Salivary Immunoendocrine and Self-report Monitoring Profiles across an Elite-Level Professional Football Season

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ABSTRACT

SPRINGHAM, M., S. WILLIAMS, M. WALDRON, A. J. STRUDWICK, C. MCLELLAN, and R. U. NEWTON. Salivary Immunoendocrine and Self-report Monitoring Profiles across an Elite-Level Professional Football Season. Med. Sci. Sports Exerc., Vol. 53, No. 5, pp. 918–927, 2021. Purpose: This investigation examined the longitudinal changes and interrelationships of salivary and self-report monitoring measures across a professional football season. Methods: Measures were collected biweekly from 18 senior professional male players across a 6-wk preseason and eight 5-wk in-season mesocycles and analyzed 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 (R² = 0.43–0.45). For these relationships, increases in cortisol were associated with compromised self-report responses, whereas increases in testosterone/cortisol were associated with improved responses. Nonlinear 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. Conclusions: 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 well-being. Key Words: WORKLOAD, MONITORING, TEAM SPORTS, IMMUNOLOGY, ENDOCRINOLOGY

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 analyzed 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 noninvasively (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). Because 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 and symptom 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 after a match in football (9,25), rugby (26–28), and Australian football (AFL) (29) cohorts, which manifests for ~24–72 h (9,25–29). Longitudinal data in football indicate an increase in C during periods of increased workload (30) and a reduction in T:C toward 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 interrelationships of s-IgA, s-AA, s-T, s-C, and ASRM measures in football players. This, despite consensus statements from both the American College of Sports Medicine (32) and the International Olympic Committee (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 interrelationships 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 yr; height, 181 ± 7.0 cm; body mass, 72.4 ± 5.2 kg) from one English Championship team participated in the investigation. Saliva samples and ASRM data were collected after recovery days across a complete 6-wk pre-season and 40-wk (48-game) in-season period. In total, 802 s-IgA, 785 s-AA, 795 s-T, 791 s-C, and 697 ASRM measures were analyzed. The in-season period was divided into eight 5-wk mesocycles (M), of which M1 indicates 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 (Fig. 1). Total player workload was recorded across the investigation using the Borg category-ratio RPE scale (CR-10) (33). CR-10 response was collected within 30 min of all training sessions and games and multiplied by session or game duration (in minutes) 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 before 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.

Saliva sampling and athlete self-report measures. Players reported to the team training facility between 0900 and 0930 h on sample collection days. Players were asked to abstain from alcohol and caffeine consumption for 24 and 12 h (respectively) before 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 then place an oral fluid collector (OFC; SOMA Bioscience, Wallingford, United Kingdom) on the tongue. With the mouth closed, 0.5 mL of saliva was collected, as indicated by a volume accuracy indicator on the OFC. The OFC was then placed into 3 mL of buffer solution in a bespoke 10-mL container (OFC Buffer; SOMA Bioscience) and mixed gently by hand for 2 min (18). Players were then asked to complete the pre- and post-exercise questionnaires. This method has been validated previously for s-IgA (13,35). Salivary IgA and s-C were determined using specifically calibrated ELISA kits (SOMA Bioscience) test strips, which captured s-IgA and s-C at test times. A 5-min incubation period, the LFI strips were inserted into a lateral flow device reader (SOMA Bioscience), which used signal intensity to provide quantifiable values for s-IgA (in micrograms per milliliter) and s-C (in nanomolars) (35). Salivary IgA and s-C were determined using specifically programmed curves assigned to the LFI strips, provided by the manufacturer (SOMA Bioscience). 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 lateral flow device method with the enzyme-linked immunosorbent assay (ELISA) method indicates strong validity for s-IgA (r = 0.93;
RESULTS

Longitudinal analysis of salivaary and ASRM monitoring variables. Descriptive data of salivaary and ASRM variables by season mesocycle are presented in Table 1.

