrix} \cdot 1} = \begin{bmatrix} .784 & .241 \\ .194 & .408 \end{bmatrix} \quad (2)$$

$$\mathbf{A}_{\Delta t=4} = e^{\begin{bmatrix} -.305 & .440 \\ .354 & -.992 \end{bmatrix} \cdot 4} = \begin{bmatrix} .503 & .251 \\ .202 & .112 \end{bmatrix} \quad (3)$$

$$\mathbf{A}_{\Delta t=7} = e^{\begin{bmatrix} -.305 & .440 \\ .354 & -.992 \end{bmatrix} \cdot 7} = \begin{bmatrix} .344 & .175 \\ .141 & .072 \end{bmatrix} \quad (4)$$

The presentation of such DT results in a time-lagged parameter plot (as per Figure 3) can assist with conceptualization and interpretation of autoregressive and cross-lagged effects, in terms of their respective strength, direction, and lag timing. Additional outputs from the CT model, as shown in Table S1, are detailed as part of the main analyses below. Other examples of CT modeling using the ctsem package, both in a frequentist and Bayesian domain, can be located elsewhere (de Haan-Rietdijk et al., 2017; Hecht et al., 2019; Hecht & Voelkle, 2019; Redhead et al., 2019; Voelkle et al., 2018). For interested readers, the work by Voelkle et al. (2012) provides mathematical and technical detail for the CT modeling

approach. Recommendations on sample size requirements for some CT models are provided by Hecht and Zitzmann (2020).

2.5.4. Estimating a bivariate CT model

Continuous-time modeling with the ctsem package can be partitioned into five steps: (1) data preparation, (2) model specification, (3) model fitting, (4) extraction of results, and (5) post-processing of model estimates. In step 1, all time points of measurement were converted such as to represent elapsed time relative to the start date of the study (i.e., 09 November 2010, 8:30 am), which was coded as $t = 0$, and data were standardized via grand-mean centering to facilitate model convergence (Driver et al., 2017). A dichotomous variable labelled "match" (non-match = 0, match = 1) was also created which indicated whether a match occurred at each time point or not. Once again, we used the exact time of each match and converted these points, relative to the starting date for this study. In step 2, a bivariate process model was specified with match entered as a time-dependent predictor, before fitting the CT model to our dataset in step 3. In step 4, model estimates were summarized with a standard error (*SE*) and 95% confidence interval (CI). To determine statistical significance, *p* values were computed from *z* scores with the significance level set to $\alpha = .05$. In step 5, the CT parameters were converted into DT parameters. Model specification, fitting and plotting can be replicated using the R code supplied.

3. Results

Initial data exploration identified 10 testosterone values (1.6% of the dataset) as outliers, based on a cut-off criteria of ±3SD from the grand mean. Subsequently, these values were winsorized to 3SD from the grand mean before CT modeling. Testosterone had a pooled $M = 149.3$ and $SD = 46.6$ pg/mL (minimum = 41.0, maximum = 289.6), whilst training motivation

had a pooled M = 6.67 and SD = 1.92 score (minimum = 1, maximum = 10). Population means (95% CI) for testosterone and training motivation are shown in Figure 2, overlaying all individual observations. Where testosterone concentration tended to rise and fall across certain weeks and matches (Figure 2A), training motivation exhibited a more consistent pattern across all weeks (Figure 2B), rising slightly before each match and falling dramatically the next day before a return to mid-week values. The individual means over time points of testosterone had an average (across participants) of M = 150.3 and SD = 24.5 pg/mL. For training motivation those statistics were: M = 6.79 and SD = 1.02. To highlight data means and dispersion at the individual level, the plotted results for each participant are provided as a supplemental file (see Figures S1 and S2).

Insert Figure 2A and 2B here.

The CT model estimates for the rugby dataset are presented in Table 1. Both CT auto-effects are significantly different from zero. Concerning the CT cross-effects, we see that training motivation at one time point is positively related to testosterone at the next time point ($p < .001$), whereas the testosterone to training motivation relationship, whilst also positive, is not significant. To better interpret the CT persistence (auto-effects) and relationship (cross-effects) estimates, both results were converted into DT parameters (see below).

