Day-to-day coordination of the stress and reproductive axes: a continuous-time analysis of within-person testosterone and cortisol relationships in athletic and healthy men

Blair T Crewther , Martin Hecht , Rachel L Grillot , Adar B Eisenbruch , Tikal Catena , Neill Potts , Liam P Kilduff , Christian J Cook , Dario Maestripieri , James R Roney

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Title: Day-to-day coordination of the stress and reproductive axes: a continuous-time analysis of within-person testosterone and cortisol relationships in athletic and healthy men

Header: HPA- and HPG-axes in athletic and healthy men

Authors: Blair T Crewther[1,2,3], Martin Hecht[4], Rachel L Grillot[5], Adar B Eisenbruch[6], Tikal Catena[5], Neill Potts[7], Liam P Kilduff[8,9], Christian J Cook[2,3], Dario Maestripieri[10], James R Roney[5]

Affiliations:

[1]Institute of Sport – National Research Institute, Warsaw, Poland

[2]School of Science and Technology, University of New England, Armidale, Australia

[3]Hamlyn Centre, Imperial College, London, UK

[4]Helmut Schmidt University, Hamburg, Germany

[5]Department of Psychological and Brain Sciences, University of California, Santa Barbara, USA

[6]Department of Psychology, Marist College, USA

[7]Western Australian Institute of Sport, Perth, Australia

[8]A-STEM, Faculty of Science and Engineering, Swansea University, Swansea, UK

[9]Welsh Institute of Performance Science (WIPS), Swansea University, Swansea, UK

[10]Department of Comparative Human Development, University of Chicago, USA

Corresponding author:

Dr. Blair Crewther

Department of Endocrinology

Institute of Sport – National Research Institute

01-982 Warsaw

POLAND

Primary email: blair.crewther@insp.pl

Secondary email: blair.crewther@gmail.com

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Highlights

- The HPA- and HPG-axes contribute to allostatic regulation, reproductive success, and survival
- Reports of positive testosterone and cortisol relationships suggest facilitative cross-talk
- Within-person testosterone and cortisol coupling were tested in athletic and healthy men
- A lagged, negative cross-effect of cortisol on testosterone emerged in both cohorts
- The non-lagged, contemporaneous cortisol and testosterone relationships were positive
- Inhibitory and facilitatory hormonal actions appear to coexist on varying timescales

Abstract

Day-to-day coordination of the stress (i.e., hypothalamic-pituitary-adrenal [HPA]) and reproductive (i.e., hypothalamic-pituitary-gonadal [HPG]) axes is central to allostatic regulation, reproductive success, and survival. Reports of positive, within-person testosterone and cortisol relationships (or coupling) suggest cross-talk of a facilitative nature, but longitudinal evidence is scarce and has methodological and analytical limitations. To address this, we used a continuous-time (CT) model to investigate day-to-day, within-person coupling of testosterone and cortisol in two male cohorts. Salivary testosterone and cortisol fluctuations were monitored in 35 athletic men across two international tournaments (M = 19.3 tests) and in 41 healthy men during normal daily living (M = 27.9 tests). Bayesian CT analysis revealed a diminishing effect of each hormone on itself as time-interval length or lag increased. In both groups, cortisol had a negative lagged effect on testosterone that persisted for around three days. The cortisol effect on testosterone peaked after 0.71 and 0.51 days in athletic (standardized estimate = -0.13) and healthy men (standardized estimate = -0.11), respectively. Further estimates of non-lagged, contemporaneous correlations revealed positive testosterone and cortisol relationships (athlete r = 0.04, healthy r = 0.46). In summary, complex within-person HPA and HPG interplay emerged in two independent male cohorts. Specifically, a rising cortisol concentration was linked to a fall in testosterone concentration at later time points, but concurrently these hormones tended to rise and fall together. Our results suggest that inhibitory and facilitatory hormonal actions coexist on varying timescales, thereby expanding knowledge of HPG and HPA cross-talk in everyday life.

