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Human Kinetics
BRIEF REVIEW

Selected Immunoendocrine Measures for Monitoring Responses to Training and Match Load in Professional Association Football: A Review of the Evidence

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Biomarkers relating to player "stress balance," immunological (ie, immunoglobulin-A), and hormonal (ie, testosterone and cortisol [T:C]) status are now commonly used in football. This article is our critical review of the scientific literature relating to the response of these measures to player load and their relationships with player health. The commonly reported relationship between immunoglobulin-A and training or match load highlights its sensitivity to changes in psychophysiological stress and the increased risk of compromised mucosal immunity. This is supported by its close relationship with symptoms of upper respiratory tract infection and its association with perceived fatigue in football players. Testosterone and cortisol concentrations and the testosterone–cortisol ratio are sensitive to changes in player load, but the direction of their response is often inconsistent and is likely influenced by player training status and non-sport-related stressors. Some evidence indicates that sustained periods of high training volume can increase resting testosterone and that sustained periods of low and high training intensity can increase resting cortisol, compromising the testosterone–cortisol ratio. These findings are noteworthy, as recent findings indicate interrelationships between testosterone, cortisol, and testosterone:cortisol and perceived measures of fatigue, sleep quality, and muscle soreness in football players. Variability in individual responses suggests the need for a multivariate and individualized approach to player monitoring. Overall, we consider that there is sufficient evidence to support the use of salivary immunoglobulin-A, testosterone, cortisol, and testosterone:cortisol measures as part of a multivariate, individualized player monitoring system in professional football.

Keywords: saliva, immunological, hormonal, soccer

Professional Association Football is a high-intensity and high-volume competitive sport,[1–4] characterized by a long competitive season with clustered periods of high game density.[5] Players are routinely exposed to high training loads to holistically prepare for these demands.[6–9]

The load–recovery relationship describes the interplay between sport-related stress (applied from single or multiple training sessions and games over time), nonsport–related stress (including any physiological or psychological stimuli or stressors outside of sport), and recovery.[10–12] Achieving stress balance can mitigate the risk of maladaptive training (denoting a negative change in a biological system in response to inappropriate loading and/or inadequate recovery), thereby reducing the risk of injury and illness.[10–12]

Authors of widely cited position and consensus statements advocate the use of biological measures to support the early detection of maladaptive training.[10–12] In football, player monitoring is conducted regularly (ie, daily[13] or biweekly[13–15]), and as such, it is preferable if methods are noninvasive and provide rapid results. Consequently, salivary measures that provide an indication of psychophysiological stress, immunological (ie, immunoglobulin-A), and hormonal (ie, testosterone, cortisol, and testosterone:cortisol [T:C]) regulation are now commonly used in practice.[13]

Despite popular use, the scientific research literature relating to immunological (Immunoglobulin-A), and hormonal (testosterone, cortisol, and T:C) monitoring in football has not been reviewed. Consequently, we reviewed the scientific literature relating to the response of these measures and their relationships with player health and well-being.

Immunological Measures

Salivary Immunoglobulin-A

Biological Role, Synthesis, and Secretary Regulation. Immunoglobulins are glycoproteins secreted by the mucosal surfaces of the gut, urogenital tract, oral cavity, and respiratory system.[16–19] Immunoglobulin secretion is the principal effector function of the mucosal immune system, providing the first line of defense against antigens and pathogens present at the mucosal surfaces. They protect against microbial pathogens by preventing adherence to and penetration across the mucosal epithelium; by neutralizing viruses within the epithelial cells during transcytosis; and by excreting locally formed immune complexes across epithelial cells to the luminal surfaces.[16–19] Salivary IgA (s-IgA) is the most abundant of the 5 secretary immunoglobulins (ie, A, D, E, G, and M), constituting ~90% of the total immunoglobulin concentration in mucosal fluid.[16–19] Therefore, inverse relationships are typically reported between s-IgA and upper respiratory tract

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infection (URTI) risk and symptoms (URTS) in athletes.[16,19–21] For example, Neville et al[20] reported a 50% increase in URTI incidence in athletes when s-IgA concentration decreased to below 40% of the individualized mean healthy concentration. Consequently, this threshold has been widely adopted in practice to indicate when URTI risk is increased.

