Summated Training and Match Load Predictors of Salivary Immunoglobulin-A, Alpha-Amylase, Testosterone, Cortisol and T:C Profile Changes in Elite-Level Professional Football Players: A Longitudinal Analysis.

Original Investigation.

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Title

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Running Title

Training and Match Load Predictors of Salivary Biomarker Changes in Professional Football Players.

Abstract

We examined how summated training and match load measures relate to salivary immunological and hormonal profile changes in professional football players. Data were collected from 18 elite-level professional male football players from one English Championship team across a complete 40 wk competitive season. Daily training (micro-technology) and match (computerised tracking) measures of total, high-speed and high-metabolic load running distance and sprint, acceleration, deceleration and sRPE load were converted into exponentially weighted moving average ‘acute’ (7d), ‘chronic’ (28d) and acute:chronic composite load measures. Bi-weekly morning saliva samples were analysed for immunoglobulin-A, alpha-amylase, testosterone, cortisol and testosterone:cortisol. A two-stage data reduction technique using partial least squares modelling and a backward stepwise selection procedure determined the most parsimonious model for each salivary variable. Testosterone had non-linear

relationships with chronic total (P=0.015; Cohen's D: large), high-metabolic load (P=0.001;small) and high-speed (P=0.001;trivial) running distance and linear relationships with chronic sRPE (P=0.002;moderate↓) and acute:chronic high-speed running distance (P=0.001; trivial↑). Cortisol had a non-linear relationship with chronic high-speed running distance (P=0.001;trivial). Testosterone:cortisol had non-linear relationships with chronic decelerations (P=0.039;small) and chronic summated acceleration and deceleration load (P=0.039;small). Non-linear relationships typically indicated optimal hormonal responses at squad mean loads. No load variables clearly related to salivary immunoglobulin-A or alpha-amylase changes. We conclude that chronic total and high-intensity load measures relate to hormonal changes and might be useful indicators of player readiness. Acute load variables were not related to immunological or hormonal changes and consequently, should not be used as surrogate measures of player readiness in isolation.

Keywords: Football; Monitoring; Stress; Saliva; Immunology; Endocrinology

Introduction

Professional association football training and match play are high-intensity, high-volume activities. The competitive season is long (i.e., 40 – 42 wks) and characterised by frequent, clustered periods of high game density (i.e., when players are required to play 2 games in 7 d) [1]. Consequently, imbalance to the relationship between summated training and match load ('load') and recovery can occur, resulting in maladaptive training; denoted by negative changes in a biological system in response to external load or inadequate recovery [2-4].

Individualised, multivariate, concurrent monitoring of internal and external training and match load, alongside biological fatigue measures (i.e., immunological or hormonal measures used to quantify the physiological response to load [2-5]), are advocated to determine the load-recovery relationship, and mitigate the risk of maladaptive training [2-4]. In football, load is readily monitored using indices derived from Global Positioning Systems (GPS), Micro-Electrical Mechanical (MEMS) sensors and computerised tracking technology [6]. Of these, total distance (TD), high-speed running distance (HSR), sprint, acceleration, deceleration and metabolic power measures (i.e., high metabolic load distance (HMLd)) are most frequently used in practice [6] and research [7-9]. It has recently been recommended that load indices should be converted into composite values to reflect 'acute' ((A) ~ 7 d average load; proposed to be analogous to player 'fatigue') and 'chronic' ((C) ~ 28 d average load; proposed to be analogous to player 'fitness') load, and the acute : chronic (A:C) load ratio in order to indicate player 'readiness' (to accept new load [3]) [3,4]. To date, composite load measures have demonstrated relationships with injury risk [7,8] and match play physical performance [9] in football players.

Biological fatigue measures can be collected around games (i.e., ~ 24 – 48 h pre- and post- match) [6] to indicate player recovery status [2-4]. Owing to high game frequencies in football, regular (often bi-weekly) monitoring is warranted to facilitate timely player load management decision making. Consequently, measures that impart minimal psychophysiological stress (i.e., those that are not fatiguing or invasive) and have fast result availability are preferable. As such, resting salivary measures of immunological

and hormonal status are popular [6] because sample collection is fast (~ 30 s), non-invasive and results are available rapidly [10-14].

Salivary immunoglobulin-A (s-IgA) and α-amylase (s-AA) are antimicrobial proteins, secreted by mucosal cells under sympathetic adrenal medullary (SAM) axis regulation [15]. Prolonged, excessive psychophysiological stress (i.e., by excessive training and/or match load or inadequate recovery) can reduce s-IgA and s-AA secretion, and compromise mucosal immunity [15]. To date, reductions in s-IgA have been associated with increases in upper respiratory tract infection (URTI) risk in football players [11], and both s-IgA and s-AA have demonstrated the ability to track changes in load in football players [13,16] and professional [17] and Paralympic [18,19] swimmers.