Keywords:

Androgens, Glucocorticoids, Neuroendocrine, State-Space Modeling, Sport, Rugby

1. Introduction

In daily life, reciprocal coordination of steroid hormones from the stress (i.e., hypothalamic-pituitary-adrenal [HPA]) and reproductive (i.e., hypothalamic-pituitary-gonadal [HPG]) axes is central to allostatic regulation, reproductive success, and survival [1-3]. Although historically viewed as being mutually antagonistic axes, whereby HPA activation (e.g., increasing cortisol secretion) inhibits or suppresses the HPG axis (e.g., lowering testosterone secretion), and vice versa [1, 2], accumulating reports now suggest cross-talk of a facilitative nature. This evidence includes positive within-person testosterone and cortisol associations (or coupling) within a day, across developmental stages, and across the lifespan [4-9].

Recent work also demonstrated positive testosterone and cortisol coupling at a pulsatile level [10, 11], subsequent to high-frequency (i.e., every 10 mins or 15-30 mins) sampling procedures within a day. As further novelty, one study on healthy older men revealed that testosterone was positively correlated to subsequent cortisol measurements with a peak lag (or time of maximum coupling) occurring at 60 mins [11]. Similarly, among healthy younger men, both testosterone and cortisol had a positive lagged effect on each other, with a peak lag observed at around 8 mins [10]. These lagged results could reflect upstream and/or downstream interconnections between the HPG- and HPA-axes that co-regulate hormone secretion via complex feedforward or feedback signals [1, 3]. Whatever the mechanism/s, the emergence of lagged, bidirectional effects of testosterone and cortisol on each other underscores more nuanced inter-axes communication in everyday life.

Longitudinal evidence of positive within-person testosterone and cortisol coupling is scarce in healthy men [12, 13], but supported by a secondary analysis of data on athletic men [14, 15]. This work is still limited by several factors, such as sparse sampling (≤10 time points) that prevents meaningful inferences at the day-to-day level, and use of models that conceptualize time as an exogenous predictor (e.g., hierarchical or mixed-effect modeling), a

common approach in this area [4-7, 9]. Considering time implicitly in the temporal order of measurements, as per continuous-time (CT) modeling to estimate the lagged effect of one process on itself (i.e., auto-effect) and on another process (i.e., cross-effect) [16], can shed light on how each hormonal signal evolves and dissipates over time, as well as bihormonal interplay that constitutes normal HPG and HPA functioning [1, 2]. Crucially, the CT auto-effects and cross-effects can be used to derive discrete-time (DT) estimates and process error variances, thereby enabling a more detailed assessment of hormone coupling. This includes estimation of lagged effects for any time interval of interest, both observed and unobserved [10], to increase the tenability of causal interpretations regarding hormonal cross-talk.

There is mounting evidence that contextual factors, like acute stress or prior stress exposure, can lead to stronger or more persistent HPG and HPA coupling [6, 9, 17, 18]. For instance, the stress of human competition promoted parallel, and correlated, increases in athlete testosterone and cortisol concentrations [18]. Elite athletes present a unique population that is exposed to acute and chronic stressors, as a normal part of the training process [19]. Athlete research indicates that positive coupling of these hormones, or hormones to performance (e.g., workloads, motivation), might be important adaptive features [14, 15, 20]. To date, no comparable study has examined elite athletes using intensive day-to-day sampling, whilst also comparing non-athletic men as a reference group. This research could extract more nuanced information on HPA and HPG interplay, as well as identify possible individual, contextual, and environmental dependencies; all of which could illuminate new signaling pathways for allostasis, reproduction, and survival.

This study investigated the day-to-day, within-person coupling of testosterone and cortisol concentrations in athletic and healthy men over periods of 29 (consecutive and non-consecutive) and 31 (consecutive) testing days, respectively. Using a CT model, we sought to describe three hormonal effects; (1) lagged *auto-effects* (i.e., effect of testosterone and cortisol

on themselves at later time points), (2) lagged *cross-effects* (i.e., effect of testosterone and cortisol on each other at later time points), and (3) non-lagged *contemporaneous* effects (i.e., testosterone and cortisol effects on matching time points) [21]. The first hypothesis was that negative auto-effects of testosterone and cortisol would materialize [10], which means that the lagged effect of each hormone on itself will decrease as time interval increases. The second hypothesis was that positive cross-effects of testosterone and cortisol would emerge [10, 11], along with positive contemporaneous relationships [4-9], whereby each hormone has a positive effect on the lagged and non-lagged measurements of the other hormone. Our final hypothesis was that the athletes, who were monitored across a stressful tournament, would exhibit stronger hormonal effects and relationships than healthy men, who were profiled during normal daily living.