Synthesis of IgA is mediated by the adaptive immune system.[16–19] In salivary glands, polymeric IgA (p-IgA) is synthesized in plasma cells and crosses adjacent acinar and ductal cells under the regulatory control of polymeric immunoglobulin receptors (p-IgR); considered the rate-limiting step of s-IgA secretion. At the apical membrane, the p-IgR–p-IgA complex splits, releasing a secretory component, which binds with p-IgA to create s-IgA in the mucosal fluid.[16–19]

Secretion of IgA is regulated by the autonomic nervous system (ANS).[16–19] Sympathetic nervous system (SNS) innervation upregulates secretion,[16–19] whereas parasympathetic nervous system (PNS) innervation increases total mucosal fluid secretion.[16–19] Consequently, PNS activity can increase or decrease s-IgA by proxy of regulating the total volume of mucosal fluid secreted.[16–19] Accordingly, s-IgA changes are proposed to indicate ANS function, stress balance, mucosal immunological status, and URTI risk in athletes.[13,16,19,21–29]

Acute Responses to Football. Few investigations have directly examined the acute s-IgA response to football match play. Thorpe and Sunderland reported equivocal pre- to postmatch changes to serum IgA in semiprofessional players.[29] However, Sari-Sarraf et al[30] reported a *small* reduction to s-IgA across 2 bouts of simulated match play, separated by 48 hours. More recently, Coad et al[23] reported a 36-hour reduction to s-IgA following Australian Rules Football (AFL) match play when player match load was high, yet no meaningful changes were observed when player match load was normal. Collectively, these findings infer a particular vulnerability of football players to mucosal immunosuppression following acute periods of high match load, that is, when 2 games are played in quick succession.

Our unpublished findings indicate equivocal postmatch changes to s-IgA during periods of normal player loading, and an increased postmatch s-IgA response during high player loading (Figure 1A). We measured s-IgA in 10 professional male outfield players around 2 league games. Game 1, during a single-game week (ie, when 1 game was played in 7 d) and game 2, the second game during a double-game week (ie, when 2 games were played in 5 d). The same players played between 75 and 90 minutes in game 1 and in both games during the double-game week. For game 1, we observed a *moderate* prematch anticipatory rise in s-IgA at −1 hour, which returned to prematch (−24 h) levels at 1 hour and 72 hours postmatch. For the double-game week, we observed *small* and *moderate* increases to s-IgA at 1 hour and 72 hours postmatch, respectively. These findings might be explained by the additional psychophysiological stress associated with playing 2 games in 5 days. This is supported somewhat by a concurrent increase in salivary cortisol (s-C) observed at the same time points (Figure 1C). The response might also be explained by the effect of nontraining–related stress on SNS activation. For example, s-IgA is known to be sensitive to lifestyle factors, including inadequate diet and psychological stress,[31] that were not quantified in the analysis.

Longitudinal Responses to Football. Several investigations have examined the s-IgA response to sustained football loading, typically reporting an inverse relationship between load and s-IgA. Morgans et al[26] reported a reduction to s-IgA in English Premier League (EPL) players across a condensed winter fixture period (7 games in 30 d), which normalized 10 days after players returned to regular game density. Similarly, Owen et al[32] reported an ~50% reduction to s-IgA during a 7-day period of intensified training. More recently, a reduction to s-IgA was also reported following 4 days of consecutive training across a national team training camp.[27] Sustained periods of high SNS activity are thought to reduce p-IgR availability and limit the transit of s-IgA into saliva.[15,22] This might explain the reductions to s-IgA observed during these periods. Importantly, such reductions to s-IgA have been associated with increased URTS in football players.[21,25] For example, both Moreira et al[25] and Dunbar et al[21] reported inverse relationships between s-IgA and URTS in professional football players.

Notwithstanding previous findings,[26,27,32] our recent study reported that s-IgA did not relate to acute (7 d) or chronic (28 d) exponentially weighted moving average (EWMA) measures of player training load.[14] However, Figueiredo et al[33] reported *large* inverse correlations for measures of training volume (ie, training duration and total distance) and training intensity (ie, number of accelerations) with s-IgA responses across 3 consecutive days of training in elite-level players. Since other research indicates that s-IgA normalizes in <3 days following match play,[23] we proposed[14] that s-IgA might not be sensitive to training and match loads quantified using time windows >3 days. Thus, on balance, it appears that s-IgA might be sensitive to recent (ie, <3 d), but not in longer term (ie, >3 d) changes to training and match volume and intensity in football players.

To date, only 2 studies have examined the cross-season s-IgA response in football players.[15,34] The researchers collected biweekly[15] and weekly[34] saliva samples across English Championship[15] and EPL[34] seasons. We[15] reported a *small* cross-season reduction to s-IgA and that s-IgA was lower in mesocycles characterized by high player load and higher in mesocycles characterized by low player load. Conversely, Dunbar et al[34] reported equivocal cross-season changes to s-IgA but increases during the winter fixture period, when game density was high. Differences in study findings might relate to contextual differences between sample leagues. For example, the English Championship has a substantially greater fixture density than the EPL.[5] Consequently, the s-IgA response observed in the English Championship[15] might be explained by a chronic load-induced suppression of p-IgR availability, resulting from frequent periods of high game density. Comparatively, the increased s-IgA response observed in the EPL cohort[34] might reflect an acute stress response to an isolated period of high game density during a period of otherwise adaptive training.

