Salivary Immunoendocrine and Self-Report Monitoring Profiles Across an Elite-Level Professional Football Season.

Original Investigation.

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Title

Salivary Immunoendocrine and Self-Report Monitoring Profiles Across an Elite-Level Professional Football Season.

Short Title

Multivariate Monitoring Profiles in Football

Abstract

Purpose

This investigation examined the longitudinal changes and inter-relationships of salivary and self-report monitoring measures across a professional football season.

Methods

Measures were collected bi-weekly from 18 senior professional male players across a six-week pre-season and eight five-week in-season mesocycles and analysed using a linear mixed-effects model.

Results

Analysis identified a *small* (P=0.003) cross-season suppression of salivary immunoglobulin-A, *small* reductions to salivary α-amylase (P=0.047) and salivary cortisol (P=0.007), and *trivial* changes to salivary testosterone (P>0.05). The testosterone:cortisol ratio typically responded inversely to changes in player workload. Self-report measures of fatigue (P=0.030), sleep quality (P=0.003) and muscle soreness (P=0.005) improved (ES=*small*) across the first half of the season. Fatigue and sleep measures were most consistently related to hormonal

measures (R^2 = 0.43 to 0.45). For these relationships, increases in cortisol were associated with compromised self-report responses, whereas increases in testosterone:cortisol were associated with improved responses. Non-linear relationships were identified for fatigue with immunoglobulin-A (*P*=0.017; ES=*trivial*) and testosterone (*P*=0.012; ES=*trivial*); for sleep quality with testosterone (*P*<0.001; ES=*trivial*); for muscle soreness with testosterone (*P*=0.012; ES=*trivial*) and for the self-report inventory sum with testosterone (*P*=0.027; ES=*trivial*). For these relationships, self-report responses were optimal at mean immunoglobulin-A and testosterone levels and very low levels (-2 SD) exerted the most compromising effects.

Conclusion

Players can experience a chronic cross-season suppression of mucosal immunity. Salivary immunoglobulin-A, testosterone, cortisol and testosterone:cortisol measures relate to self-report measures of fatigue, sleep quality and muscle soreness. In-season reductions in testosterone, cortisol and testosterone:cortisol or increases in cortisol among elite football players could be used to indicate the need for reduced workload, which might lead to improved wellbeing.

Key Words

Workload; Monitoring; Team Sports; Immunology; Endocrinology;

Introduction

Professional Association Football (football) players are exposed to high workloads (1) and congested fixture schedules (2). Consequently, achieving a balance between workload and recovery is not always possible (3), which can lead to player stress, immune and hormonal imbalances (4-9) and subsequent increases in injury and illness risk (1, 3, 10, 11).

To mitigate injury and illness risk, the football training environment necessitates valid and reliable player monitoring methods with fast data availability (12). Athlete self-report measures (ASRM) of perceived fatigue, sleep quality, stress, mood and muscle soreness (13) are widely used in practice (12) because they correspond with changes in training load (8, 14) and can be deployed and analysed rapidly (12, 13). Biological fatigue markers are often used alongside ASRM to provide objective understanding of the workload-recovery relationship (12, 13, 15). Of these, salivary biomarkers are particularly popular (12) because samples can be easily obtained non-invasively (16) and results for entire squads (~ 25 players) can be available in ~ 30 min using point of care analysis systems (4-6, 12, 17-19).

Salivary immunoglobulin-A (s-IgA) and α-amylase (s-AA) are antimicrobial proteins secreted by mucosal cells under sympathetic nervous system (SNS) control (20). Under normal circumstances, both are rhythmically secreted and play a role in mucosal immunity (7). Since SNS activity stimulates s-IgA and s-AA secretion, both are indicative of acute stress (7, 13, 20) and can be used to track changes in workload in football players (5-7) and athletes (21-23). In response to prolonged ‘stressful’ stimuli, such as increased physical training demands, secretion of s-IgA and s-AA can be reduced, which is associated with an increase in upper respiratory tract infection (URTI) and symptom (URTS) risk in football players (19, 24).