Relative to baseline, high workloads were observed in mesocycles 2 \( (P \leq 0.001; \text{ES} = \text{small}) \), 4 \( (P = 0.005; \text{ES} = \text{small}) \), 5 \( (P \leq 0.001; \text{ES} = \text{small}) \), 7 \( (P \leq 0.001; \text{ES} = \text{small}) \), and 8 \( (P \leq 0.001; \text{ES} = \text{moderate}) \) and low workload was observed in mesocycle 6 \( (P = 0.047; \text{ES} = \text{small}) \); Fig. 3A). Salivary IgA was lower than baseline across all mesocycles \( (\text{ES} = \text{trivial to small}) \) and lowest during mesocycle 5 \( (P = 0.003; \text{ES} = \text{small}) \); Fig. 3B). s-AA reduced to below baseline across mesocycles 4 to 8 \( (\text{ES} = \text{trivial to small}) \). This effect was significant for mesocycle 8 \( (P = 0.047; \text{ES} = \text{small}) \); Fig. 3C). Salivary C was highest during preseason \( (P = 0.006; \text{ES} = \text{small}) \) and reduced t0 below baseline across mesocycles 5 t0 8 \( (\text{ES} = \text{trivial to small}) \). This effect was significant for mesocycle 8 \( (P = 0.007; \text{ES} = \text{small}) \); Fig. 3D). No significant changes to s-T were observed \( (P = 0.030; \text{ES} = \text{small}) \); Fig. 3E). Salivary T/C was lowest during preseason \( (P = 0.011; \text{ES} = \text{small}) \) and highest during mesocycles 6 \( (P = 0.011; \text{ES} = \text{small}) \) and 8 \( (P \leq 0.001; \text{ES} = \text{small}) \); Fig. 3F).

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 5 \( (P = 0.030; \text{ES} = \text{small}) \); for sleep quality in mesocycles 4 \( (P = 0.011; \text{ES} = \text{small}) \), 5 \( (P = 0.003; \text{ES} = \text{small}) \), 6 \( (P = 0.009; \text{ES} = \text{small}) \), 7 \( (P = 0.025; \text{ES} = \text{small}) \), and 8 \( (P = 0.040; \text{ES} = \text{small}) \); for muscle soreness in mesocycles 4 \( (P = 0.017; \text{ES} = \text{small}) \), 5 \( (P = 0.005; \text{ES} = \text{small}) \), 6 \( (P = 0.007; \text{ES} = \text{small}) \), 7 \( (P = 0.021; \text{ES} = \text{small}) \), and 8 \( (P = 0.030; \text{ES} = \text{small}) \); and for the ASRM total in mesocycles 5 \( (P = 0.008; \text{ES} = \text{small}) \) and 6 \( (P = 0.019; \text{ES} = \text{small}) \); Figs. 4A–C, F).

![FIGURE 2—Example data collection schedule relative to team match and training activities for single (A) and double (B) game weeks across the investigation. Black bars, match day; gray bars, training day; hollow bars, recovery session; gaps, recovery day (off). *Saliva sample and ASRM data collection.](http://www.acsm-msse.org)
TABLE 1. Descriptive data for player workload, salivary, and ASRM monitoring variables by season mesocycle.

| Season | Workload, AU | s-IgA, μg·mL⁻¹ | s-C, g·mL⁻₁ | s-T, pg·mL⁻¹ | s-C, nM | Fatigue, AU | Soreness, AU | Mood, AU | ASRM Total, AU |
|--------|-------------|----------------|-------------|--------------|---------|------------|-------------|---------|---------------|
| 1st    | 8418 (1136), 7964 – 8411 | 282 (271), 149 – 230 | 19.0 (18.8), 15.6 – 15.6 | 17.7 (17.2), 14.0 – 14.0 | 6.1 (5.5), 5.1 – 5.1 | 1.90 (0.70), 1.75 – 2.05 | 2.03 (2.1), 1.85 – 2.24 | 1.50 (0.60), 1.34 – 1.76 | 9.48 (8.55), 7.75 – 9.45 |
| 2nd    | 8125 (1379), 7739 – 8511 | 282 (271), 149 – 230 | 19.0 (18.8), 15.6 – 15.6 | 17.7 (17.2), 14.0 – 14.0 | 6.1 (5.5), 5.1 – 5.1 | 1.90 (0.70), 1.75 – 2.05 | 2.03 (2.1), 1.85 – 2.24 | 1.50 (0.60), 1.34 – 1.76 | 9.48 (8.55), 7.75 – 9.45 |
| 3rd    | 8535 (1540), 8179 – 8891 | 282 (271), 149 – 230 | 19.0 (18.8), 15.6 – 15.6 | 17.7 (17.2), 14.0 – 14.0 | 6.1 (5.5), 5.1 – 5.1 | 1.90 (0.70), 1.75 – 2.05 | 2.03 (2.1), 1.85 – 2.24 | 1.50 (0.60), 1.34 – 1.76 | 9.48 (8.55), 7.75 – 9.45 |
| 4th    | 8199 (1595), 7895 – 8502 | 282 (271), 149 – 230 | 19.0 (18.8), 15.6 – 15.6 | 17.7 (17.2), 14.0 – 14.0 | 6.1 (5.5), 5.1 – 5.1 | 1.90 (0.70), 1.75 – 2.05 | 2.03 (2.1), 1.85 – 2.24 | 1.50 (0.60), 1.34 – 1.76 | 9.48 (8.55), 7.75 – 9.45 |
| 5th    | 8199 (1595), 7895 – 8502 | 282 (271), 149 – 230 | 19.0 (18.8), 15.6 – 15.6 | 17.7 (17.2), 14.0 – 14.0 | 6.1 (5.5), 5.1 – 5.1 | 1.90 (0.70), 1.75 – 2.05 | 2.03 (2.1), 1.85 – 2.24 | 1.50 (0.60), 1.34 – 1.76 | 9.48 (8.55), 7.75 – 9.45 |
| 6th    | 8535 (1540), 8179 – 8891 | 282 (271), 149 – 230 | 19.0 (18.8), 15.6 – 15.6 | 17.7 (17.2), 14.0 – 14.0 | 6.1 (5.5), 5.1 – 5.1 | 1.90 (0.70), 1.75 – 2.05 | 2.03 (2.1), 1.85 – 2.24 | 1.50 (0.60), 1.34 – 1.76 | 9.48 (8.55), 7.75 – 9.45 |
| 7th    | 8125 (1379), 7739 – 8511 | 282 (271), 149 – 230 | 19.0 (18.8), 15.6 – 15.6 | 17.7 (17.2), 14.0 – 14.0 | 6.1 (5.5), 5.1 – 5.1 | 1.90 (0.70), 1.75 – 2.05 | 2.03 (2.1), 1.85 – 2.24 | 1.50 (0.60), 1.34 – 1.76 | 9.48 (8.55), 7.75 – 9.45 |