Nonetheless, our findings[15] are consistent with a cross-season analysis in AFL players,[22] where a *large* reduction to s-IgA was reported, linked to preceding player load. Such results are also consistent with Moreira et al,[25] who reported that a 2-week end-of-season prophylactic period facilitated s-IgA recovery in football players. Interestingly, we[15] also reported a relationship between s-IgA and perceived fatigue, supporting the efficacy of s-IgA as a broader objective measure of player fatigue status. Collectively, existing longitudinal data indicate that football players might be vulnerable to a cross-season suppression of mucosal immunity and that short-term (~2 wk) alleviations to player load facilitate immunological recovery.

In summary, there is evidence of short-term reductions to s-IgA following high isolated match loads and chronic reductions to s-IgA during sustained periods of high load in football players.

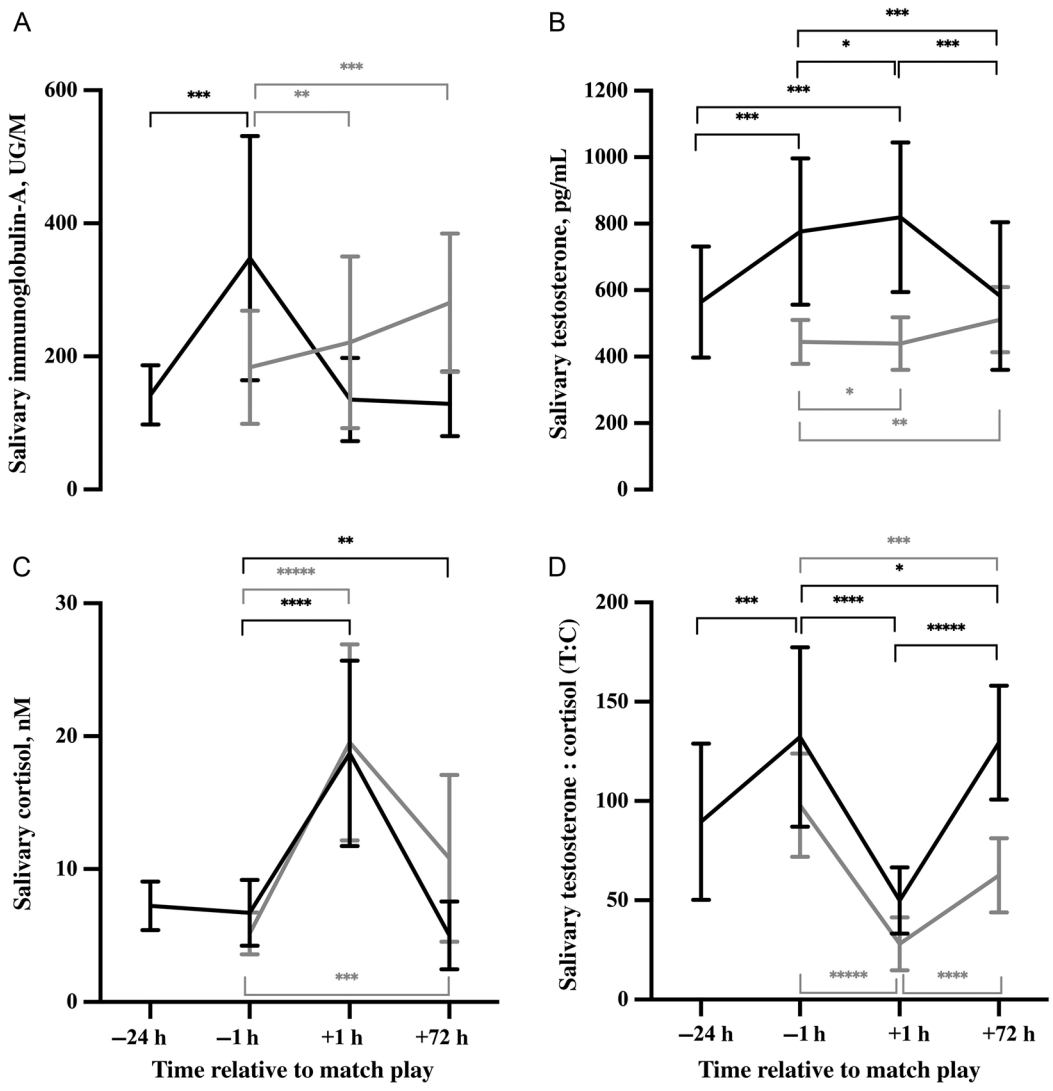

Figure 1 — (A) Salivary immunoglobulin-A (s-IgA), (B) salivary testosterone (s-T), (C) salivary cortisol (s-C), and (D) salivary testosterone:cortisol ratio (s-T:C) responses to professional football match play during single- (black line) and double-game (gray line) game weeks. Error bars denote SD. Symbols denote the clinical significance of biomarker changes using Cohen *d* effect sizes and thresholds proposed by Hopkins et al[85]: *0.0 to 0.2 = *trivial*; **0.2 to 0.6 = *small*; ***0.6 to 1.2 = *moderate*; ****1.2 to 2 = *large*; *****>2 = *very large*. Note: In-house unpublished data.