Testosterone (T) and cortisol (C) are steroid hormones, detectable in saliva (s-T, s-C) [15], that reflect anabolic (s-T) and catabolic (s-C) balance (s-T:C) [20]. Their secretion is regulated by the hypothalamic pituitary adrenal (HPA) (s-T and s-C) and hypothalamic pituitary gonadal (HPG) (s-T) axes. Football match play typically induces acute increases in C, equivocal changes to T but reductions in T:C, signalling a catabolic state, that can manifest for ~ 24 – 72 h [20]. Longitudinally, 25% and 35% increases in C have been reported during sustained periods of increased load [21] and game density [22] in football players. Since muscular recovery is augmented in anabolic environments [23], s-T, s-C and s-T:C are considered as useful indicators of athletic readiness [15]. Collectively, owing to their reactivity to SAM, HPA and HPG axis activation, salivary immunological (s-IgA, s-AA) and hormonal (s-T, s-C, s-T:C) measures are considered as useful indicators of holistic stress balance (i.e., from the psychophysiological stress

derived from both sport and non-sport means [2-4]) and the load-recovery relationship in athletes [15] and football players [13,16,20-22,24-26].

High acute loads have been associated with increased injury risk [7,8] and compromised match play physical performances in football players [9]. Conversely, high chronic loads have been associated with reduced injury risk [7,8] and improved high-intensity match play physical performances [9]. These findings have typically been attributed to the effects of 'fatigue' and 'fitness', based on the premise that 'acute' and 'chronic' load indices are analogous to 'fatigue' and 'fitness' status. However, the relationships between composite load indices and biological fatigue measures are yet to be empirically evaluated to test these assumptions. Indeed, no longitudinal empirical investigations have examined if composite load indices relate to immunological (s-IgA, s-AA) or hormonal (s-C, s-T, s-T:C) profile changes in football players. To optimally support player health and performance, it is clearly important to understand how composite load measures relate to biological fatigue measures. Accordingly, the aim of this investigation was to investigate how composite measures of summated training and match load (TD, HSR, sprint, acceleration, deceleration and HMLd) relate to biological fatigue measures (s-IgA, s-AA, s-T, s-C and s-T:C) in elite-level professional football players.

Materials and Methods

Study design

Daily training and/or match load measures and bi-weekly resting saliva samples were collected from 18 senior professional male outfield players (age = 24 $\pm$ 4 years; height

= 181 ± 7.0 cm, body mass = 72.4 ± 5.2 kg) from one English Championship (EC) team across one complete season. Informed consent was obtained from all participants prior to data collection and an ethics declaration was approved for this investigation by the Edith Cowan University (Australia) Human Research Ethics Office.

Training load

Training load was recorded for all pre-season and in-season training sessions. External load was measured using sports GPS and MEMS sensors (Statsports Viper 2, Belfast, Northern Ireland, UK), sampling at 10 Hz (GPS) and 100 Hz (tri-axial accelerometer, gyroscope and magnetometer). Typical error for distance and speed for this device are < 3% and < 2% [27] respectively. A software application (www.gnssplanning.com) reported previously [9], was used to identify a geographical point (ground station) based on the latitude and longitude coordinates of the team training facility. This determined the mean number of satellites and horizontal dilution of precision for GPS data across the sample period, which equated to 8.7 ± 1.0 and 0.66 ± 0.08 % respectively; indicating optimal conditions for satellite transmissions [28].

Players wore the same GPS device for all training sessions. Devices were worn in a neoprene vest, between the scapulae as per manufacturer guidelines. Load variable selection was based on use in practice [6] and similar scientific research literature relating to load quantification in elite level professional football [7-9]. Total distance – (total distance completed (m)); high-speed running (HSR) – (total distance completed between 5.5 m/s and 80% of individualised maximal linear running velocity (m)); high metabolic load distance (HMLd) – (distance covered when energy consumption per kilogram per second is > 25 W/kg^{-1} (m)); number of sprints (total number of sprint

efforts > 80% of individualised maximal linear running velocity); and high intensity variables: total number of accelerations (ACC), decelerations (DEC) and changes to speed (ACC+DEC) were recorded. Acceleration and DEC efforts were identified according to manufacturer guidelines as a change in player velocity of > 0.5 m/s^2 maintained for > 0.5 s. Efforts were zone-banded based on the peak magnitude of ACC or DEC with thresholds set at > 3 m/s^2 and > -3 m/s^2 respectively. These thresholds are consistent with those reported elsewhere in the football science research literature [9,29-32]. Training load data were extracted from GPS devices using manufacturer software (Statsports Viper, Belfast, Northern Ireland, UK). Internal load was recorded using player rating of perceived exertion (RPE) from the CR-10-scale [33]. CR-10 response was collected within 30 min of all training sessions and multiplied by session duration (min) to provide an arbitrary unit (AU) of session load, denoted as sRPE. This method has been validated for use in football previously [34]. Data collection and analysis was completed by the same investigator across the entire sample period.