Data are presented as mean ± SD, 95% CI.

The most important finding of this investigation was the chronic cross-season suppression of s-IgA relative to baseline measures (Fig. 3B). 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 upper respiratory tract infection and symptoms in elite-level professional football players (19,24). In the current investigation, baseline s-IgA was calculated as the average of values measured after a recovery day, during single game weeks in mesocycle 1. We reasoned that this was the most appropriate representation of optimal player “fitness” (i.e., after preseason), when “fatigue” was low (i.e., early in the competitive season, after 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, 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. Because 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 English Championship has the highest

No changes ($P > 0.05$) to perceived stress level or mood were observed (Fig. 4D, E).

**Relationships between salivary and ASRM monitoring variables.** S-IgA shared a quadratic relationship with perceived fatigue ($P = 0.017$; ES = trivial; Table 2; Fig. 5A). 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; Figs. 5B–E). S-C shared linear relationships with perceived fatigue ($P = 0.031$; ES = trivial $\uparrow$) and sleep quality ($P = 0.031$; ES = trivial $\downarrow$; Table 2). S-T:C shared linear relationships with perceived fatigue ($P = 0.014$; ES = trivial $\downarrow$) and sleep quality ($P = 0.031$; ES = trivial $\uparrow$; Table 2).

**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 (Fig. 3B). 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 upper respiratory tract infection and symptoms in elite-level professional football players (19,24). In the current investigation, baseline s-IgA was calculated as the average of values measured after a recovery day, during single game weeks in mesocycle 1. We reasoned that this was the most appropriate representation of optimal player “fitness” (i.e., after preseason), when “fatigue” was low (i.e., early in the competitive season, after 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, 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. Because 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 English Championship has the highest
fixture density of all the major European Leagues (2), and the current cohort were regularly exposed to fixture densities >1 game per week (Fig. 1).

The lowest s-IgA values were observed during mesocycle 5 (Fig. 3B), which coincides with the Christmas fixture period (Fig. 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 ($\leq 1$ 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 (Figs. 3D, F).