2. Material and Methods

2.1. Participants

The athlete group comprised 35 professional male rugby players (M = 27.8, SD = 3.1 years), who were selected for an international training squad. Each athlete received a regular medical and health assessment in this environment. Written informed consent was given after the athletes received a briefing on the study protocols, risks, and benefits. Ethical approval was granted from the Human Research Ethics Committee at Swansea University, Swansea, as part of a larger project investigating elite athlete performance and recovery in tournament competition. The healthy group consisted of 41 male students (M = 20.2, SD = 2.2 years), who were recruited from a subject pool for a project examining hormonal correlates of men's psychology and behavior. The participants were pre-screened for any health and medical disorders, before providing written informed consent. Ethical approval was provided by the University of California (UC), Santa Barbara, Institutional Review Board.

2.2. Monitoring procedures

The athletic men were monitored intermittently across two international tournaments played in 2010 and 2011, where eight rugby matches were played over a 19-week period (from 9th November in 2010 to 20th March in 2011). This sampling period corresponded to the late Autumn, Winter, and early Spring months. Specific information regarding the weekly training and competition schedule for this team are detailed elsewhere [20]. Briefly, during each week of competition, a pre-breakfast (from 0800 to 0900 hours) saliva sample was taken across 1-3 training days (Monday to Friday), on the morning of competition (played on a Saturday or Sunday), and the recovery day that followed. The total number of sampling days was 29, but dispersed across consecutive and non-consecutive testing days. Samples were stored in a standard freezer after collection, before being shipped to the laboratory for long-term storage in a -80° C freezer. Not all athletes were monitored every week, because of variable team selections for each contest and governing rules (15 players + 8 substitutes), as well as unforeseen injuries and/or illness that excluded some participants. Additionally, no samples were taken during a competitive match played away from home, as international travel prohibited the storage and freezing of any samples collected. A total of 674 samples were collected for hormone determination (participant M = 19.3, SD = 7.9, 5 to 29 range).

The healthy men were scheduled for monitoring under normal living conditions over 31 consecutive days (from 27th January to 27th February in 2013), with this sampling period corresponding to the Winter months in the country of origin. On weekdays, the participants submitted a saliva sample at the laboratory, with the time of collection recorded (M = 1330 hours, SD = 1.12 hours). Please note that this time period differed from athletes, who were sampled in the early morning. On weekends, samples were self-collected by participants and stored in home freezers until delivery to the laboratory at their next visit. Due to a data

collection error, no information on sampling timing was recorded during the weekend. This detail was subsequently imputed, based on the mean times recorded for each participant, using the imputeTS package (version 3.2) in R [22]. To prevent samples thawing, instructions were given to place the frozen samples in a bag of ice and minimize transit time during delivery. Due to the frequent nature of testing, it was anticipated that some samples would be missed. A total of 1143 samples were collected for hormone testing (participant M = 27.9, SD = 2.5, 18 to 30 range).

2.3. Hormone testing

For both male cohorts, all saliva samples were self-collected into polypropylene tubes using a passive drool technique without artificial stimulation [6, 10, 12]. Similar instructions were given to stop eating at least 30 mins before scheduled sampling and, if required, a quick mouth rinse with water was permitted to improve saliva flow. Adhering to recommended guidelines [23], the samples collected were stored in a -80 ºC freezer and assayed within a month of collection. If a freezer was not immediately available, the samples were refrigerated for no more than 24 hours before freezing.