Match load

Match load was recorded for all home and away games. External load variables were measured using 6 fixed semi-automated high definition motion cameras (Chyronhego TRACKAB, London, UK). Following games, raw TRACKAB player position data were converted to equivalent training load variables using manufacturer software (Statsports Viper, Belfast, Northern Ireland, UK). This method has been described previously [35], and is widely used in practice and scientific research literature [7,9,35]. Strong relationships are reported between Statsports Viper and TRACKAB for TD (r^2 = 0.98) and HSR (r^2 = 0.98) [35] and our unpublished data indicate strong relationships for HMLd (r^2 = 0.93), ACC (r^2 = 0.94), DEC (r^2 = 0.95) and number of sprints (r^2 = 0.97)

196 using this method during elite-level professional football match play. Internal match
197 load was calculated using the same sRPE method as was used following training.
198
199 ***Composite load indices***
200 For each load variable, the pooled (summated training and match derived measures)
201 7 d absolute sum, 28 d absolute sum, exponentially weighted moving average
202 (EWMA) acute load, EWMA chronic load and the EWMA acute : chronic load ratio
203 (A:C) were calculated. EWMA indices were calculated using equations by Williams
204 and colleagues [36]:
205
206 $$EWMA_{today} = Load_{today} * \lambda_a + ((1 - \lambda_a) * EMWA_{yesterday})$$
207
208 Where λ_a represents the degree of time decay. Time decay was calculated using:
209
210 $$\lambda_a = 2/(N + 1)$$
211
212 Where *N* is the chosen time decay constant. Decay factors representing time
213 constants for 7 d (acute) and 28 d (chronic) were used. These equated to 0.25 and
214 0.069 respectively.
215
216 ***Saliva Sampling***
217 Saliva samples were collected the morning after rest and / or recovery days across
218 the sample period. Typically, this was two days prior (i.e., match day (MD) -2) and two
219 days after (i.e., MD +2) games during both single and double game weeks. Baseline
220 saliva measures were calculated for individual players as the mean of MD-2 data

collected during single game weeks in the first 5-week in-season mesocycle. We reasoned that this best represented when player 'fitness' was high (i.e., following pre-season), when 'fatigue' was low (i.e., early in the competitive season, following a recovery day during single game weeks) and thus when player holistic stress balance was optimal. Players reported to the team training facility between 09:00 and 09:30 on sample collection days. They were asked to abstain from caffeine consumption prior to sample collection and samples were collected prior to breakfast and training. They were asked to sit quietly, swallow existing saliva in the mouth and to then place an oral fluid collector (OFC; SOMA Bioscience, Wallingford, UK) on the tongue. With the mouth closed, 0.5 ml of saliva was collected, as indicated by a volume adequacy indictor on the OFC. The OFC was then placed into 3 ml of buffer solution in a bespoke 10 ml container (OFC Buffer; SOMA Bioscience, Wallingford, UK) and mixed gently by hand for 2 min.

Salivary IgA and Cortisol

Two drops of the OFC sample were applied to two lateral flow immunochromatographic (LFI; SOMA Bioscience, Wallingford, UK) test strips: which captured s-IgA and s-C at test and control reagent lines within a solid base nitrocellulose membrane. After a 5 min incubation period, the LFI strips were inserted into a lateral flow device reader (LFD; SOMA Bioscience, Wallingford, UK), which used signal intensity to provide quantifiable values for s-IgA (μg/ml) and s-C (nM). These were determined using specifically programmed curves assigned to the LFI strips, provided by the manufacturer (SOMA Bioscience, Wallingford, UK). Analysis of s-IgA and s-C was conducted by the same researcher across the entire sample period; who had ~ 10 years' experience in sample collection and analysis using this method in the

applied football environment. Comparison of the LFD method with the enzyme-linked immunosorbent assay (ELISA) method indicates strong validity for s-IgA (r = 0.93; *P* < 0.001) [10] and s-C (r^2 = 0.79) [37] . Repeated sampling indicates strong reliability for s-IgA (ICC r = 0.89, *P* < 0.001 and CV = 9.4%) [10] and s-C (CV = 6.8%) [37].

Salivary α-Amylase and Testosterone

The remaining OFC buffer solution was sealed and taken to a private laboratory (SOMA Bioscience, Wallingford, UK) where s-AA (μg/ml) and s-T (pg/ml) were measured by ELISA using enzyme immunoassay test kits (EIA; SOMA Bioscience, Wallingford, UK), and an automated analyser (Tecan Nanoquant, Tecan, Männedorf, Switzerland) as per manufacturer guidelines. Following analysis, s-T was converted to its molar value to calculate s-T:C. All analysis was completed by the same laboratory technician. All samples were analysed within 24 h of collection. The intra- and inter- assay CV for s-AA and s-T analysis using this method is 4.71% and 11.4%; and 7.94% and 9.4% respectively; as reported in other applied environments [38].

Statistical analysis

Statistical analysis was conducted using *R* (version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria). Individual salivary measures were associated with the EWMA 7 d ‘acute’ and 28 d ‘chronic’ load measures summated up to the end of the previous day. The season was divided into nine equal 5-wk mesocycles (one preseason and eight in-season phases). ‘Phase of season’ was then modelled as a re-scaled linear effect to represent the linearised effect of ‘readiness’ for each salivary variable across the season. This was then included as a covariate to help to control for any potential longitudinal effects (i.e., to changes in player ‘readiness’ across the

season). A two-stage data reduction process was then used to determine the most parsimonious model for each salivary biomarker.