Testosterone (T) and cortisol (C) are steroid hormones, detectable in saliva (s-T, s-C) (20), that reflect anabolic (s-T) and catabolic (s-C) balance when expressed as s-T:C (9). Previous research has reported acute increases in C, equivocal changes to T and a reduction in T:C post-match in football (9, 25), rugby (26-28) and Australian football (AFL) (29) cohorts, which manifests for ~ 24 - 72 hrs (9, 25-29). Longitudinal data in football indicate an increase in C during periods of increased workload (30) and a reduction in T:C towards the end of the season (31). However, previous longitudinal investigations are limited by infrequent data collection (30, 31) and short sampling periods (31), which reduce their capacity to sensitively describe seasonal changes to hormonal status.

Limited empirical data are available to describe the seasonal changes and inter-relationships of s-IgA, s-AA, s-T, s-C, s-T:C and ASRM measures in football players. This, despite consensus statements from both the American College of Sports Medicine (ACSM) (32) and the International Olympic Committee (IOC) (10, 11) recommending longitudinal multivariate monitoring in the support of elite athletes. Such data will help to refine monitoring methods in practice in football. Accordingly, the aims of this study were to investigate: a) the longitudinal changes; and b) the inter-relationships of s-IgA, s-AA, s-T, s-C, s-T:C and ASRM measures across an elite-level professional football season.

Methods

Study design

Eighteen senior professional male outfield players (age = 24 ± 3.8 years; height = 181 ± 7.0 cm, body mass = 72.4 ± 5.2 kg) from one English Championship (EC) team participated in the

investigation. Saliva samples and ASRM data were collected following recovery days across a complete 6-week preseason and 40-week (48-game) in-season period. An example of the typical training and sample collection schedule for the team is provided in Figure 2. In total, 802 s-IgA, 785 s-AA, 795 s-T, 791 s-C and 697 ASRM measures were analysed. The in-season period was divided into eight 5-week mesocycles (M); of which M1 = games 1 – 5; M2: 6 – 11; M3: 12 – 17; M4: 18 – 23; M5: 24 – 31; M6: 32 – 36; M7: 37 – 40 and M8: 41 – 48 (Figure 1). Total player workload was recorded across the investigation using the CR-10-scale (33). CR-10 response was collected within 30 min of all training sessions and games and multiplied by session or game duration (min) to provide an arbitrary unit (AU) of workload. This method has been validated for use in elite professional football training previously (34). Informed consent was obtained from all participants prior to collection of any data used in this investigation. An ethics declaration (project approval number: 21995) was approved by the Edith Cowan University (AU) Human Research Ethics Office.

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

Saliva Sampling and Athlete Self Report Measures

Players reported to the team training facility between 09:00 and 09:30 on sample collection days. Players were asked to abstain from alcohol and caffeine consumption for 24 hrs and 12 hrs (respectively) prior to sample collection. This was confirmed verbally with players at the point of sample collection. None of the players were smokers. Players 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 (18). Players were then asked to complete an ASRM inventory (13), composed of five questions relating to: fatigue, sleep quality, muscle soreness, stress level and mood. Responses were scored on a Likert scale of 1-5, where 1, 2, 3, 4 and 5 = *very good*, *good*, *normal*, *bad* and *very bad*, respectively (13).

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 lines. After a five 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) (35). Salivary IgA and s-C 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 sample period. This method has been validated previously for s-IgA (18, 36) and s-C (36, 37). Indeed, comparison of the LFD method with the enzyme-linked immunosorbent assay (ELISA) method indicates strong validity for s-IgA ($r = 0.93$; $P < 0.001$) (18) and s-C ($r^2 = 0.79$) (38) . Repeated sampling indicates strong reliability for s-IgA (ICC $r = 0.89$, $P < 0.001$ and CV = 9.4%) (18) and s-C (CV = 6.8%) (38).

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 manufacturers guidelines. Validity and reliability data are unavailable for these measures. 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 hours 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 (39).

Team Match, Training and Data Collection Schedule

Saliva and ASRM data were collected bi-weekly following recovery days or days 'off'. Typical team training, match and data collection schedules are provided in Figure 2. Baseline saliva and ASRM measures were calculated as the mean of MD-2 data collected during single game weeks in mesocycle one (Figure 2). Mesocycle one was used as baseline for workload.