Furthermore, some research indicates that s-IgA relates to URTI, URTS, and perceived fatigue status in football players, supporting its use in applied practice.

Hormonal Measures

Periods of excessive training load,[31,35–44] competition,[31,40,45–49] and psychological stress[31,39,44,45,48,50–53] can reduce testosterone (T), and/or increase cortisol (C) in athletes, giving rise to a compromised hormonal balance (T:C). Consequently, hormonal monitoring has been advocated to support the identification of maladaptive training in athletes.[11,12,31,44]

Salivary Versus Hematological Measures

Salivary steroid hormone measures provide a reliable reference value for their respective blood concentrations.[31] For example, strong correlations are reported between serum (C) and salivary (s-C)-derived measures of cortisol during rest,[31,54,55] following high-intensity exercise[31,56,57] and following football match play.[31,58] Similarly, strong correlations have also been reported between resting serum (T) and salivary (s-T) measures of testosterone.[31,59,60] However, since salivary hormone concentrations characterize only the free concentration of steroid hormones in blood, they represent only the biologically active portion of each hormone.[31,61] For example, free-, rather than protein-bound- hormones are considered the biologically active components in blood. Since protein-bound hormones are typically too large to transit through salivary glands, only free hormone concentration is measured in saliva. Consequently, salivary measures are thought to provide a more accurate reflection of biologically active hormone concentration than blood. Thus, there might be greater merit in monitoring salivary as opposed to serum hormones in athletes.[31] Indeed, exercise-induced changes in cortisol[31,62,63] and testosterone[31,64] concentrations are more pronounced in saliva than serum.

Salivary Testosterone

Biological Role, Synthesis, and Secretary Regulation. Testosterone is the primary androgenic steroid hormone in males.[31,44,65] It is mostly synthesized from cholesterol in the Leydig cells of the testes under the intermediary control of several other hormones, including progesterone, dehydroepiandrosterone, and androstenedione.[65] To a smaller extent, it is synthesized in the zona reticularis of the adrenal cortex. The principal role of testosterone is to exert anabolic and anticatabolic effects to stimulate protein synthesis and inhibit protein degradation.[65] Since hormonal balance influences glycogen resynthesis,[46] it is also considered to have an important role in muscular and metabolic recovery.[31,44,46,65]

Secretion is principally regulated by the hypothalamic–pituitary–gonadal (HPG) axis in males.[31,44,65] This is initiated by direct innervation of the hypothalamus from the central nervous system (CNS) at the onset of exercise, which stimulates the secretion of gonadotropin-releasing hormone. This, in turn, stimulates the secretion of luteinizing hormone from the gonadotrophic cells of the anterior pituitary gland. Luteinizing hormone binds to G-protein-coupled membrane receptors on the Leydig cells, induced by protein kinase A. This stimulates the synthesis of testosterone, which is released into the systemic circulation.[65]

Acute Responses to Football. Football match play is reported to exert equivocal[66] or increasing[29,67,68] effects on testosterone. For example, Ispirlidis et al[66] reported equivocal pre- to postmatch changes to T.[66] More recently, Thorpe and Sunderland[29] reported a 44% increase to s-T immediately postmatch,[29] and Rowell et al[68] reported postmatch increases to s-T for ~18 hours. Match-induced increases to CNS activity increased hemoconcentration and decreased metabolic clearance, and match running activities were proposed to explain the response.[29] For example, since acute increases in T are widely reported following resistance-type training that induces muscle damage,[31,69,70] Thorpe and Sunderland[29] proposed that muscle damage resulting from sprint activity might exert a similar effect on the postmatch T response. Indeed, a similar "rebound anabolic response" was previously reported following international rugby match play.[47]

Direct analyses of the football load to s-T response relationship yield inconclusive findings. For example, we recently reported that EWMA acute load measures did not relate to s-T responses.[14] Indeed, only coupled (ie, "acute" relative to "chronic" load [A:C]) for high-speed running distance was retained as a predictor of the s-T response, exerting only a *trivial* effect. Conversely, Rowell et al reported an increase to s-T when acute (3 d smoothed average) sRPE load increased by 1 SD, in central defenders.[71] Of note, this response was not observed in the other outfield positional groups.

Consistent with previous reports,[29,67,68] our unpublished findings indicate *moderate* increases to s-T at 1 hour postmatch during normal game density (game 1), (Figure 1B). This response is likely explained by match-induced increases to CNS activity.[10–12] However, during high game density (game 2), we observed only *trivial* (−1 h to +1 h) to *small* (−1 h to +72 h) pre- to postmatch increases to s-T, and an overall suppression of s-T at −1 hour (*large*), 1 hour (*large*), and 72 hours (*small*) compared with game 1. This suggests a downregulation of the HPG axis during periods of increased player loading, signaling a fatigued or otherwise maladaptive training state.[10–12] Importantly, we also observed disparity in individual player responses for s-T (Figure 2B), supporting the need for individualized monitoring in practice.