First, the 'multivariate methods with unbiased variable selection' ('*MUVR*') algorithm [39] was used to identify the minimal-optimal candidate load predictor variables for each salivary variable. The MUVR package is an algorithm for multivariate modelling, aimed at finding associations between predictor data (an X matrix) and a response (a Y vector) via partial least squares modelling. MUVR is useful for handling data that has large numbers of variables and few observations, and constructs robust, parsimonious multivariate models that generalize well, minimize overfitting and facilitate interpretation of results [39].

Second, the candidate training and match load predictor variables identified for each salivary measure were entered into a backward stepwise selection procedure to identify the best-fitting overall model. Quadratic polynomials and interaction effects between predictors were considered as part of this process. Quadratic models explored the possibility of non-linear relationships by including a squared predictor term in the model; if this term was significant and improved the model fit (based on likelihood ratio tests), the quadratic term was retained and presented as such. If not, then a linear model was used to assess the relationship between the predictor and outcome variable. Player identity was included as a random effect to account for repeated observations within players. Effects were deemed to be statistically significant at an alpha level of $P < 0.05$. Data are presented as means and 95% confidence intervals (CI), alongside Cohen's *d* effect sizes (ES) [40]. These were estimated from the estimated marginal means and the 'sigma'/SD taken from the

random effects term of the mixed model. Thresholds for ES were: 0.0-0.2 = *Trivial*; 0.2-0.6 = *Small*; 0.6-1.2 = *Moderate*; 1.2-2 = *Large*; >2 = *Very Large*. The conditional R^2 value (which considers both fixed and random effects in the model) is also provided as a goodness-of-fit measure for these relationships. Data for non-linear relationships is presented as means and 95% CI with estimated salivary variable responses at typically very low (-2 SD), low (-1 SD), mean, high (+ 1 SD) and very high (+2 SD) values of each training and match load predictor variable .

Results

Predictors of Salivary Proteins

s-IgA

Only a linear effect of phase of season (P = 0.011, ES = *Trivial* ↑) (*Supplementary Table 1*) was retained from the variable selection process for s-IgA.

s-AA

Only a linear effect of phase of season (P < 0.001, ES = *Small* ↓) (*Supplementary Table 2*) was retained from the variable selection process for s-AA.

Predictors of Salivary Hormones

s-T

Six variables were retained from the variable selection process for s-T (Table 1). Linear effects were identified for phase of season (P = 0.004, ES = *Trivial* ↓), chronic sRPE (P = 0.002, ES = *Moderate* ↓) and A:C HSR (P = 0.011, ES = *Trivial* ↑). Non-linear effects were identified for chronic TD (P = 0.015, ES = *Large*) (Figure 1, Panel

A), chronic HSR (*P* = 0.001, ES = *Trivial*) (Figure 1, Panel B) and chronic HMLd (*P* = 0.001, ES = *Small*) (Figure 1, Panel C). For TD, s-T was highest at very high chronic load (+2 SD). For HSR, s-T was highest at very low (-2 SD) and very high (+2 SD) chronic load. For HMLd, s-T was highest at squad mean chronic load.

****Insert Table 1 Here****

****Insert Figure 1 Here****

s-C

Two variables were retained from the variable selection process for s-C (Table 2): a linear effect for phase of season (*P* < 0.001, ES = *Small* ↓) and a non-linear effect for chronic HSR (P = 0.001, ES = *Trivial*). For chronic HSR, s-C was lowest at squad mean chronic load and highest at very low (-2 SD) and very high (+2 SD) chronic load (Figure 1, Panel D).

****Insert Table 2 Here****

s-T:C

Four variables were retained from the variable selection process for s-T:C (Table 3). Linear effects were identified for phase of season (*P* = < 0.001, ES = *Small* ↑) and chronic HSR (*P* = 0.554, ES = *Trivial* ↑). Non-linear effects were identified for chronic DEC (*P* = 0.039, ES = *small*) (Figure 1, Panel E) and chronic ACC+DEC (*P* = 0.039, ES = *Small*) (Figure 1, Panel F). For chronic DEC, s-T:C was highest at squad mean

chronic load. For ACC+DEC, s-T:C was highest at very low (-2 SD) and very high (+2 SD) chronic load.

Insert Table 3 Here

Discussion

The aim of this investigation was to examine the relationships between composite load measures and salivary immune (s-IgA, s-AA) and hormone (s-T, s-C, s-T:C) profile changes in elite-level professional football players. Chronic (for TD, HSR, HMLd and sRPE) and acute:chronic (for HSR) load variables related to hormonal profile changes (s-T, s-C, s-T:C), exerting *trivial* to *large* effects. No load variables were associated with s-IgA or s-AA profile changes. Results indicate that chronic total and high-intensity load measures might be useful indicators of player readiness because they relate to hormonal profile changes, which has been identified as an important element of the holistic stress balance model [2-4]. However, acute load variables did not relate to immunological or hormonal profile changes, which questions their use as contributing measures of player readiness in isolation.