****** Insert Figure 2 Here******

Statistical Analysis

All estimations were made using the *lme4* package (40) with *R* (version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria). Baseline saliva and ASRM data were used as the reference point to determine longitudinal changes across the season. A linear mixed-effects model was used to model the effect of season mesocycle on the dependent variables whilst adjusting for the baseline value for each player and total workload in each season phase. The random effects were player identity (differences between players' mean s-IgA, s-AA, s-T, s-C and s-T:C values), player identity × season mesocycle (variability in the effect of season on s-IgA, s-AA, s-T, s-C and s-T:C values across players), and the residual. The *lmerTest* package (41) was used to conduct Bonferroni-adjusted pairwise comparisons for the main effect of saliva variables and season mesocycle, and their interactions. Data are presented as means and 95% confidence intervals (CI), alongside Cohen's *d* effect sizes (42). 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*. A linear mixed-effects model was also used to model the relationship between ASRM measures (as the dependent variable) and saliva variables (as the fixed effect), with total workload included as a covariate and player identity included as a random effect. Polynomial terms were included in the model to accommodate non-linear responses and were retained if statistically significant. Separate analyses were performed for each ASRM measure. The conditional R^2 value (which considers both fixed and random effects in the model) is provided as a goodness-of-fit measure for these relationships. Data for non-linear relationships is presented as means and 95% CI with estimated ASRM responses at typically very low (-2 SD), low (-1 SD), mean, high (+ 1 SD) and very high (+2 SD) values of each salivary predictor variable (43).

Results

Longitudinal Analysis of Salivary and ASRM Monitoring Variables

Descriptive data of salivary and ASRM variables by season mesocycle are presented in Table 1.

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

Relative to baseline, high workloads were observed in mesocycles two (*P* = < 0.001; ES = *small*), four (*P* = 0.005; ES = *small*), five (*P* = < 0.001; ES = *small*), seven (*P* = < 0.001; ES =small) and eight (*P* = < 0.001; ES = *moderate*) and low workload was observed in mesocycle six (*P* = 0.047; ES = *small*), (Figure 3, Panel A). Salivary IgA was lower than baseline across all mesocycles (ES = *trivial* to *small*) and lowest during mesocycle five (*P* = 0.003, ES = *small*), (Figure 3, Panel B). Salivary AA reduced to below baseline across mesocycles four to eight

(ES = *trivial* to *small*). This effect was significant for mesocycle eight (*P* = 0.047; ES = *small*), (Figure 3, Panel C). Salivary C was highest during preseason (*P* = 0.006; ES = *small*) and reduced to below baseline across mesocycles five to eight (ES = *trivial* to *small*). This effect was significant for mesocycle eight (*P* = 0.007; ES = *small*), (Figure 3, Panel D). No significant changes were observed to s-T (Figure 3, Panel E). Salivary T:C was lowest during preseason (ES = *trivial*) and highest during mesocycles six (*P* = 0.011; ES = *small*) and eight (*P* = < 0.001; ES = *small*), (Figure 3, Panel F).

******Insert Figure 3 Here******

Perceived measures of fatigue, sleep quality and muscle soreness reduced to below baseline across the first half of the season and remained thereafter. This effect was significant for fatigue in mesocycle five (*P* = 0.030; ES = *small*); for sleep quality in mesocycles four (*P* = 0.011; ES = *small*), five (*P* = 0.003; ES = *small*), six (*P* = 0.009; ES = *small*), seven (*P* = 0.025; ES = *small*) and eight (*P* = 0.040; ES = *small*); for muscle soreness in mesocycles four (*P* = 0.017; ES = *small*), five (*P* = 0.005; ES = *small*), six (*P* = 0.007; ES = *small*), seven (*P* = 0.021; ES = *small*) and eight (*P* = 0.030; ES = *small*) and for the ASRM total in mesocycles five (*P* = 0.008; ES = *small*) and six (*P* = 0.019; ES = *small*) (Figure 4, Panels A, B, C and F). No changes (*P* > 0.05) were observed to perceived stress level or mood (Figure 4, Panels D and E).

******Insert Figure 4 Here******

Relationships Between Salivary and ASRM Monitoring Variables

S-IgA shared a quadratic relationship with perceived fatigue (*P* = 0.017; ES = *Trivial*), (Table 2; Figure 5, Panel A). S-T shared quadratic relationships with perceived fatigue (*P* = 0.012; ES

= *Trivial*), sleep quality (*P* = < 0.001; ES = *Trivial*) muscle soreness (*P* = 0.012; ES = Trivial) and ASRM Total (*P* = 0.027; ES = *Trivial*), (Figure 5, Panels B, C, D and E). S-C shared linear relationships with perceived fatigue (*P* = 0.031; ES = *Trivial* ↑) and sleep quality (*P* = 0.031; ES = *Trivial* ↑) (Table 2). S-T:C shared linear relationships with perceived fatigue (*P* = 0.014; ES = *Trivial* ↓) and sleep quality (*P* = 0.031; ES = *Trivial* ↑) (Table 2).