Insert Table 1 here.

The converted DT autoregressive effects and cross-lagged effects were plotted (see Figure 3) for a time interval length ranging from 0 to 30 days. For an interval of 2 days, the

autoregressive effect for testosterone is $a^*_{2_{\text{Testosterone}}} = .79$, indicating strong persistence for this time period (Figure 3A). The corresponding autoregressive effect for the training motivation process, $a^*_{2_{\text{Motivation}}} = .21$, indicates weak persistence for the same time interval. Both the testosterone and motivation autoregressive effects approached a value of virtually zero around an interval length of 30 days and 10 days, respectively. The DT cross-lagged effect of training motivation on testosterone showed a relationship peak after 2.83 days, $a^*_{3_{\text{Motivation}\rightarrow\text{Testosterone}}} = .27$ (this value equates to .25 as a within-person standardized effect[2]), before slowly dissipating to approach a zero value after about 30 days (Figure 3B).

Insert Figure 3A and Figure 3B here.

The diffusion variances represent the latent process error variations. As both testosterone and training motivation were controlled for (rather substantive) measurement error, the diffusion variances appear as quite small. However, when inspecting the intraclass correlation coefficients, $\text{ICC}_{\text{Testosterone}} = .56$ and $\text{ICC}_{\text{Motivation}} = .69$, it becomes apparent that roughly a third of the total latent variance is located at the within-person level. The significant diffusion covariance represents the non-zero random error covariation of both processes and highlights the extent to which they might share common causes.

The CT intercepts, which determine the average process means, are also significantly different from zero and therefore suggest that the process means differ from zero as well. The asymptotic trait variances are significantly different from zero. Subsequently, there are between-person differences in the individual process means for testosterone and training motivation. The negative asymptotic trait covariance (p = .019) signifies that persons with a

[2] The within-person standardized effect = estimate × $\frac{\sqrt{wpp}}{\sqrt{wpr}}$, where *wpp* denotes the within-person variance of the predictor variable and *wpr* denotes the within-person variance of the response variable.

higher process mean on one variable exhibit a lower process mean on the other variable. The significant non-zero measurement error variances for both processes (both $p < .001$) indicates that there was substantive noise due to the below perfect reliability of the employed instruments (i.e., the single-item motivation question, and the testosterone assay kit). By controlling for this unreliability of the measurement instruments in our model, the previously more obscured effects are carved out more clearly.

Match occurrence or onset had a significant impact on both testosterone concentration and training motivation compared to "baseline" data (i.e., all time points on non-match days). Whereas the match association with testosterone concentration was positive ($m_{\text{Testosterone}} = .62$), it is negative regarding motivation to train ($m_{\text{Motivation}} = -2.96$). When expressed as a standardized effect[3], match onset correlates with an average testosterone increase of 1.78 SDs and a decrease in training motivation of –9.34 SDs. Based on reviewer feedback, we also modeled data from those athletes selected to play each week. The primary cross-effect (training motivation to testosterone) and match effect (on testosterone) were no longer significant, which we attribute primarily to a lack of statistical power. Due to convergency problems, we were unable to model data taken from non-match selections each week.

Our results can be summarized as follows: first, the persistence of the testosterone and training motivation processes both decreased at longer time lag intervals, but at a much faster rate for motivation than for testosterone; second, a higher rating of training motivation was related to a higher testosterone concentration at the next time point, although in a non-linear manner with longer lag intervals, whereas the testosterone to motivation relationship was non-significant; third, match onset was related to both processes with testosterone strongly increasing and training motivation strongly decreasing, relative to non-match data.

[3] The within-person standardized effect = $\frac{Estimate}{\sqrt{wpr}}$, where *wpr* denotes the within-person variance.