The most important finding from this investigation is the *large* non-linear relationship identified between chronic TD and s-T. For this relationship, increases in chronic TD were associated with increases in s-T, with the greatest s-T values observed at +2 SD of chronic TD (Table 1 and Figure 1, Panel A). 'Chronic' measures of load indicate medium-to-long-term training and match load exposure (28 d) and are proposed to be analogous to 'fitness' status [41]. Since TD is a global measure of training volume [6], this

relationship suggests that chronic training *volume* might be an important regulator of T concentration in football players. Previous studies have demonstrated an unclear relationship between load and T concentration in this population. For example, sustained periods of high load have been associated with equivocal [21], increasing [26] and decreasing [22,25] effects on T concentration in football players. However, these investigations are somewhat limited by infrequent hormonal sampling [21,22,25], short sampling periods [22,25], limited load variable reporting [21,22,25,26] or the use of sub-elite players [25]. Comparatively, the current investigation employed daily multivariate load monitoring and bi-weekly hormonal sampling across a complete competitive season in elite-level professional players. Accordingly, the study design and methods employed herein might facilitate a more sensitive analysis. Our result is consistent with findings from other researchers, reporting increases in resting T among elite-level professional rugby union players following periods of high chronic load (21 d) [42]. It is possible that a high chronic training volume up-regulates the HPG axis, serving to increase T concentration. Indeed, this mechanism has previously been proposed to explain temporal increases in s-T in football players [20], and might also help to explain the significant, albeit *small* 'U' shaped relationship identified herein between chronic ACC+DEC and s-T:C (Table 3 and Figure 1, Panel F), for which *very high* loads were associated with optimal s-T:C responses.

Interestingly, our analysis also identified a *moderate* negative linear relationship between chronic sRPE load and s-T (Table 1), suggesting that high chronic *internal* load compromised s-T concentration. This result contrasts recent findings, indicating a positive linear relationship between these variables [26]. Rowell and colleagues [26] suggested that high chronic internal loads might facilitate increases in s-T secretion,

but did not propose an explanatory mechanism. Session RPE is an internal training load measure, used to quantify training stress by multiplying perceived effort and session duration [33,41]. Of note, *excessive* load and / or inadequate recovery are implicated as the dominant causes of maladaptive training [2-4], which in-turn, can disturb HPG axis function and reduce T secretion [24]. Therefore, it is possible that our finding is explained by a disturbance to HPG axis function during periods of excessive internal load across the sample period. Maladaptive training is most likely to occur during sustained periods of high game density or training load in football [13], both of which are commonplace in the English Championship [1]. Collectively, the relationship between chronic TD and s-T indicates that high chronic training volume might increase s-T concentration, while the chronic sRPE – s-T relationship indicates that excessive chronic *internal* load might compromise the response. Thus, chronic *high-intensity* training volume might have important interactive effects on T secretion in football players.

We also observed *small* non-linear (inverted 'U' shaped) relationships between chronic HMLd and s-T (Table 1 and Figure 1, Panel C) and between chronic DEC and T:C (Table 3 and Figure 1, Panel E). For these relationships, s-T and s-T:C responses increased across *very low* to *mean* chronic HMLd and DEC loads but decreased thereafter through *high* to *very high* loads. Collectively, these relationships suggest optimum loading 'zones' (at approximately squad mean chronic load, herein) for determining player T and T:C profiles. Of note, HMLd accounts for acceleration, deceleration, sprinting and HSR activity (in any combination), and consequently, is considered a 'global' measure of high-intensity load [9]. Chronic DEC is a measure of exposure to negative change in speed, which has a very high mechanical demand at

the threshold employed herein (> - 3 m/s^2) [29]. Thus, these relationships also implicate chronic high-intensity training volume as an important moderating factor for T and T:C profile changes in football players. Moreover, these findings indicate merit in ensuring that players are exposed to appropriate chronic HMLd and DEC loads to optimise T and T:C responses, but to avoid *excessive* chronic HMLd and DEC loads (i.e., +1 and +2 SD of chronic HMLd and DEC training and match load herein), since these scenarios might compromise the hormonal response.

The notion that chronic high-intensity training volume can exert an important influence on hormonal profile is also supported somewhat by the significant, albeit *trivial*, 'U'-shaped relationship identified between s-C and chronic HSR (Table 2 and Figure 1, Panel D). For this relationship, the s-C response was highest at *very low* and *very high* chronic HSR load and was lowest at approximately the squad mean HSR load. Cortisol is secreted in response to HPA axis activation and is used as a quantitative stress biomarker in athletes [17]. Accordingly, this suggests that periods of low 'fitness' (i.e., when chronic HSR is *very low*) and high 'fatigue' (i.e., when chronic HSR is *very high*) exert compromising effects on C concentration in football players. Interestingly, this finding is consistent with previous research, reporting increases in s-C during periods of increased training intensity [12] and load [21] in football players. Practically, the nature of this relationship indicates merit in exposing players to an appropriate chronic HSR load (to optimise the C response) but to avoid excessively low (i.e., -2 and -1 SD) and high (i.e., +1 and +2 SD) chronic HSR loads, since this might compromise the C response.