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

******Insert Figure 5 Here******

Discussion

The first aim of this study was to investigate the longitudinal changes to salivary and ASRM monitoring variables across a professional football season. Longitudinal changes were observed in all salivary variables, with s-IgA, s-C and s-T:C responding to changes in the workload of players across mesocycles. Improvements in ASRM measures were observed across the first half of the competitive season and were generally maintained thereafter. The second aim of this study was to investigate the interrelationships of salivary and ASRM measures. Relationships were identified between s-IgA and fatigue; s-T and fatigue, sleep quality and muscle soreness; s-C and fatigue and sleep quality and s-T:C and fatigue and sleep quality.

The most important finding of this investigation was the chronic cross-season suppression of s-IgA relative to baseline measures (Figure 3, Panel B). Salivary IgA is the most abundant antimicrobial protein in saliva and is indicative of mucosal immunological status (17). Indeed,

reductions in s-IgA are associated with an increased risk of URTI and URTS in elite level professional football players (19, 24). In the current investigation, baseline s-IgA was calculated as the average of values measured following a recovery day, during single game weeks in mesocycle one. We reasoned that this was the most appropriate representation of optimal player 'fitness' (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 holistic stress balance was optimal. Repeated exposure to training and match-play places significant stress on the SNS, and prolonged SNS activation is thought to reduce s-IgA secretion by reducing the availability of polymeric immunoglobulin receptors (p-IgR), which initiate the transit of s-IgA to saliva (17). Recent research has demonstrated cross-season reductions in s-IgA in AFL players (17) and reductions to s-IgA in response to high fixture densities (5) (> 1 game per week) and workload (7) in football. Since baseline measures herein relate to the physiological status of players during single game weeks, the suppression of s-IgA likely reflects the supplementary effect that high fixture densities (> 1 game per week) exert on s-IgA. Indeed, the EC has the highest fixture density of all the major European Leagues (2), and the current cohort were regularly exposed to fixture densities > 1 game / week (Figure 1).

The lowest s-IgA values were observed during mesocycle five (Figure 3, Panel B), which coincides with the Christmas fixture period (Figure 1). This mesocycle includes sequential double- and treble-game weeks, and has been shown to cause a transient reduction in s-IgA (5). Morgans and colleagues (5) reported that s-IgA returned to baseline ~ 10 d after a return to regular match density (≤ 1 game per week). Interestingly, we observed a similar trend for s-IgA recovery in mesocycle seven, when match density was lowest. Our results indicate that periods of intensified match load can suppress s-IgA, and that subsequent alleviations can mitigate this response. That s-IgA was low during preseason might reflect the low training

status and stress tolerance of players expected at this time (24). Indeed, an increase in s-C and a decrease in s-T:C were also observed during preseason training (Figure 3, Panels D and F).

Salivary C and s-AA followed similar transient reductions during the second half of the season (Figure 3, Panels C and D). Values were lowest in mesocycle eight, when workload (Figure 2, Panel A) and fixture density (Figure 1) were highest. Cortisol is secreted from the adrenal cortex via the hypothalamic pituitary adrenal axis (HPA) (21), and exerts catabolic effects to reduce protein synthesis and increase protein degradation (20). Salivary AA is secreted by mucosal cells via the sympathetic adrenal medullary (SAM) axis (21), and contributes to digestion and mucosal immunity (16). Owing to their reactivity to HPA and SAM axis stimulation, both are used as quantitative stress markers in athletes (21). Strong correlations are reported between s-C and total workload (4) and increases in cortisol are reported at the end of the competitive season (31) and during periods of increased workload (30) in football players. Similarly, s-AA is reported to increase during periods of intensified competition (22) and workload (23). Accordingly, our results contrast previous findings (4, 22, 23, 30, 31) and might indicate an adaptive training state across the season. Indeed, player ASRM responses during the second half of the season herein were consistent with adaptive training (13). Alternatively, recent research suggests hyposensitivity of the HPA axis and a reduced cortisol response to stress testing in overtrained athletes (44). Accordingly, it is also (conversely) possible that our result indicates maladaptive training. However, to date, there are no reports of this response in professional football players.