Longitudinal Responses to Football. Longitudinal investigations have reported equivocal,[15,38] increasing,[36] and decreasing[42] cross-season changes to T in football players. Early investigations measured serum T at 3,[42] 4,[38] and 6[36] time points across the season and reported player load by proxy of average game density,[38] or descriptively.[36,42] More recently, we[15] measured s-T twice a week across a 45-week season and reported cross-season changes to mesocycle average s-T, game density, and sRPE load. Interestingly, despite reporting varying directions for the T response, all investigations reported an inverse relationship between player load, game density, and T.

Notwithstanding previous observational findings,[15,36,38,42] direct examination of the s-T response to chronic football loading indicates a complex relationship.[14,71] For example, we recently reported a *large* positive relationship between EWMA chronic (28 d) total distance and s-T.[14] Similarly, Rowell et al[71] reported increases to s-T following a 28-day period of high load in football players, and Gleeson et al[40] reported an increase to s-T following a 21-day period of high load in international rugby players. Collectively, these findings indicate an upregulation of the HPG axis in response to high training volumes; giving rise to increases in s-T, during periods of otherwise adaptive training.[14]

Evidence is also available to indicate that chronic high-intensity training volume can exert an effect on s-T in football players. For example, we reported a *moderate* inverse relationship between EWMA chronic sRPE load and s-T; and a *small* nonlinear relationship between EWMA chronic high metabolic load distance (HMLd; considered a "global" measure of high-intensity load) and s-T.[14] For the latter relationship, the optimal s-T response was observed at the mean chronic HMLd load, with compromised responses observed at both very low and very high loads. We concluded that these relationships might indicate disturbance to the HPG axis during sustained periods of excessive player loading, signaling a fatigued or maladaptive training state.

In summary, there is good evidence of short-term increases to s-T following football match play and that this effect might be compromised during periods of high player training or match load. There is also evidence that s-T can increase in response to long-term increases in training volume and that excessive high-intensity training volume can compromise this response. Recent findings that s-T measures relate to perceived measures of fatigue, sleep quality, and muscle soreness in football players support the efficacy of s-T as a broader measure of player recovery status. However, practitioners should be aware of high individual variability in the response.[15]

Salivary Cortisol

Biological Role, Synthesis, and Secretary Regulation. Cortisol is a steroid hormone that principally exerts catabolic effects to reduce protein synthesis and increase protein degradation. Metabolically, cortisol increases lipid metabolism and the rate of gluconeogenesis, but inhibits glucose uptake into skeletal muscle by decreasing the translocation of glucose receptors to the cell membrane. Importantly, cortisol inhibits components of inflammatory and immunological function[31,72] and as such is a widely used biomarker of recovery status in athletes.[29,31,36–38,40,42,44,46,52,53,58,72–75]

Cortisol synthesis and secretion are governed by the hypothalamic–pituitary–adrenal (HPA) axis, under ANS control. Psychological or physiological stress stimulates corticotropin-releasing hormone secretion from the paraventricular nucleus of the hypothalamus. This, in turn, stimulates the secretion of adrenocorticotropic