Samples from athletic men were tested for testosterone and cortisol concentrations using commercial enzyme-linked immunoassay (ELISA) kits (Salimetrics LLC, USA). The intra-assay coefficient of variation (CV) was < 3% on duplicate determinations with inter-assay CVs of < 12%. These assays were performed by the lead investigator at Swansea University. Samples from healthy men were shipped on dry ice for testing at the Hominoid Reproductive Ecology Laboratory, University of New Mexico. Testosterone was determined with the Salimetrics kit, with intra-assay and inter-assay CVs of < 3% and < 12.5%, respectively. Cortisol was measured with ELISA protocols (cortisol antibody, R4866) from the UC Davis Clinical Endocrinology Laboratory. The respective intra-assay and inter-assay

CVs were < 5.0% and < 13.2%. Each participants' samples were assayed in a single plate to eliminate inter-assay variance in hormone concentrations.

2.4. Analytical procedures

The study data were analyzed in the R (version 4.1.1) programming environment [24]. Descriptive statistics were computed for each hormonal variable after averaging the within-person results in each group. To identify the source (i.e., between- and within-person) of hormonal variability, an intraclass correlation coefficient (ICC) was calculated from the variances of a linear mixed-effect model [25]. As a further preliminary step, we tested whether testosterone is predictive of cortisol across concurrent (same day) measurements. To disaggregate the between- and within-person effects of testosterone, we followed recommendations to create two additional metrics [25, 26]. Testosterone was first centered between persons (testosterone_cb) to reflect the overall average score for each participant across all measurements, and then centered within a person (testosterone_cw) to reflect changes from each participant's average score on all measurements. Both metrics were entered simultaneously into a linear mixed-effects model, specified with a random slope (testosterone_cw) and random intercept (participant id), to predict cortisol.

As the main analysis, a Bayesian CT model was fitted separately to each male dataset using the ctsem package (version 3.6.0) in R [27]. Specifically, we fitted a first order, bivariate process model of testosterone and cortisol with two chains and 10000 iterations. To account for assay imprecision, we chose the same fixed measurement error variances for testosterone (0.102) and cortisol (0.139), based on prior work [10]. Other Bayesian CT settings are detailed in a supplemental file. Prior to analysis, each individual time series was within-person centered, to eliminate between-person variance, and then standardized (within a person) to aid model convergence [27]. Consequently, all CT parameters represent a pooled

average of within-person effects. Some data missingness was evident in the studied groups that could lead to biased parameter estimates. To counter this, the ctsem package uses full information maximum likelihood estimation to handle missing data [27]. We used ctsem's default priors, which are intended as weakly informative for typical conditions in the social sciences [28].

The key CT parameters of interest are the within-person *auto-effects* of testosterone and cortisol (i.e., lagged effect on themselves), and within-person *cross-effects* of testosterone and cortisol (i.e., lagged effect on each other). Secondary outcomes include; *diffusion variances* that reflect process error variance, or equivalently the residual or unexplained error variance (as in regular regression) in a time-series prediction model, and *diffusion covariances* that show interdependence in this process error variance. All parameters are presented with a 95% credibility interval (CI). A result was deemed meaningful or substantial if zero was not included in the 95% CI. The diffusion covariances were back-transformed into a contemporaneous correlation representing the average within-person, day-to-day correlation between testosterone and cortisol. To aid interpretation of the lagged outcomes, the CT auto-effects and cross-effects were converted into DT estimates for plotting at different time interval lengths. When the auto- and cross-effects from the CT framework are transformed into corresponding parameters in the DT framework, they are respectively termed *autoregressive* and *cross-lagged* effects [see Table 1 in Hecht and Voelkle [29] for an overview of terms for corresponding CT and DT parameters].

3. Results

Mean testosterone concentrations were found to be similar among athletic and healthy men, after averaging the participants results within each group (Table 1). However, the male athletes presented a much lower (~45%) mean cortisol concentration than healthy men, which

was likely due to a different ELISA kit. The within-person centering and standardization procedure (see above) accounted for this difference in our main CT analyses. The athlete ICC results indicate that only a small amount of the testosterone (16%) and cortisol (9%) variation originated at the between-person level; this being less than that observed among healthy men (testosterone ICC = 51%, cortisol ICC = 23%).

Insert Table 1 here.