We observed negligible cross-season changes to s-T (Figure 3, Panel E), but an increase in s-T:C during mesocycle six (Figure 3, Panel F) when workload was low, resulting from a trivial increase in s-T (Figure 3 Panels D and E). Testosterone is a steroid hormone secreted from the

testes and adrenal glands via the hypothalamic pituitary gonadal (HPG) (testicular) and HPA (adrenal gland) axes (20). It exerts anabolic effects to increase protein synthesis and decrease protein degradation (20). Research to date indicates acute quantitative changes to T and C, signalling a catabolic state in relation to the intensity and duration of preceding workload (9, 45, 46). Previous longitudinal investigations have reported equivocal changes to T and decreases in T:C at the end of the season and during periods of increased workload (30, 31). Accordingly, our finding for s-T is consistent with previous research (30, 31). That s-T:C appears to have increased in response to low workload is also consistent with previous research (30, 31) and indicates that mid-season reductions in workload can improve hormonal balance in players. The increase in s-T:C at the end of the competitive season is contrary to previous research (30, 31), and might be explained by differences in end of season game density, training loads and other inter-team factors between investigations. In the current investigation, this change was related to a concurrent reduction in both s-T and s-C in mesocycle eight, suggesting a maladaptive training state. This might be explained by increases in psychophysiological stress (20) related to the particularly high game density (Figure 1) and workload (Figure 3, Panel A) during this phase of the season.

Perceived player fatigue, sleep quality and muscle soreness improved across the first half of the season (Figure 4, Panels A, B and C). Equivocal changes were observed for perceived stress level and mood (Figure 4, Panels D and E). The ASRM used herein are typically sensitive to daily, within-weekly and seasonal changes in training load in English Premier League (EPL) and AFL players (14, 15, 47, 48) and correlate with daily training load during pre-season and in-season mesocycles (8, 14, 15). In the current investigation, fatigue, sleep quality and muscle soreness were worst during preseason, which might reflect the low training status expected at this time (24). However, in-season ASRM did not appear to respond to changes in workload

or game density. Previous scientific literature suggests that ASRM measures might not account for the effect of all (non-training related) stressors (49). As such, it is possible that non-training stressors during the in-season phase disguised ASRM changes related to workload and game demands, and might also explain the large standard deviations observed in the ASRM measures (Figure 4). As reported previously, it is also possible that players manipulated ASRM responses for their own benefit (i.e. team selection) during the in-season phase (12). However, in accordance with previous recommendations (12), we educated players regarding ASRM prior to the investigation. Accordingly, the temporal improvement in ASRM observed herein suggests an adaptive training state.

Salivary cortisol and s-T:C were linearly related to perceived fatigue. For these relationships, increases in s-C were associated with increased fatigue, whereas increases in s-T:C were associated with reduced fatigue (Table 2). Furthermore, s-T shared a quadratic relationship with perceived fatigue, whereby very low levels of s-T (- 2 SD) were associated with the most compromising effects (Figure 5, Panel B). To date, cortisol has demonstrated equivocal (46) or negative (21) effects on perceived fatigue in athletes and is associated with increases in anxiety and depressive state (20). Conversely, increases in testosterone and T:C have been reported to improve perceived fatigue (46). Accordingly, our results are consistent with previous research and indicate that s-C, s-T and s-T:C monitoring can objectively determine fatigue status in professional football players.

Sleep quality was linearly related to s-C and s-T:C (Table 2). Increases in s-C were associated with compromised sleep quality, whereas increases in s-T:C were associated with an improved response. Also, s-T shared a non-linear relationship with sleep quality, whereby very low levels of s-T (-2 SD) were associated with the most compromising effects (Figure 5, Panel C). Our

findings contrast recent research that demonstrated unclear relationships between s-C, s-T, s-T:C and sleep quality (50). Serpell and colleagues (50) used a wrist actigraphy measure of sleep quality across a short (4 d) pre-season rugby training camp. Our contrasting findings might relate to differences in the training and competition demands of rugby and football, differences in the relative fitness and fatigue profiles of the players at the point of data collection and / or the methods used to measure sleep quality. Notwithstanding, sleep quality is thought to share an intricate relationship with the HPA axis, and excessive HPA axis activation is thought to compromise sleep quality as a consequence of increases in systemic cortisol and catecholamine concentrations (51). Moreover, sleep quality is proposed to be an important mediator of testosterone, since most testosterone secretion occurs during night-time sleep (50). To that end, the negative association of s-C and the beneficial association of s-T with sleep quality herein are unsurprising. These findings support attempts to improve player sleep quality in practice (52), since sleep quality evidently relates to hormonal balance in professional football players.