4. Discussion

This study investigated the interrelatedness between testosterone and training motivation in elite male athletes under ecological conditions, where both processes were characterised by transient fluctuations each week. In this environment, a stronger and more persistent temporal association on successive time points was identified for testosterone than for motivation. As hypothesized, a positive lagged association between training motivation and subsequent testosterone concentration was identified, whilst no corresponding link between testosterone and ensuing motivation transpired. Additionally, we uncovered a match effect that was positive for testosterone and negative for training motivation at match onset.

Our primary finding was a positive cross-lagged (within-person) relationship between training motivation and subsequent testosterone concentration. Crucially, this interplay followed a non-linear trend, increasing steadily from a zero-time lag to peak with a lag of 2.83 days, before declining over longer time intervals. Sports research indicates that shifts in emotional state and motivated behaviors, induced experimentally (e.g., video presentations with coach feedback) or naturally (e.g., contest win-loss effects), can affect testosterone secretion and, in some cases, athletic performance several hours or days later (Booth et al., 1989; Cook & Crewther, 2012; Mazur & Lamb, 1980). One study reported similar lagged responses among male rugby players following a “positive” and “negative” motivational strategy, each performed twice, after a professional rugby match (Crewther & Cook, 2012). The strategy designed to enhance motivation produced a larger testosterone stress response (3 days later) and a higher pre-match testosterone concentration (6-7 days later), which coincided with better match performance. Lab-based competition provides further support for lagged behavioral effects on testosterone (Carré et al., 2013; Mehta & Josephs, 2006; Zilioli & Watson, 2014). The actual mechanism/s involved are still unclear, especially as the

linkage identified herein spanned (existed) across a wide time-lagged continuum, but could entail one or more structural, functional or developmental connections between testosterone and brain reward centres (e.g., basolateral amygdala, meso-limbic and meso-cortical, prefrontal-amygdala coupling / decoupling) that drive social approach and avoidance behaviors (Enter et al., 2016; Spielberg et al., 2015; Terburg & van Honk, 2013), including effects mediated by the dopamine and stress systems.

The within-person testosterone relationship with subsequent training motivation, although in the hypothesized (positive) direction, was not statistically significant. Such a finding could be explained by several overlapping features. One being the pulsatile secretion of testosterone (Beaven et al., 2010) that we could not adequately profile with a single saliva sample per session. Alternatively, a stronger hormone to behavior association (or vice versa) might arise when employing objective (i.e., number, type and intensity of physical actions), rather than subjective (i.e., single rating), indicators of training motivation that perhaps better capture athlete level of engagement, effort and persistency in a given task. Situational cues, or lack thereof, is another consideration in sport. If sampling occurs closer to a competitive activity, pre-encounter factors (e.g., mood, expectations) may contribute to both testosterone secretion and motivational state (Chichinadze et al., 2012) and, in turn, their modeled relationship. Recent work also highlighted the role of stable / unstable hierarchies in moderating testosterone's time-dependent effect on competitive motivation in men (Losecaat Vermeer et al., 2020), such that a stronger effect might be seen among athletes whose status within a team could be deemed relatively unstable. Regardless, there is reasonable evidence that testosterone correlates with dominance or status outcomes in sporting competition (Casto & Edwards, 2016; Cook & Crewther, 2012; Gaviglio et al., 2014; Salvador et al., 2003; Suay et al., 1999), especially when testosterone is indexed via pre- and post-competition measures.

A positive match association with athlete testosterone concentration was also demonstrated, after controlling for sampling (time) differences. In other words, within-person testosterone concentration increased at the onset of an international rugby match, relative to all non-match data when normalized for time of day. Athletes often exhibit a higher testosterone concentration on the day of competition, compared to time-matched samples taken on a control day (Bateup et al., 2002; Casto & Edwards, 2016; Salvador et al., 2003; Suay et al., 1999). This hormone response likely reflects the psychological anticipation of impending competition, coupled with other emotional and confidence factors (Casto & Edwards, 2016; Chichinadze et al., 2012). In contrast, within-person motivation to train declined at match onset versus non-match data. We attributed this to a shift in athlete focus to competitive readiness, not training to perform, on the day competition and likely reinforced by a combination of environmental (e.g., pre-competition routines) features (Cook & Crewther, 2012) and unconscious primers (e.g., verbal cues from staff and other athletes) (Vallerand, 2012). It is noteworthy that the magnitude of the match effect on both outcomes was exceptionally large. Converting our estimates into predicted means[4] at match onset yielded a testosterone concentration of 180 pg/mL and a motivational score of 1.6; both are plausible and within the measurement range for each outcome.