Surprisingly, no training and match load variables related to s-IgA or s-AA profile changes. This contrasts previous research, indicating that s-IgA and s-AA measures are sensitive to changes in load in football players [13,14,16] and professional [17] and Paralympic [18,19] swimmers. Indeed, existing data typically indicate reductions in s-IgA in response to acute [14] and chronic [13,16] periods of increased load in football players. We propose several explanations for this finding. First, consistent with previous recommendations, [36] we quantified 'acute' load using an EWMA 7 d decay factor, spanning 168 hr of training and competition time. Though equivalent data are unavailable for s-AA, s-IgA is reported to normalise in ~ 18 to 60 h following training and match-play [43] in football players, respectively. Thus, it is possible that s-IgA measures are not sensitive to load accrued > 60 h preceding sample collection. Indeed, s-IgA measures might indicate short-term (i.e., 1-3 d), but not long-term (i.e., 4-7 d) stress balance within-microcycles in football players. It is also possible that our finding is explained somewhat by the effect of non-training related stress on SNS activation. Indeed, lifestyle factors and other sources of psychophysiological stress that were not quantified in the current investigation could have 'masked' load-induced secretory changes to s-IgA and s-AA. For example, it is known that both s-IgA and s-AA are sensitive to changes in psychological stress [15]. Importantly, since acute load variables did not relate to any of the salivary biomarkers, it is evident that EWMA 7 d 'acute' and A:C measures should not be used as surrogate measures of player 'fatigue' status in isolation.

Strengths and Limitations

The strengths of this investigation relate to the participation level of the cohort, the study duration and the sampling frequency. Indeed, load was measured daily and salivary variables were analysed bi-weekly in a sample of 18 elite-level professional football players for ~ 1 year. However, the authors acknowledge several limitations. Firstly, data were collected from a single team and we acknowledge that players from other cohorts might respond differently owing to intra- and inter- team factors (i.e., variance in individual and team physical, technical, tactical and psychological preparation methods, and exposure to non-sport related stressors). Secondly, we acknowledge the relatively high variability of some point of care salivary analysis variables and recognise that this might account for some trivial interactions reported. Thirdly, as per manufacturer guidelines, we did not screen saliva samples for blood contamination, but acknowledge that this might affect the accuracy and validity of some findings. Accordingly, some caution is advised when interpreting these results. Fourthly, the authors acknowledge recent scientific literature proposing methodologic limitations of using 'acute' and 'chronic' load monitoring variables as surrogate measures of 'fatigue' and 'fitness' status (respectively) [44]. Accordingly, when interpreting the results herein, some caution is advised relating to the interchangeability of these terms. Finally, that we only included male participants limits the application of these findings to female players.

Practical Applications

Chronic EWMA TD, HMLd, HSR, DEC and ACC+DEC load measures exerted important interactive effects on hormonal profile changes in football players: a linear relationship was identified between chronic TD load and s-T; non-linear 'U' shaped

relationships were identified between chronic HSR load and both s-T and s-C and between chronic ACC+DEC load and s-T:C; and inverse 'U' shaped relationships were identified between chronic HMLd load and s-T and between chronic DEC load and s-T:C. For all non-linear relationships, the optimal hormonal response was observed at squad mean loads. Accordingly, coaches and practitioners should attempt to manage player exposure to these load variables and avoid excessively 'low' (i.e., -1 to -2 SD below squad mean) or excessively 'high' (i.e., -1 to -2 SD above squad mean) levels. Indeed, these scenarios might compromise hormonal responses; which are linked to player readiness, and in-turn, injury and illness risk and performance potential [2-4].

No relationships were identified between the EWMA acute load variables and salivary biomarkers. Therefore, at present, we recommend that EWMA acute and A:C load variables should not be used in isolation as surrogate measures of player readiness. Indeed, as per previous recommendations [2,3], regular immunological and hormonal profile monitoring appears to still be warranted to identify momentary readiness in football players.

We acknowledge that other response to load measures are widely used in practice to help to identify player readiness (i.e., measures of metabolic, neuromuscular, and inflammatory status) [6]. Consequently, further research is also warranted to examine how EWMA load variables relate to these measures. This will improve current understanding relating to the efficacy of training and match load measures to indicate player readiness in football.

Conclusion

Measures of chronic EWMA training volume and high intensity training volume are associated with salivary hormone profile changes; but acute EWMA variables do not relate to salivary immunological or hormonal profile changes in elite-level professional football players.

Disclosure of Interest

The authors report no conflict of interest.

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661

662 ***Supplementary Table 1***. Predictors of Salivary Immunoglobulin-A (s-IgA).
663

	s-IgA				
Predictors	*Estimates*	*ES*	*CI*	*Standardized CI*	*P*
(Intercept)	89.71		43.8 – 135.7		**<0.001**
Phase of Season	34.8	*Trivial* ↑	8.1 – 61.6	0.02 – 0.13	**0.011**
Random Effects					
σ^2	18274				
$T_{00\ Player\_ID}$	1357				
ICC	0.07				
N_{Player_ID}	18				
Observations	1154				
Marginal R^2	0.123				
Conditional R^2	0.184				

664

Supplementary Table 2. Predictors of Salivary α-amylase (s-AA).