A quadratic relationship was observed between s-T and muscle soreness (Figure 5, Panel D), for which very low (- 2 SD) levels of s-T were associated with the most compromising effects. We also observed a trend for s-C to relate to muscle soreness (Table 2), for which increases in s-C were associated with a compromised response (P = 0.057; ES = *Trivial*). These relationships might reflect the positive anabolic effects of testosterone (20, 21) and the negative catabolic consequences of cortisol (20, 21) on muscular recovery following training and match play. Indeed, muscle recovery is augmented in anabolic as opposed to catabolic environments (53).

We also observed a quadratic relationship between s-IgA and fatigue (Figure 5, Panel A) whereby very low (-2 SD) and very high (+2 SD) s-IgA were associated with compromised

fatigue. That low s-IgA was associated with compromised fatigue might be explained by reductions in s-IgA during periods of sustained, excessive SNS activation (17). Indeed, this is likely during prolonged periods of high workload (7) or game density (5), such as mesocycle five. That very high s-IgA was associated with compromised fatigue might be related to periods of high acute workloads inducing increases in SNS activity, s-IgA secretion (20) and player fatigue. Equally, it is also possible that incidences of very high s-IgA are explained by infection and that concurrent increases in perceptual fatigue are explained by increases in Interleukin-1 (IL-1) as part of an infection-related immune response (54). Recent investigations indicate relationships between s-IgA and perceived player wellness, energy level, readiness to train, and muscle soreness in football players (55). To our knowledge, this is the first investigation to report the relationship between s-IgA and fatigue in professional football players.

Limitations

This investigation was conducted using a single homogenous sample but acknowledge that other cohorts might respond differently owing to situational, contextual and inter-team factors. We did not screen saliva samples for blood contamination and acknowledge that this is a limitation that could affect the accuracy and validity of the findings. Accordingly, we recommend that future research should screen saliva samples for blood contamination and control for behaviours that might induce saliva sample blood contamination (i.e. tooth brushing). We also acknowledge that the absence of a control group challenges the capacity to discern between workload-induced and normal seasonal variation in the salivary biomarkers. This should be considered when interpreting the results. Finally, we acknowledge that the in-house ELISA method employed herein lacks independent scientific validation, and thus advise the reader that some caution should be applied when interpreting the s-AA and s-T results.

Practical Applications

Our results indicate a chronic suppression of mucosal immunity and accordingly, practitioners should adopt practices to promote immune function. Practical recommendations to promote immune function in athletes have been provided previously (10).

Periods of high game density and workload exacerbated disturbances to mucosal immunity, whereas reductions mitigated the response. Accordingly, planned periods of reduced workload or squad rotation should be considered around demanding mesocycles to accommodate immunological and hormonal recovery. Our results indicate that this might be particularly important around the Christmas fixture period and towards the end of the season.

Our findings indicate merit in the use of s-IgA, s-T, s-C and s-T:C monitoring in professional football players. These measures responded to changes in game density and workload and related to perceived fatigue, sleep quality and / or muscle soreness. Practitioners should consider reducing player workload in cases where s-T and s-T:C measures are < -1 SD below baseline or when s-C is > 1 SD above baseline, since these values were associated with compromised wellbeing. Similar considerations should be afforded to players that present with particularly low (< -1 SD below baseline) or high (> 1 SD above baseline) s-IgA measures.

Conclusion

Football players can experience a chronic suppression of mucosal immunity and s-IgA, s-T, s-C and s-T:C measures are influenced by changes in workload and / or game density and relate to perceived measures of fatigue, sleep quality and muscle soreness.

Conflict of Interest

None. The results of the present study do not constitute endorsement by ACSM. The results of the present study are presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation.