Many studies have examined whether testosterone affects, or responds to, shifts in motivation, dominance or social status in sports training (Cook et al., 2013; Cook et al., 2018; Crewther et al., 2016; Crewther & Cook, 2018; Serpell et al., 2018) and competitive settings (Bateup et al., 2002; Booth et al., 1989; Edwards et al., 2006; Salvador et al., 2003; Suay et al., 1999). Extending this work, we combined data from both settings and explored more nuanced associations via reciprocal, time-lagged analyses. The possibility of a time-delayed

[4] Predicted mean at match onset = $wpm + \frac{wpsd}{\sqrt{wpr}} \times$ match effect, where *wpm* denotes the descriptive within-person mean, *wpsd* denotes the descriptive within-person SD, and *wpr* denotes the descriptive within-person variance.

influence of motivation on testosterone has implications for study planning (e.g., timing of tests) to capture this relationship and how to model this interplay given inherent complexities (e.g., reciprocity, time dependency) of social endocrinology. On a practical level, this information could guide the timing of motivational strategies across the training week to optimize the hormonal milieu for key activities. A further possibility exists to refine theoretical models by considering how time might give rise to distinction in the relationship between each process. Our data partly supports theory (i.e., Biosocial Model of Status) (Mazur, 1985) that athlete perceptions of, and effort towards, activities that serve to achieve or preserve social status could manifest hormonally, but perhaps this connection emerges over several days and persists for longer periods (up to 1-2 weeks) than previously thought. This pattern could explain the highly variable testosterone response to wins and losses in athletic competition (Casto & Edwards, 2016). The predicted rise in testosterone level at match onset also aligns to the Challenge Hypothesis (Wingfield et al., 1990) when applied to competitive interactions among young men (Archer, 2006). Potentially, athlete intentions to engage in a sporting activity, as an indicator of intrinsic motivation (Vallerand, 2012), might affect testosterone dynamics and aggressive behaviors in male-to-male competition later in the week.

The current findings must be balanced against several constraints, over and above those described earlier. Such results may not replicate in lesser-trained athletes who often present weaker within-person testosterone associations with motivational outcomes than either highly-trained men (elite beta = .86, non-elite beta = non-significant) (Crewther et al., 2016) or women (elite $r \geq .70$, non-elite $r \leq .50$) (Cook et al., 2018). Our estimates were also based on all available athletes and contexts (i.e., training, competition, and recovery days); a necessary approach to increase statistical power and ensure model convergence. Moreover, although the self-report instrument followed expected trends, and reliably so across each

week of competition, it only reflects a basic subscale of engagement and has yet to be formally validated. Further bias might arise from the verbal collection of this data, but this approach ensured greater compliance and consistency in a challenging and stressful environment. As a further limitation, we only sought to describe natural fluctuations in testosterone concentration and training motivation over time and, like all observational datasets, causality is never clear. Combining longitudinal profiling with one or more experimental manipulations of athlete testosterone and motivational state could prove fruitful in both verifying, and providing etiological insight into, this dynamic relationship.

In summary, a positive relationship between fluctuations in training motivation and testosterone concentration at a later point was identified in an elite rugby environment. The hypothesized bidirectional interplay between these processes did not, however, materialize. Nevertheless, the non-linear, time-lagged nature of the observed relationship supports, and extends, theoretical models linking testosterone and competitive human behaviors, as does the predicted match effect on both outcomes. As a working exemplar, we also highlight the utility of CT modeling for examining two relevant concepts in social endocrinology, reciprocity and time dependency, with added flexibility for complex longitudinal designs.

Competing interest

The authors of this paper have no competing interests.