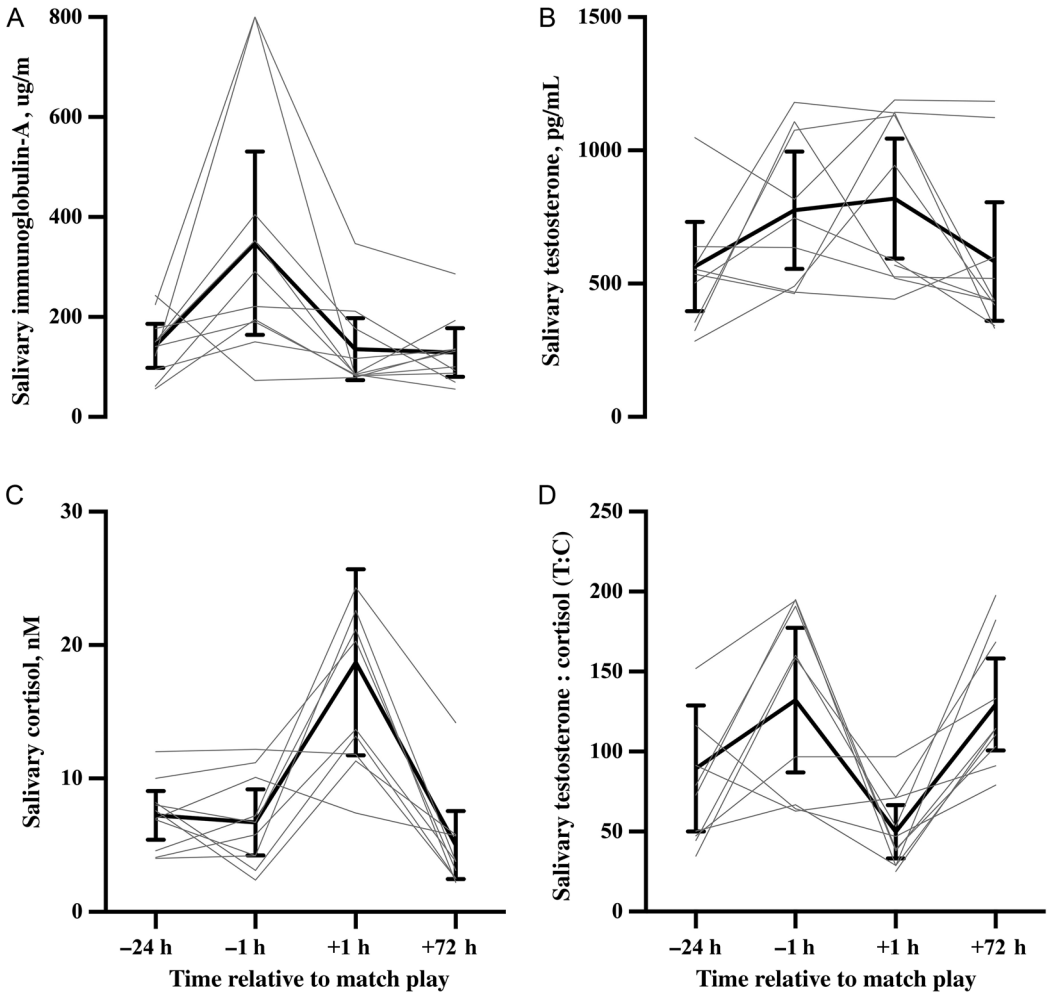

Figure 2 — Group mean and individual player responses for (A) salivary immunoglobulin-A (s-IgA), (B) salivary testosterone (s-T), (C) salivary cortisol (s-C), and (D) salivary testosterone:cortisol ratio (s-T:C) to professional football match play during a single-game week. Error bars denote SD. Note: In-house unpublished data.

hormone from the anterior pituitary gland, which increases cholesterol concentration and the cellular activity of desmolase in the inner mitochondrial membrane of the adrenal gland. Cholesterol is then converted to pregnenolone and progesterone, which converts to 17-A-hydroxyprogesterone, 11-deoxycortisol, and then cortisol, which is secreted into the systemic circulation. Regulation of cortisol secretion is mediated by a negative feedback mechanism governed by mineralocorticoids (MR) and glucocorticoids (GR) receptors in the hypothalamus, which reduce secretion of corticotropin-releasing hormone, and adrenocorticotropic hormone and, therefore, cortisol. Owing to the reactivity of the HPA axis to psychophysiological stress, cortisol is considered to indicate holistic stress balance in athletes.[46,73]

Cortisol exerts its cellular effects by binding to MR and GR. Since MR have a ~10-fold higher affinity for C than GR, MR are considered to govern baseline homeostatic actions, whereas GR only become occupied by C during phasic peaks.[76] Thus, *moderate* C concentrations are considered to "prime" the immune system in anticipation of a threat via MR, whereas *high* concentrations dampen inflammation via GR.[77] GR regulate homeostatic corrections to illness and injury,[78] with insufficient C release leading to unrestrained inflammation.[79] Thus, C secretion is a key corrective mechanism, and dysfunction in secretion will inhibit the restoration of homeostasis.

Acute Responses to Football. Football match play is reported to induce equivocal[58] or increasing[66,68,80,81] effects on cortisol for up to 72 hours postmatch. For example, Ispirlidis et al,[66] Carli et al,[80] and Silva et al[81] reported postmatch increases to C that returned to prematch levels at 45 minutes,[80] 24 hours,[66] and 72 hours[81] postmatch. More recently, Rowell et al[68] reported increases to s-C at 30 minutes postmatch in players with "low," "medium," and "high" match loads. Interestingly, s-C reduced to below prematch levels at 42 hours postmatch in players with medium and high match loads. Similar acute increases to cortisol have also been reported following rugby[47,82] (~36 h) and AFL[46] (~24 h) match play. Of note, 2 of these investigations also reported lower C at 36 hours[47] and 96 hours[46] postmatch, relative to prematch. Cunniffe et al[47] described this as a "rebound anabolic response," since it was coupled with a concurrent increase in T and proposed that it might reflect the physiological requirement to repair match-induced muscle damage.

Again, our unpublished findings indicate that game density influences the postmatch s-C response. For example, consistent

with previous findings, we observed *large* and *very large* increases to s-C (−1 h to + 1 h) during periods of normal (game 1) and high (game 2) game density, respectively (Figure 1C). Interestingly, s-C recovered to below prematch levels at + 72 hours following game 1 (−1 h to + 72 h; ES = *small*) but remained elevated after game 2 at the same time point (−1 h to + 72 h; ES = *moderate*). The latter response likely relates to the additional psychophysiological stress of playing 2 games in 5 days and might indicate that longer recovery periods are required during phases of high game density to accommodate hormonal recovery.