Fluctuations in athlete testosterone concentration did not significantly predict cortisol, with regards to the person-averaged scores (testosterone_cb b = 0.00 [se = 0.00], beta = -0.01 [se = 0.05], $p = 0.799$) and the person-centered changes (testosterone_cw b = 0.00 [se = 0.01], beta = 0.02 [se = 0.06], $p = 0.795$). Among healthy men, the same analyses revealed significant positive effects of both between-person centered testosterone (b = 0.04 [se = 0.01], beta = 0.29 [se = 0.07], $p < 0.001$) and within-person centered testosterone (b = 0.04 [se = 0.01], beta = 0.35 [se = 0.04], $p < 0.001$) on cortisol. Hence, men with a higher mean testosterone level tended to have a higher mean cortisol level than men with a lower mean testosterone level; within-men, days with higher testosterone concentrations tended to have higher cortisol concentrations than days with lower testosterone levels. To aid interpretation, prediction plots are depicted for athletic (Figure 1A) and healthy men (Figure 1B) showing the marginal mean effects of testosterone_cb and testosterone_cw on cortisol concentrations.

Insert Figure 1 here.

The CT parameters are presented in Table 2. Within both groups, the negative auto-effects of testosterone and cortisol were found to be substantial, thereby indicating that

consecutive values of each process were related to itself to some degree. For the cross-effect parameters, the cortisol to testosterone association was meaningful in both groups, but not vice versa. This shows that cortisol measured at one time point is negatively associated with testosterone measured at a later time point. For each cohort, positive and substantial random error (diffusion variance) was seen in the testosterone and cortisol processes. The diffusion covariances were also positive and meaningful, indicating some commonality in process noise. Accordingly, and consistent with the mixed-effect models, the contemporaneous testosterone and cortisol correlation was positive and moderate (r = 0.46) in healthy men. The corresponding correlation was positive, but very weak (r = 0.04), in athletic men.

Insert Table 2 here.

The DT estimates for athletic (Figures 2A) and healthy men (Figure 2B) revealed similar general trends in the autoregressive effects of testosterone and cortisol. Each process was strongly and positively related to itself over shorter time intervals (e.g., cortisol concentration at a given time point is strongly related to measurements taken within the next 8-16 hours), before declining steadily to reach a zero value after ~3 days. The DT plots also allude to population-specific effects. That is, among athletic men, the testosterone effect on itself was less pronounced (i.e., steeper decline) than the cortisol-on-cortisol effect, with a reversal of this pattern seen in healthy men. The DT cross-lagged effect of cortisol on testosterone followed a U-shaped pattern, representing a negative association that was similar in magnitude and timing across both samples of men. When considering the 95% CI, the cross-lagged effect of cortisol on testosterone was found to be negative and substantial for up to ~3 days. The peak effect materialized at an interval length of around 0.71 days

(standardized estimate = -0.132) in male athletes and around 0.51 days (standardized estimate = -0.110) in the healthy group.

Insert Figure 2 here.

4. Discussion

This study represents an unprecedented investigation into day-to-day, within-person coupling of HPG and HPA hormones in athletic and healthy men. In partial support of our hypotheses, we found (1) an autoregressive effect of testosterone and cortisol on themselves, (2) a negative cross-lagged effect of cortisol on testosterone, but not vice versa, and (3) a positive contemporaneous relationship between these hormones. These results were consistent in both male cohorts. Contrary to expectations, no evidence emerged of stronger cross-lagged effects and contemporaneous relationships among athletic (vs. healthy) men.

The first major finding was a negative cross-lagged effect of cortisol on testosterone, whereby a rising cortisol concentration was followed by a fall in testosterone concentration at later time points. The positioning of this result in literature is difficult, as lagged analyses of HPA and/or HPG hormones are rare and limited to diurnal or circadian models [10, 11, 30]. This could explain the positive lagged effects of cortisol and testosterone on each other, as reported by some [10, 11], but not replicated in this work. Nevertheless, our results are consistent with the well-documented inhibitory or suppressive effect of glucocorticoids on the HPG axis [1, 3]. Both cohorts presented a similar cortisol effect on testosterone that persisted for up to ~3 days, with a peak response seen a little after half a day or more. Timewise, these connections are compatible with the biological activity of glucocorticoids [31] and androgens [32], which span a broad time range via non-genomic (i.e., secs to mins) and genomic (i.e., hours, days, and weeks) pathways. The exact mechanisms involved are still unclear, as

cortisol can affect testosterone production at different levels (e.g., organum vasculosum of the lamina terminals, anterior pituitary, gonads) of the HPG axis [1], or it could reflect upstream hypothalamic factors (e.g., arginine vasopressin, corticotropin-releasing hormone) that also target the HPG axis and regulate downstream cortisol release [1].