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Data availability

The research data is unavailable due to a confidentiality agreement, but a simulated dataset has been created based on the CT modeled results. The simulated dataset can be sourced online and loaded directly into the R programme (see supplementary code provided).

Supplementary data

Supplementary data associated with this article can be found online.

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Table 1. Continuous-time estimates for a bivariate (Testosterone / Training motivation) model with rugby match as a predictor

				95% CI		
Parameter name	Parameter	Estimate	*SE*	Lower	Upper	*p* value
Auto-effect	$a_{\text{Testosterone}}$	–.13	.05	–.23	–.03	.011
	$a_{\text{Motivation}}$	–.83	.10	–1.02	–.64	< .001
Cross-effect	$a_{\text{Testosterone}\rightarrow\text{Motivation}}$	.08	.11	–.14	.30	.497
	$a_{\text{Motivation}\rightarrow\text{Testosterone}}$	.31	.09	.14	.48	< .001
Diffusion variance	$\sigma^2_{\text{Testosterone}}$	.04	.02	.01	.07	.016
	$\sigma^2_{\text{Motivation}}$	.17	.07	.02	.31	.023
Diffusion covariance	$\sigma_{\text{Testosterone}\leftrightarrow\text{Motivation}}$	–.05	.03	–.11	.00	.048
Continuous-time intercept	$b_{\text{Testosterone}}$	–.10	.04	–.18	–.02	.015
	$b_{\text{Motivation}}$	.32	.08	.17	.48	< .001
Trait variance (asymptotic)	$\sigma^{2*}_{\mu_{\text{Testosterone}}}$	.15	.05	.05	.25	.004
	$\sigma^{2*}_{\mu_{\text{Motivation}}}$	.22	.06	.11	.33	< .001
Trait covariance (asymptotic)	$\sigma^{*}_{\mu_{\text{Testosterone}\leftrightarrow\text{Motivation}}}$	–.10	.04	–.19	–.02	.019
Measurement error variance	$\sigma^2_{\varepsilon_{\text{Testosterone}}}$	.69	.05	.59	.79	< .001
	$\sigma^2_{\varepsilon_{\text{Motivation}}}$	.33	.04	.24	.41	< .001
Match effect	$m_{\text{Testosterone}}$	.62	.19	.25	.99	.001
	$m_{\text{Motivation}}$	–2.96	.22	–3.40	–2.52	< .001

Note 1: n = 643 observations for testosterone and 916 observations for training motivation
Note 2: Estimates of variances and covariances are of the full variance-covariance matrices, not Cholesky decompositions
Note 3: Standard errors (*SE*) were approximated using the Delta method

Figure 1. Monitoring timeline across each training week of international rugby competition with matches played either on a Saturday (schedule A) or Sunday (schedule B).

Figure 2. Means and a 95% bootstrapped CI for the salivary testosterone (2A) and self-reported training motivation (2B) measures in each testing session. The grey bars reflect training days, the clear bars represent match day, and blue bars indicate a recovery day. Each rugby match (dashed line) started ~7 hours after the morning test. Please note that matches were played on consecutive and non-consecutive weeks, but are presented sequentially for comparative purposes only.

Figure 3. (3A) Discrete-time plot for the autoregressive effects (i.e., persistence of testosterone and motivation) at time interval lengths up to 30 days. A value closer to 1 indicates a high autoregressive effect = strong persistence, whereas a value closer to 0 represents a low autoregressive effect = weak persistence. For both processes, persistency decreased as the lag interval increased. (3B) Discrete-time plot for the cross-lagged effects (i.e., association between training motivation and testosterone at the subsequent time point, and between testosterone and training motivation at the subsequent time point) depending on time interval length. The interrelationships between both processes improved with longer lag intervals, peaking after 2.83 days (indicated by the dashed vertical line) before decreasing for longer intervals beyond this point.