	s-AA				
Predictors	*Estimates*	*ES*	*CI*	*Standardized CI*	*P*
(Intercept)	169.8		111.4 – 228.4		**<0.001**
Phase of Season	-163.0	*Small* ↓	-197.5 – -128.6	-0.30 - -0.20	**<0.001**
Random Effects					
σ^2	29408				
$T_{00\ Player\_ID}$	3711				
ICC	0.11				
N_{Player_ID}	18				
Observations	1136				
Marginal R^2	0.212				
Conditional R^2	0.300				

Table 1. Predictors of Salivary Testosterone (s-T). *EWMA, exponentially weighted moving average; TD, total distance; HSR, high-speed running; HMLd, high metabolic load distance; sRPE, session rating of perceived exertion; A:C, acute:chronic; [2], denotes a non-linear relationship.*

	s-T				
Predictors	*Estimates*	*ES*	*CI*	*Standardized CI*	*P*
(Intercept)	208.2		5.58 – 410.8		**0.044**
Phase of Season	-73.9	*Trivial* ↓	-124.8 – -23.0	-0.12 - 0.01	**0.004**
EWMA chronic TD^2	0.00	*Large*	0.00 – 0.00	0.03 – 0.28	**0.015**
EWMA chronic HSR^2	0.02	*Trivial*	0.01 – 0.03	0.08 – 0.30	**0.001**
EWMA chronic HMLd^2	0.00	*Small*	-0.01 – -0.00	-0.53 – -0.14	**0.001**
EWMA chronic sRPE	-1.32	*Moderate* ↓	-2.13 – -0.50	-0.61 – -0.14	**0.002**
EWMA A:C HSR	16.9	*Trivial* ↑	3.87 – 29.98	0.03 – 0.20	**0.011**
Random Effects					
σ^2	40663				
$T_{00\ Player\_ID}$	3396				
ICC	0.08				
$N_{\ Player\_ID}$	18				
Observations	1093				
Marginal R^2	0.087				
Conditional R^2	0.157				

673 **Table 2.** Predictors of Salivary Cortisol (s-C). *EWMA, exponentially weighted moving average; HSR,*

674 *high-speed running; [2], denotes a non-linear relationship*

675

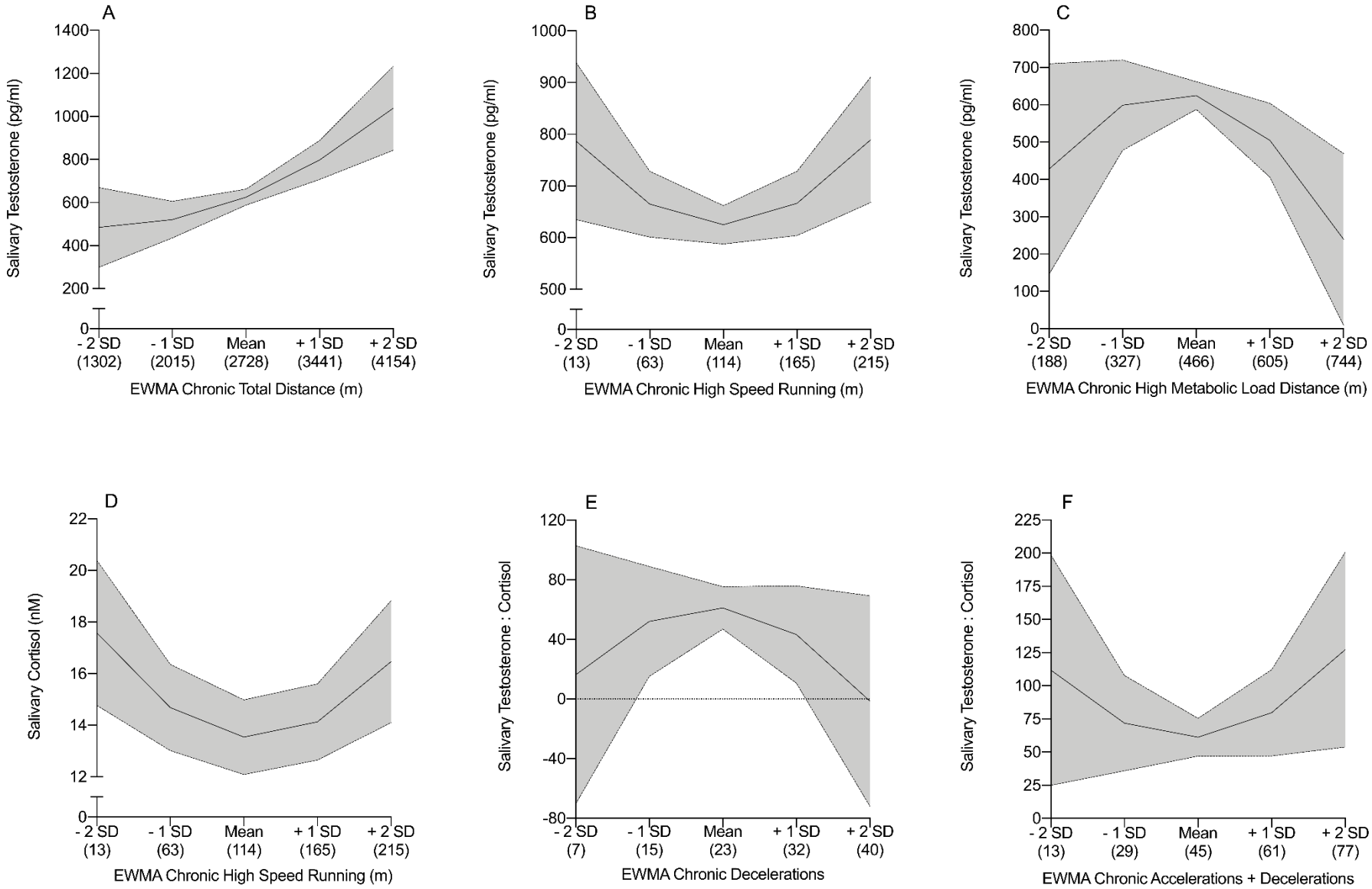
			s-C		
Predictors	*Estimates*	*ES*	*CI*	*Standardized CI*	*P*
(Intercept)	12.79		8.47 – 17.12		**<0.001**
Phase of Season	-8.53	*Small* ↓	-10.5 – -6.6	-0.34 – -0.22	**<0.001**
EWMA chronic HSR[2]	0.00	*Trivial*	0.00 – 0.00	0.04 – 0.15	**0.001**
Random Effects					
σ^2	74.24				
$\tau_{00\ Player\_ID}$	5.29				
ICC	0.07				
N_{Player_ID}	18				
Observations	1083				
Marginal R^2	0.138				
Conditional R^2	0.195				