Direct analyses of the load to s-C response relationship yield less consistent findings. For example, Dunbar et al[37] reported a strong correlation between acute (7 d average) HMLd load and the s-C response in EPL players. However, we recently reported that EWMA acute load variables, including HMLd, did not relate to s-C responses.[14] Discrepancies might be explained by methodological differences relating to the calculation of acute load, and by cohort-specific factors.

Longitudinal Responses to Football. Cross-season investigations report equivocal[36] or temporal changes to cortisol that positively relate to player load.[37,38,42] Indeed, Filaire et al[38] reported a mid-season peak in C when match load was high and Handziski et al[42] reported a peak in C during the preseason phase. Findings are likely explained by increased HPA axis activity during periods of increased psychophysiological stress and/or changes to receptor sensitivity or expression. More recently, we reported a *small* increase to s-C during the preseason phase, but a *small* reduction to s-C during the final mesocycle of the season, when game density and player load were high.[15] We proposed that this might indicate that players can maintain an adaptive training state across the competitive season. Indeed, this was reported in AFL players.[73] However, we also cautiously proposed that the response could indicate hyposensitivity of the HPA axis, consistent with maladaptive training.[51] Indeed, previous scientific literature discusses that ANS disturbance might downregulate the adrenalin response and therefore the C response to stress.[83]

We also recently reported that s-C was nonlinearly related to EWMA chronic high-speed running load in football players.[14] For this relationship, s-C was highest at very low and very high loads, with the optimal response observed at the mean. We proposed that this might indicate an effect of training status on s-C. For example, increased psychophysiological stress might be expected during periods of low player "fitness" (ie, when chronic load is very low) and high player "fatigue" (ie, when chronic load is very high), giving rise to increased s-C. Similarly, Rowell et al[71] reported increases to s-C when chronic (28 d) sRPE load increased from low to- high in football players. On balance, findings indicate that s-C measures are sensitive to in-season changes in chronic load and relate to player training status.

In summary, there is evidence that s-C is sensitive to football match play and longer term changes in load. Recent reports that s-C shares linear relationships with perceived fatigue and sleep quality in football players also support the efficacy of s-C as indicator of player recovery status.[15]

The Testosterone–Cortisol Ratio

The testosterone–cortisol ratio (T:C) describes overall anabolic (T) and catabolic (C) balance.[29,35] Since muscular recovery is attenuated in anabolic environments,[29] T:C is considered to be a useful indicator of athletic readiness.[29,31,36,38,42,44,46,52,66,68,71,73–75,84] Efficient muscular recovery is of particular importance to football players, owing to condensed training and match schedules. Consequently, T:C monitoring is thought to have merit in practice.[29] Fatigue or maladaptive training might be indicated by a reduction in T:C, driven by an increase in C, a reduction in T, or both.[46,73]

Acute Responses to Football. Football match play is reported to exert equivocal[29,67] or decreasing[68,81] effects on T:C for up to ~48 hours. Thorpe and Sunderland[29] reported a similar T:C 1 hour before and immediately after match play, owing to concurrent increases in both hormones. It was proposed that this might be explained by some conversion of dehydroepiandrosterone into T, which is secreted in response to the same adrenocorticotropic hormone as C (pregnenolone).[29,67] Indeed, Edwards et al[67] attributed similar findings to the same mechanism. Notwithstanding, Rowell et al[68] reported an immediate reduction to s-T:C following match play, driven by increases to s-C, which normalized in ~18 hours. Of note, the magnitude of this response was greater in players with moderate and large match loads, than in players with low match load. Similarly, Silva et al[81] reported a postmatch reduction to T:C for ~48 hours, owing to postmatch increases to C. Findings are broadly consistent with reports from rugby[47,82,84] and AFL[46] cohorts, where ~14 to 72 hours postmatch reductions to T:C are typical.

Consistent with previous reports,[68,81] our unpublished findings indicate *large* and *very large* reductions to s-T:C at 1 hour postmatch during normal (game 1) and high (game 2) game density scenarios, respectively (Figure 1D). Consistent with previous research,[68,81] this response was driven by postmatch increases to s-C in both scenarios (Figure 1C) and to the additional effect of suppressed s-T during game 2 (Figure 1B). Importantly, for game 1, s-T:C recovered to prematch (−1 h) levels at 72 hours postmatch but remained suppressed at 72 hours postmatch following game 2 (*moderate*). This likely reflects the greater psychophysiological stress of playing 2 games in 5 days and indicates that longer recovery periods are required to restore hormonal balance during periods of high game density.