Supplementary file: A longitudinal investigation of bidirectional and time-dependent interrelationships between testosterone and training motivation in an elite rugby environment

```
#########################################################################
#          R code for running the continuous-time model with R package ctsem          #
#########################################################################

## load/install packages
# used ctsem version: 3.0.9
# => get latest ctsem version: install.packages( "ctsem" )
library( ctsem )

# load data, where ctTestMot is the dataset name
load( url( "http://amor.cms.hu-berlin.de/~psymetho/ctTestMot/ctTestMot.Rdata" ) )

# data are in long format: participant identification (id) × time of measurement (time);
# variables: salivary testosterone (Testosterone), motivation to train (Motivation),
# standardized measures of motivation (Motivation_stand) and testosterone
# (Testosterone_stand), and occurrence of a match (Game)

## construct a bivariate continuous-time model with match as a predictor

# drift matrix equals default drift matrix, but with explicit naming for interpretability
A <- matrix( c( "drift_Testosterone",
                "drift_Testosterone}Motivation",
                "drift_Motivation}Testosterone",
                "drift_Motivation"),
                  nrow=2, ncol=2 )

# set up model
m  <- ctModel( n.latent = 2,
                latentNames = c("Testosterone","Motivation"),
                n.manifest = 2,
                manifestNames = c("Testosterone_stand","Motivation_stand"),
                n.TDpred = 1,
                TDpredNames = "Game",
                Tpoints = max( tapply( ctTestMot$time, list( ctTestMot$id ), length ) ),
                DRIFT = A,
                CINT = matrix( c("CINT_Testosterone", "CINT_Motivation"), 2, 1 ),
                TRAITVAR = "auto",
                LAMBDA = diag( 2 ),
                MANIFESTMEANS = matrix( 0, nrow=2, ncol=1 ),
                type="omx" ) # frequentist estimation

# start time
start <- Sys.time()
```

```
## fit the continuous-time model (frequentist estimation)
# run time: roughly 10 minutes

set.seed( 4321 ) # for reproducible standard errors
r <- ctFit( dat = ctTestMot,
            dataform = "long", # long format data
            ctmodelobj = m,
            stationary = "all" )

# run time
print( runtime <- Sys.time() - start )

# extract results
print( summary( r )$ctparameters )

# plot autoregressive (AR) effects depending on interval lengths 0 to 30 days
ctPlot(r, plotType = "AR",
                xlim=c(0, 30),
                ylim=c(0, 1),
                ylab="Autoregressive effect",
                xlab="Time interval (days)")

# plot unstandardized cross-lagged effects depending on interval lengths 0 to 30 days
ctPlot(r, plotType = "CR",
                xlim=c(0, 30),
                ylim=c(0, 0.3),
                ylab="Cross-lagged effect",
                xlab="Time interval (days)")

# plot standardized cross-lagged effects depending on interval lengths 0 to 30 days
ctPlot(r, plotType = "standardiseCR",
                xlim=c(0, 30),
                ylim=c(0, 0.3),
                ylab="Cross-lagged effect",
                xlab="Time interval (days)")

# calculate unstandardized discrete-time parameter estimates for a time interval of 3 days
expm( summary( r )$DRIFT*3 )

# calculate standardized discrete-time parameter estimates for a time interval of 3 days
summary(r, verbose = TRUE, timeInterval = 3)["discreteDRIFTstd"]
```

Supplementary file: A longitudinal investigation of bidirectional and time-dependent interrelationships between testosterone and training motivation in an elite rugby environment

Table S1. Continuous-time estimates for a bivariate model on a simulated rugby dataset.