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Figure 1. Overview of the sample period showing game distribution and average game density (average number of games per week, per mesocycle) relative to in-season mesocycles and calendar month. Vertical lines indicate the distribution of competitive games. *PS, preseason*; *M, mesocycle.*

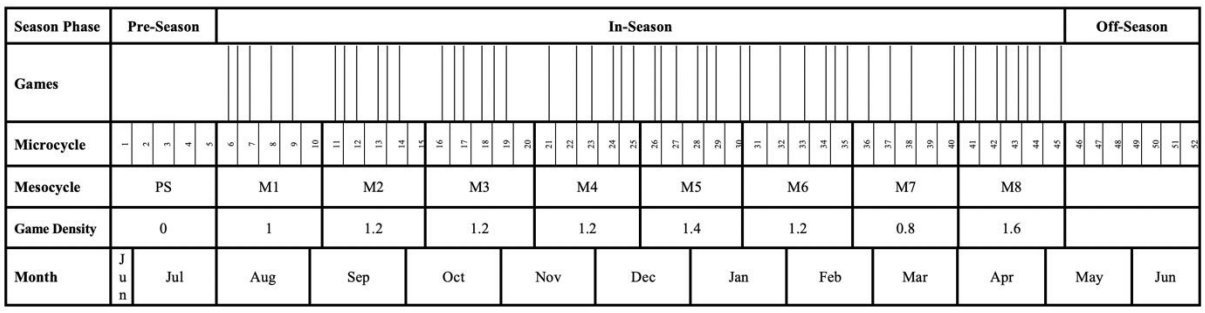


Figure 2. Example data collection schedule relative to team match and training activities for A) single and B) double game weeks across the investigation. Black bars, match day; Grey bars, training day; Hollow bars, recovery session; Gaps, recovery day (off) and *, saliva sample and ASRM data collection.

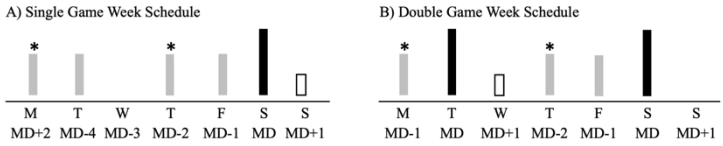
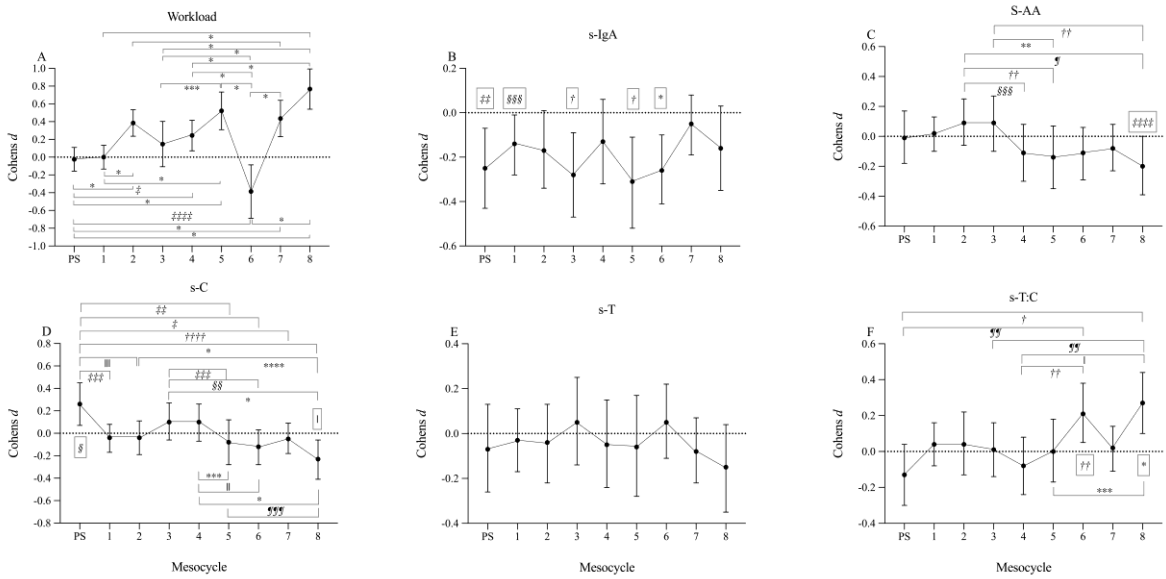