Positive testosterone and cortisol coupling was verified in both athletic (weak effect) and healthy (moderate effect) men, based on the contemporaneous correlations. This finding is congruent with longitudinal studies on untrained, non-elite and elite trained men (*r* values = 0.13 to 0.33) when time-matched samples were modeled [12-15]. Therefore, it appears that male testosterone and cortisol concentrations can rise and fall together, at least on a day-to-day level and when analyzing concurrent hormonal measurements. One important consideration is that acute stress can activate the HPG and HPA axes in similar ways, such that hormones might covary during a moment-to-moment assessment. Competitive sport, for example, often promotes a parallel and correlated rise in testosterone and cortisol concentrations [18, 33], as can a competitive learning environment [34], a brief encounter with potential mates [35], and a solo music recital with an audience [36]. The current study design, where participants provided a single daily sample and in the absence of proximal stress, likely circumvented any interpretive issues with momentary covariance in both hormones.

We found no evidence of stronger lagged or non-lagged hormonal interplay in the athletic population, relative to healthy men. This prediction was based on evidence that acute stress or chronic stress exposure can amplify, or more strongly maintain, coupled testosterone and cortisol responses [6, 9, 17, 18, 37, 38]. Our results do not refute this argument, as the male athletes were not exposed to any situational stress at time of sampling (i.e., before breakfast) and most samples were taken on training and recovery days. Attempts to model, separately, data on training-recovery days and competition were unsuccessful due to

convergency issues (e.g., insufficient observations) that preclude a model fitting to the data. Another consideration is that elite athletes undergo years of intensive training, both physiological and psychological, to ensure they tolerate well sport-related stressors [19]. Some cohort differences did surface with regards to the autoregressive effects of testosterone and cortisol, as well as the source of hormonal variance. Still, with athletes sampled in the early morning and healthy men in the afternoon, it's unclear whether these results are true population effects or simply differences in diurnal sampling and related factors (e.g. larger morning hormone secretion, time of awakening, amount of sleep).

The discovery of negative (cross-lagged) and positive (non-lagged) testosterone and cortisol relationships, across two independent male cohorts, is unique in this area. This implies that inhibitory and facilitatory cross-talk between the HPG- and HPA-axes might co-exist, and is perhaps another example of flexible responding seen in vertebrates [1, 3]. Whilst this seems to be counterintuitive, it is conceivable that cortisol and testosterone fluctuations might covary during a momentary assessment (e.g., due to co-secretion of pulses or similar baseline shifts), and yet an elevation in cortisol secretion might lead to a decline in testosterone secretion several hours or days later (e.g., due to mechanisms governing feedback control). In fact, the non-genomic actions of glucocorticoids, like cortisol, may well prepare target cells for chronic adaptations and induce early adaptive changes that are opposite to genomic effects [31]. Being able to signal divergent (yet complementary) responses on varying time scales, as we demonstrated, offers a myriad of strategies to achieve allostatic regulation, reproductive success, and ultimately survival.

This study underscores the potential of CT modeling to characterize the coevolution of the stress and reproductive axes with respect to time, which itself offers a new lens for exploring and interpreting the diverse nature of inter-axes communication in everyday life. Because stress was only an implied construct in this work, it would be prudent to expand the

bivariate CT framework to include a population-specific measure of stress (e.g., training loads for athletes, burnout among office workers, academic stress in students) to ascertain how it affects, and is affected by, HPG and HPA hormones. Studies utilizing hair cortisol as a biomarker would clearly benefit from CT models, due to a temporally delayed effect of stress on hair cortisol concentration that might manifest over several weeks [39]. Sports research indicates that daily or phase shifts in testosterone and cortisol concentrations (in both sexes) are related to broad functional outcomes, like voluntary effort [14], motivation to train [15, 20], and stress reactivity [40]; hence, including relevant indices of performance could also prove useful in deconstructing pathways to optimal physical condition or general well-being.