				95% CI		
Parameter name	Parameter	Estimate	*SE*	Lower	Upper	*p* value
Auto-effect	$a_{\text{Testosterone}}$	–.31	.09	–.48	–.13	.001
	$a_{\text{Motivation}}$	–.99	.13	–1.25	–.74	< .001
Cross-effect	$a_{\text{Testosterone}\rightarrow\text{Motivation}}$	.35	.11	.13	.58	.002
	$a_{\text{Motivation}\rightarrow\text{Testosterone}}$	.44	.12	.21	.67	< .001
Diffusion variance	$\sigma^2_{\text{Testosterone}}$	.09	.04	.00	.18	.040
	$\sigma^2_{\text{Motivation}}$	.21	.08	.05	.37	.010
Diffusion covariance	$\sigma_{\text{Testosterone}\leftrightarrow\text{Motivation}}$	–.06	.04	–.15	.02	.129
Continuous-time intercept	$b_{\text{Testosterone}}$	–.09	.06	–.20	.02	.126
	$b_{\text{Motivation}}$	.28	.10	.09	.48	.005
Trait variance (asymptotic)	$\sigma^{2*}_{\mu_{\text{Testosterone}}}$	.10	.05	.00	.19	.044
	$\sigma^{2*}_{\mu_{\text{Motivation}}}$	.21	.05	.10	.31	< .001
Trait covariance (asymptotic)	$\sigma^{*}_{\mu_{\text{Testosterone}\leftrightarrow\text{Motivation}}}$	–.12	.03	–.19	–.05	< .001
Measurement error variance	$\sigma^2_{\varepsilon_{\text{Testosterone}}}$	.63	.05	.53	.73	< .001
	$\sigma^2_{\varepsilon_{\text{Motivation}}}$	.28	.04	.21	.35	< .001
Match effect	$m_{\text{Testosterone}}$	.82	.28	.28	1.36	.003
	$m_{\text{Motivation}}$	–3.34	.33	–3.98	–2.70	< .001

Supplementary file: A longitudinal investigation of bidirectional and time-dependent interrelationships between testosterone and training motivation in an elite rugby environment