Figure 3. Standardised changes to workload and salivary biomarkers across preseason (PS) and eight six-week in-season mesocycles. *s-IgA, salivary immunoglobulin-A; s-AA, salivary α-amylase; s-C, salivary cortisol; s-T, salivary testosterone; s-T:C, salivary testosterone : cortisol ratio*. Boxed symbols indicate a difference between season phase and baseline (salivary variables only). Horizontal lines indicate pairwise differences between season phases. **, P < 0.001; †, P = 0.003; ‡, P = 0.005; §, P = 0.006; |, P = 0.007; ¶, P =0.008; **, P = 0.009; ††, P = 0.011; ‡‡, P = 0.012; §§, P = 0.017; ||, P = 0.019; ¶¶, P = 0.022; ***, P = 0.025; †††, P = 0.026; ‡‡‡, P = 0.027; §§§, P =0.035; |||, P = 0.036; ¶¶¶, P = 0.042; ****, P = 0.044; ††††, P =0.045; ‡‡‡‡, P = 0.047.*



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706 **Figure 4**. Standardised changes to perceived fatigue, sleep quality, muscle soreness, stress
707 level, mood and athlete self-report measures total score (ASRM total) across pre-season (PS)
708 and eight six-week in-season mesocycles. Boxed symbols indicate a difference to baseline
709 measures. Horizontal lines indicate a difference between season phases. *, *P =0.003; †, P =*
710 *0.005; ‡, P = 0.007; §, P = 0.009; |, P = 0.011;* ¶, *P = 0.013;* **, *P = 0.017; ††, P = 0.021;*
711 *‡‡, P = 0.025; §§, P = 0.030; ||, P = 0.038;* ¶¶, *P = 0.040.*

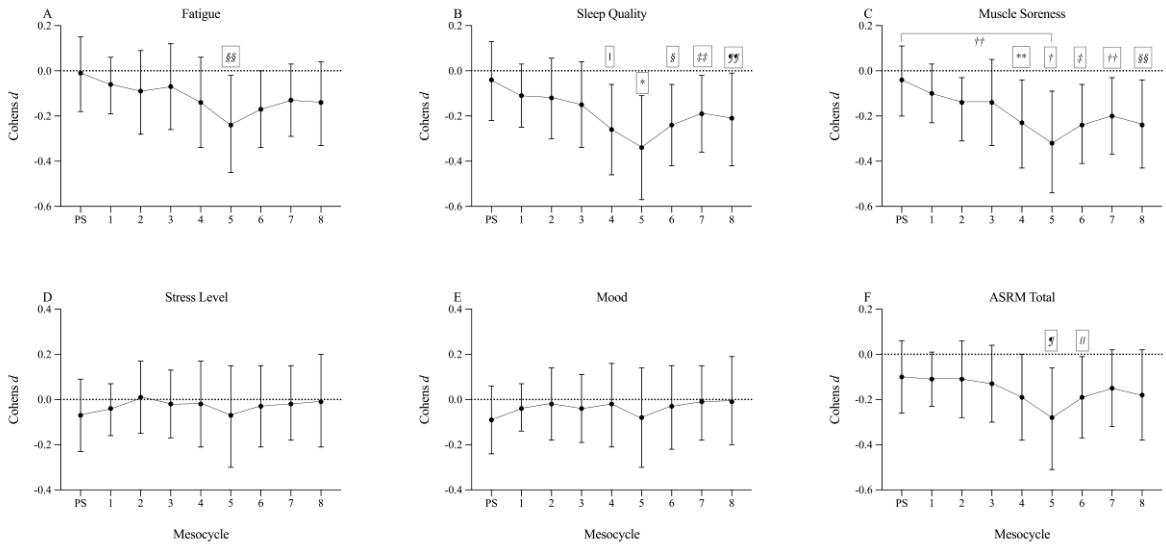

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Figure 5. Non-linear relationships between ASRM and salivary monitoring variables. Data are presented as mean ± 95% CI bands, denoted by grey areas on the curve. Figures demonstrate predicted player ASRM responses at very low (-2 SD), low (-1 SD), mean, high (+1 SD) and very high (+2 SD) s-IgA (panel A) and s-T (panels B, C, D and E) levels. Model-predicted s-IgA and s-T values at -2 SD, -1SD, mean, +1 SD and +2 SD are also provided in brackets on the X-axis.