Whilst this work advances knowledge and assessment of daily HPG and HPA interplay, some limitations do apply. The descriptive nature of data collection limits strong causative claims and only a single daily sample was taken for hormone profiling. Moreover, the cross-lagged peaks should be interpreted with care, given their estimation within the shortest sampling interval of ~1 day. A recent report [41] also highlighted the importance of process dissipation rates, when interpreting cross-lagged networks with a different sign from correlated processes. An in-depth discussion is beyond the scope of this work; see Driver [41] for more details. The small number of participants per group is another limitation, but was compensated for by the large number of repeated observations, thereby increasing CT model estimation performance [42]. Definitive sample size recommendations for popular CT models are not yet available. Finally, parameter comparisons between groups were qualitative in nature. Although a single CT model can be constructed for multiple groups using ctsem software, this approach was not feasible due to sampling differences. Indeed, other factors preclude direct comparisons, such as participant age (i.e., athletes were older than healthy men – $p < 0.001$), differences in sampling times, the sampling period and seasonality (see methods), as well as possible time-of-day effects on hormone coupling.

The use of salivary hormones is another consideration. A comprehensive study [43] compared four commercial ELISAs (DSL, Salimetrics, IBL, DRG) and an in-house method (DELFIA) for assessing salivary cortisol in the same samples. Differences in mean cortisol concentration, expressed in nmol/L, were substantial (DSL M = 48.13, SD = 44.48; Salimetrics M = 17.12, SD = 22.82; IBL M = 23.46, SD = 23.38; DRG M = 22.86, SD = 24.24; DELFIA M = 15.97, SD = 20.12) and could explain the large cortisol difference observed herein. These results were also inflated compared to reference cortisol values (M = 13.44, SD = 20.65 nmol/L) determined using tandem liquid chromatography-mass spectrometry, as demonstrated by others [44]. Such findings confirm measurement bias in cortisol determination using different ELISA kits, as well as immunoassay overestimation of salivary cortisol from reference data, due to a combination of different calibrator set points, cross-reactivity and protein interference effects [43, 44]. We eliminated this fixed bias by within-person standardization of both hormones prior to CT modeling. Relative bias appears to be less of an issue, as all salivary cortisol measurements were strongly interrelated (e.g., Pearson r = 0.86-0.99) in this work [43, 44].

5. Conclusions

The CT modeling of densely-sampled data on athletic and healthy men revealed complex HPG and HPA interplay, with distinct outcomes emerging when lagged (i.e., negative effect of cortisol on testosterone) and non-lagged (i.e., positive cortisol and testosterone relationship) within-person relationships were estimated. The co-existence of inhibitory and facilitatory hormonal actions is a novel, yet plausible, feature of HPG and HPA cross-talk. These findings also underscore CT analyses as a flexible framework to decode when, and how, different hormonal processes communicate in everyday life.

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Conflicts of interest

The authors report that there are no competing interests to declare.

Data availability

The research data collected are unavailable due to confidentiality agreements.

Supplemental materials

The R code used for the Bayesian CT analyses are provided as a supplemental file.

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Table 1. Descriptive statistics for the testosterone and cortisol concentration measures in athletic and healthy men.

	Athletic men (N = 35)			Healthy men (N = 41)		
Variable	Mean	SD	ICC	Mean	SD	ICC
Testosterone (pg/mL)	148.9	41.9	0.16	145.8	38.1	0.51
Cortisol (ng/mL)	3.69	1.70	0.09	6.75	3.66	0.23

Note: ICC = intra-class correlation coefficient.

Table 2. Continuous-time parameter estimates for the bivariate processes of testosterone and cortisol among athletic and healthy men. Data are presented as a posterior 50% median with a 95% credibility interval (CI).