676

677 **Table 3.** Predictors of Salivary Testosterone : Cortisol (s-T:C). *EWMA, exponentially weighted moving*
678 *average; DEC, deceleration; ACC+DEC, combined acceleration and deceleration; HSR, high-speed*
679 *running; ^{2}, denotes a non-linear relationship.*
680

			s-T:C		
Predictors	*Estimates*	*ES*	*CI*	*Standardized CI*	*P*
(Intercept)	59.4		30.1 – 88.7		**<0.001**
Phase of Season	48.9	*Small* ↑	34.7 – 63.0	0.17 – 0.30	**<0.001**
EWMA chronic DEC^2	-0.20	*Small*	-0.39– -0.01	-0.41 – -0.01	**0.039**
EWMA chronic ACC+DEC^2	0.06	*Small*	0.00 – 0.11	0.01 – 0.44	**0.039**
EWMA chronic HSR	0.05	*Trivial* ↑	-0.12 – 0.23	-0.10 – 0.18	0.554
Random Effects					
σ^2	3447				
$T_{00\ Player\_ID}$	669				
ICC	0.16				
N_{Player_ID}	18				
Observations	1064				
Marginal R^2 /	0.066				
Conditional R^2	0.218				

681


A
Salivary Testosterone (pg/ml)
1400
1200
1000
800
600
400
200
0
- 2 SD (1302)
- 1 SD (2015)
Mean (2728)
+ 1 SD (3441)
+ 2 SD (4154)
EWMA Chronic Total Distance (m)
B
Salivary Testosterone (pg/ml)
1000
900
800
700
600
500
0
- 2 SD (13)
- 1 SD (63)
Mean (114)
+ 1 SD (165)
+ 2 SD (215)
EWMA Chronic High Speed Running (m)
C
Salivary Testosterone (pg/ml)
800
700
600
500
400
300
200
100
0
- 2 SD (188)
- 1 SD (327)
Mean (466)
+ 1 SD (605)
+ 2 SD (744)
EWMA Chronic High Metabolic Load Distance (m)
D
Salivary Cortisol (nM)
22
20
18
16
14
12
0
- 2 SD (13)
- 1 SD (63)
Mean (114)
+ 1 SD (165)
+ 2 SD (215)
EWMA Chronic High Speed Running (m)
E
Salivary Testosterone : Cortisol
120
80
40
0
-40
-80
- 2 SD (7)
- 1 SD (15)
Mean (23)
+ 1 SD (32)
+ 2 SD (40)
EWMA Chronic Decelerations
F
Salivary Testosterone : Cortisol
225
200
175
150
125
100
75
50
25
0
- 2 SD (13)
- 1 SD (29)
Mean (45)
+ 1 SD (61)
+ 2 SD (77)
EWMA Chronic Accelerations + Decelerations

682

683 **Figure 1.** Non-linear relationships between exponentially weighted moving average
684 (EWMA) chronic total distance and salivary testosterone (panel A), EWMA chronic
685 high-speed running distance and salivary testosterone (panel B), EWMA chronic high-
686 metabolic load distance and salivary testosterone (panel C), EWMA chronic high-
687 speed running distance and salivary cortisol (panel D), EWMA chronic decelerations
688 and salivary testosterone : cortisol (panel E) and EWMA chronic summated
689 accelerations and decelerations and salivary testosterone : cortisol (panel F). Data are
690 presented as mean ± 95% CI bands, denoted by grey areas on the curves. Figures
691 demonstrate predicted hormonal responses at very low (-2 SD), low (-1 SD), mean,
692 high (+1 SD) and very high (+2 SD) EWMA workloads. Model-predicted EWMA
693 workload values at -2 SD, -1SD, mean, +1 SD and +2 SD are provided in brackets on
694 the X-axis.
695