Longitudinal Responses to Football. Longitudinal investigations have reported equivocal,[38] increasing,[15,36] and decreasing[42] cross-season changes to T:C in football players. Filaire et al[38] reported equivocal cross-season changes, but a reduction to T:C during the middle of the season when match load was high. Similarly, Handziski et al[42] reported a reduction to T:C at the end of the season when match load was high. Reductions to T:C in both investigations were attributed to concurrent increases to C and decreases to T. Inversely, we[15] (saliva) and Kraemer et al[36] (serum) reported increases in T:C when match load was low; attributed to increases in T. Interestingly, these findings suggest that in-season reductions to training load can restore hormonal balance in football players. Moreover, we[15] also reported a low s-T:C during the preseason phase, attributed to increases in s-C when player fitness and thus stress tolerance are low. This led us to propose that s-T:C measures have merit in indicating player training status.

In summary, there is good evidence that s-T:C measures are sensitive to football match play and longer term (approximately 10–28 d) changes to training load. This is supported by studies directly examining the load - s-T:C response in football players.[14,71] For example, Rowell et al[71] reported *small* to *large* reductions to in-season T:C measures when 10-day to 14-day average sRPE load increased from *low* to *high*. Similarly, we[14] reported that EWMA chronic deceleration and summated acceleration and deceleration

load were related to s-T:C responses. Recent reports that s-T:C measures are linearly related to perceived fatigue and sleep quality also support the use of s-T:C as a measure of postmatch recovery and training status in football players.[15]

Practical Applications

Research on the acute and longitudinal response of serum- and salivary-derived measures of IgA, T, C, and T:C to football loading demonstrates the efficacy of these biomarkers for player monitoring. Salivary measures might be particularly useful in practice because they are noninvasive and typically provide faster results. This might facilitate a higher frequency of sampling in the applied environment and serve to improve the precision of player monitoring.

Immunoendocrine responses to football loading are complex and likely to be influenced by contextual factors including training status, recent loading, recent game density, and nonsport–related stress. Consequently, a multivariate approach to individualized player monitoring is advised, whereby measures of player load and nonsport stress (ie, perceived well-being reviews) are used to contextualize immunoendocrine measures. Since data indicate high individual variability for T in particular; the optimal approach to determining player readiness is likely to consider the overall hormonal balance (T:C) in football players.

Practically, immunoendocrine measures can be used to inform player load planning. Current evidence indicates that postmatch immunoendocrine responses necessitate ~48 hours and ~72 hours to normalize during periods of normal and high game density, respectively. In cases where sustained compromised s-IgA or hormonal responses are observed, 2- to 5-week periods of reduced player loading are shown to improve mucosal immunity and hormonal balance in professional football players.

Limitations

The investigations discussed herein typically report CVs in the region of ~6% to 10% for s-IgA, s-T, and s-C when measured using lateral flow or the enzyme-linked immunosorbent assay method. Importantly, random error can be introduced by a researcher or practitioner- (ie, standardization of sample collection and analysis methods), and the measured player (ie, compliance with standardized presample and sample provision guidelines) related factors. Accordingly, practitioners should be appropriately trained, and sample collection and analysis methods should be strictly standardized. The latter should afford consideration, particularly for sample collection location in the mouth (ie, under the tongue, where saliva naturally pools), player dietary habits (ie, abstaining from caffeine consumption) prior to sampling and time of day (ie, to mitigate the effect of diurnal variation). S-IgA, s-T, and s-C typically follow a diurnal pattern of early morning elevation (peaking at ~06:00–09:00), followed by transient reductions across the day. Consequently, time of day can exert meaningful effects on concentration and should be standardized for longitudinal monitoring purposes. In practice, and applied research studies alike, samples are most commonly collected before training (ie, ~09:00–10:00), under resting conditions, thus permitting time for analysis prior to training, which offers further insight into player "readiness" to train.

For hormonal measures, reliability might also be influenced by blood contamination. Consequently, it is advised to control for behaviors that might induce this (ie, toothbrushing), and to screen samples for contamination prior to analysis. Finally, though s-IgA concentration in unstimulated saliva can be influenced by flow rate, measuring flow rate necessitates timely sample collection methods (ie, ~5 min to collect ~1.8 mL of saliva via the passive drool method), which might limit practicality in time-sensitive environments. Consequently, rapid oral fluid collection methods (ie, swab-based systems that collect ~0.5 mL of oral fluid in ~20 s) are more commonly utilized in practice. However, readers are advised that further research is required to examine how flow rate affects-IgA concentration in low volume (ie, 0.5 mL) samples and that not measuring flow rate might account for some variability when using these methods.

Overall, practitioners should consider the validity and reliability data available for each biomarker alongside the practicality of their deployment. In-house variability should then be established to help support the identification of meaningful change in player physiological status.

Unfortunately, there is a lack of scientific research literature available to describe the immunoendocrine responses to football loading in female players. We consider this work to be of urgent importance.

Conclusions

Salivary IgA relates to URTS risk in football players, and s-IgA, s-T, and s-T:C respond to football match play, chronic changes to player load, and relate to perceived measures of player recovery status. Consequently, there is evidence to support the use of these measures as part of an individualized multivariate player monitoring system in elite-level professional football players.

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