	Athletic men			Healthy men		
Parameter	Est.	SD	[95% CI]	Est.	SD	[95% CI]
Auto-effects						
Testosterone → Testosterone	-1.85	0.14	[-2.15, -1.59]	-1.23	0.17	[-1.59, -0.94]
Cortisol → Cortisol	-1.23	0.11	[-1.45, -1.01]	-2.58	0.47	[-3.56, -1.73]
Cross-effects						
Testosterone → Cortisol	-0.03	0.06	[-0.15, 0.08]	0.06	0.24	[-0.43, 0.52]
Cortisol → Testosterone	-0.55	0.06	[-0.66, -0.44]	-0.59	0.31	[-1.19, -0.02]
Diffusion variance						
Testosterone	1.75	0.19	[1.41, 2.15]	1.61	0.08	[1.45, 1.77]
Cortisol	1.37	0.16	[1.11, 1.72]	2.04	0.18	[1.71, 2.41]
Diffusion covariance						
Testosterone ↔ Cortisol	0.12	0.03	[0.06, 0.18]	0.33	0.04	[0.24, 0.41]

The arrows represent the direction of the estimated effect.

Figure 1. Prediction plots for the testosterone and cortisol concentration measures in athletic (1A) and healthy men (1B). Each plot shows the marginal main effect of testosterone (solid blue line) with a 95% confidence interval (shaded area). Note that the testosterone-centered values represent deviation from the between- and within-person means. Testosterone_cb = testosterone centered between persons, Testosterone_cw = testosterone centered within a person.

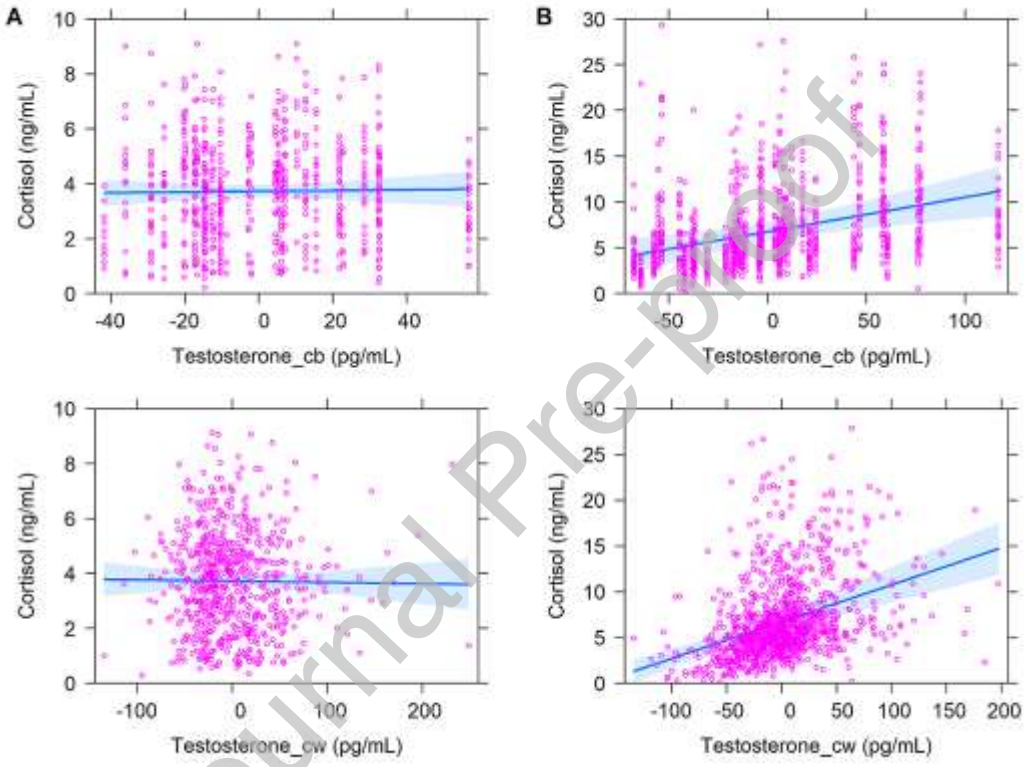

Figure 2. Discrete-time autoregressive effects of testosterone and cortisol (on themselves) and standardized cross-lagged effects of testosterone and cortisol (on each other) among athletic (2A) and healthy men (2B). Data are plotted as a posterior median (solid line) with a 95% credibility interval (dotted lines). The arrows represent the direction of the estimated effect.