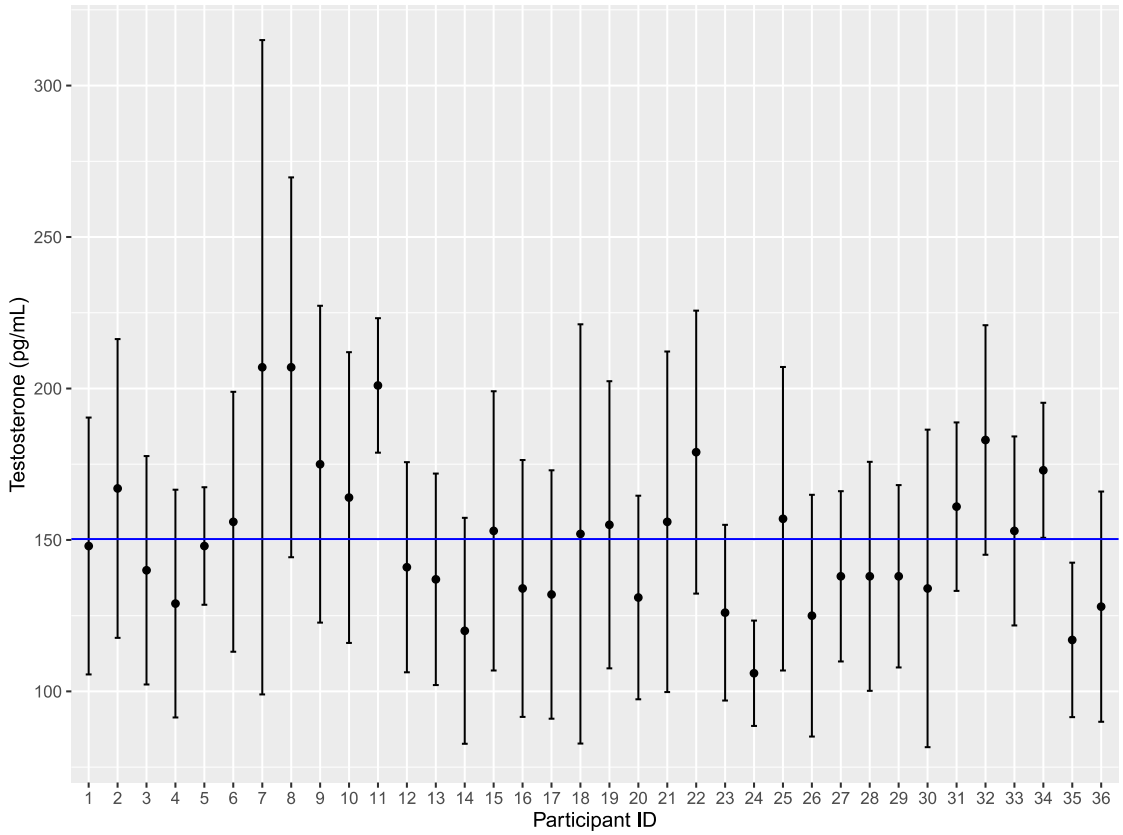

Figure S1. Mean (±*SD*) salivary testosterone concentrations for each participant pooled across all study observations. The horizontal blue line represents the individual mean averaged across persons.

Supplementary file: A longitudinal investigation of bidirectional and time-dependent interrelationships between testosterone and training motivation in an elite rugby environment

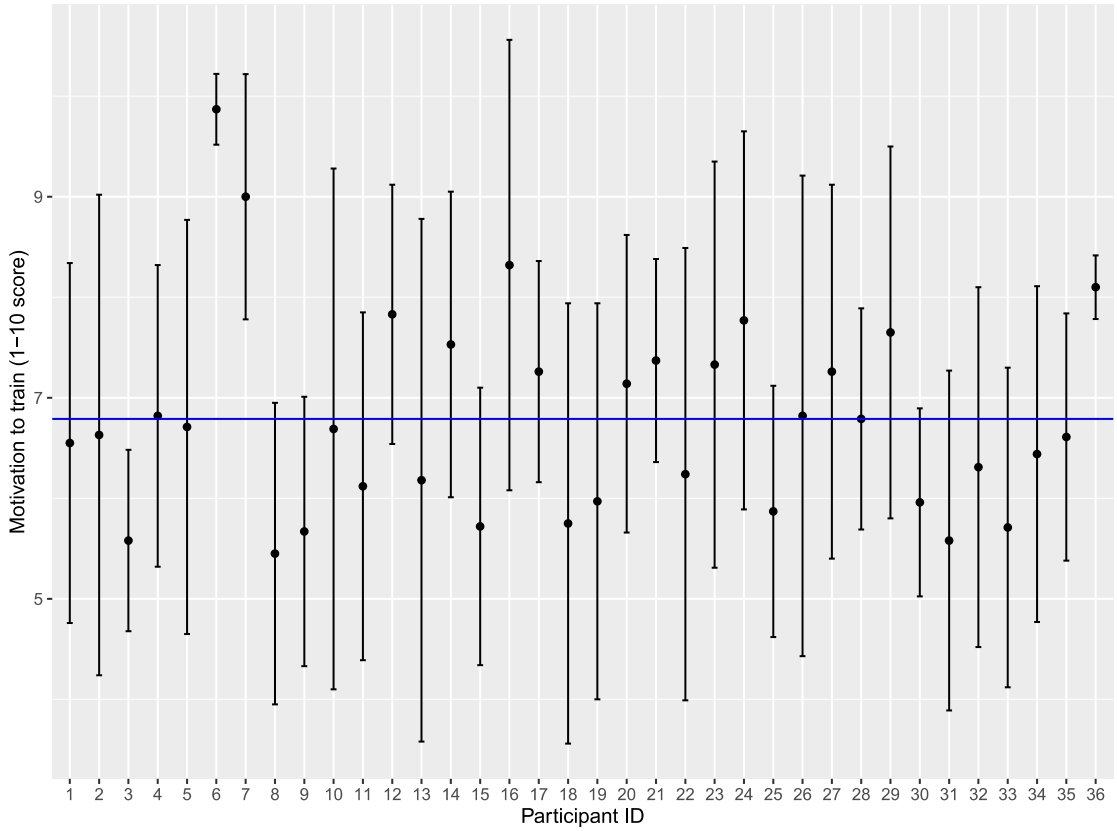

Figure S2. Mean (±*SD*) motivation to train ratings for each participant pooled across all study observations. The horizontal blue line represents the individual mean averaged across persons.