Salivary C and s-AA followed similar transient reductions during the second half of the season (Figs. 3C, D). Values were lowest in mesocycle 8, when workload (Fig. 2A) and fixture density (Fig. 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). s-AA is secreted by mucosal cells via the sympathetic adrenal medullary axis (21) and contributes to digestion and mucosal immunity (16). Because of their reactivity to HPA and sympathetic adrenal medullary 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 (Fig. 3E), but an increase in s-T:C during mesocycle six (Fig. 3F) when workload was low, resulting from a trivial increase in s-T (Figs. 3D, E). Testosterone is a steroid hormone secreted from the testes and adrenal glands via the hypothalamic–pituitary–gonadal (testicular) and HPA (adrenal gland) axes (20). It

**FIGURE 3**—Standardized changes to workload (A) and salivary biomarkers across preseason (PS) and eight 6-wk in-season mesocycles: s-IgA (B), s-AA (C), s-C (D), s-T (E), and s-T:C (F). 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.
exerts anabolic effects to increase protein synthesis and decrease protein degradation (20). Research to date indicates acute quantitative changes to T and C, signaling 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 seems to have increased in response to low workload is also consistent with previous research (30,31) and indicates that midseason 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 interteam factors between investigations. In the current investigation, this change was related to a concurrent reduction in both s-T and s-C in mesocycle 8, suggesting a maladaptive training state. This might be explained by increases in psychophysiological stress (20) related to the particularly high game density (Fig. 1) and workload (Fig. 3A) during this phase of the season.

Perceived player fatigue, sleep quality, and muscle soreness improved across the first half of the season (Figs. 4A–C). Equivocal changes were observed for perceived stress level and mood (Figs. 4D, E). The ASRM used herein are typically sensitive to daily, within-weekly and seasonal changes in training load in English Premier League and AFL players (14,15,47,48) and correlate with daily training load during preseason 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 seem to respond to changes in workload or game density. Previous scientific literature suggests that ASRM measures might not account for the effect of all (nontraining related) stressors (49). As such, it is possible that nontraining stressors during the in-season phase disguised ASRM changes related to workload and game demands and might also explain the large SD values observed in the ASRM measures (Fig. 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),

### TABLE 2. Relationships between salivary and ASRM monitoring variables.

| Fatigue | Sleep Quality | Muscle Soreness | Stress Level | Mood | ASRM Total |
|---------|---------------|-----------------|--------------|------|------------|
| s-IgA   | Trivial, 0.44, 0.017* | Trivial, 0.42, 0.305a | Trivial, 0.27, 0.254 | Trivial, 0.51, 0.471* | Trivial, 0.51, 0.526a | Trivial, 0.52, 0.373a |
| s-AA    | Trivial, 0.41, 0.105* | Trivial, 0.46, 0.219a | Trivial, 0.35, 0.255 | Trivial, 0.51, 0.517 | Trivial, 0.51, 0.253 | Trivial, 0.59, 0.314a |
| s-T     | Trivial, 0.44, 0.012* | Trivial, 0.45, <0.001* | Trivial, 0.38, 0.012 | Trivial, 0.52, 0.707 | Trivial, 0.52, 0.822* | Trivial, 0.53, 0.027a |
| s-C     | Trivial, 0.43, 0.031 | Trivial, 0.43, 0.018 | Trivial, 0.37, 0.057 | Trivial, 0.51, 0.305 | Trivial, 0.52, 0.116 | Trivial, 0.51, 0.212a |
| s-T:C   | Trivial, 0.42, 0.014 | Trivial, 0.42, 0.007 | Trivial, 0.36, 0.152 | Trivial, 0.51, 0.776 | Trivial, 0.52, 0.472 | Trivial, 0.51, 0.121 |

Data presented as ES (arrows indicate direction of linear relationships), conditional $R^2$, and $P$ values (significant $P$ values are highlighted in bold).

*Nonlinear relationship.
we educated players regarding ASRM before the investigation. Accordingly, the temporal improvement in ASRM observed herein suggests an adaptive training state.

s-C 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 (Fig. 5B). 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 states (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 nonlinear relationship with sleep quality, whereby very low levels of s-T (−2 SD) were associated with the most compromising effects (Fig. 5C). 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) preseason rugby training camp. Our contrasting findings might relate to differences in the training and competition demands of rugby and football, and 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 because most testosterone secretion occurs during nighttime 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) because sleep quality evidently relates to hormonal balance in professional football players.

A quadratic relationship was observed between s-T and muscle soreness (Fig. 5D), 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 \((P = 0.057; ES = \text{trivial})\). These relationships might reflect the positive anabolic effects of testosterone (20,21) and the negative catabolic consequences of cortisol (20,21) on muscular recovery after 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 (Fig. 5A) 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 5. 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 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 homogeneous sample but acknowledges that other cohorts might respond differently owing to situational, contextual, and interteam 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 behaviors 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 used herein lacks independent scientific validation, and thus, we 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 toward 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 respond 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, as these values were associated with compromised well-being. 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.

CONCLUSIONS

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.

The authors report no conflict of interest. The study is unfunded. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the present study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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