Selected immunoendocrine measures for monitoring responses to training and match load in professional association football: a review of the evidence.

Running Head: Immunoendocrine Measures in Football

Invited Review Article.

Abstract

Biomarkers relating to player 'stress-balance', immunological (i.e., immunoglobulin-A) and hormonal (i.e., testosterone and cortisol) 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 inter-relationships between testosterone, cortisol, 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

individualised 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, individualised player monitoring system in professional football.

Keywords

Monitoring; Saliva; Immunological; Hormonal; Soccer

Introduction

Professional Association Football is a high-intensity and high-volume competitive sport, [1-4] characterised 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), non-sport 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 (i.e., daily [13] or bi-weekly [13-15]), and as such, it is preferable if methods are non-invasive and provide rapid results. Consequently, salivary measures that provide an indication of psychophysiological stress, immunological (i.e., immunoglobulin-A) and hormonal (i.e., testosterone, cortisol and testosterone:cortisol) 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 testosterone:cortisol [T:C]) monitoring in football has not been reviewed. Consequently, we reviewed the scientific literature relating to the response of these measures football and their relationships with player health and wellbeing.

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 defence 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 neutralising 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 five secretary immunoglobulins (i.e., 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 infection (URTI) risk and symptoms (URTS) in athletes. [16,19-21] For example, Neville and colleagues [20] reported a 50% increase in URTI incidence in athletes when s-IgA concentration decreased to below 40% of the individualised 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 adaptative immune system. [16-19] In salivary glands, polymeric IgA (p-IgA) is synthesised 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 secretary component (SC), 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 innervation up-regulates secretion, [16-19] whereas parasympathetic 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-post match changes to serum IgA in semi-professional players. [29] However, Sari-Sarraf and colleagues [30] reported a *small* reduction to s-IgA across two bouts of simulated match play, separated by 48 h. More recently, Coad and

colleagues [23] reported a 36 h 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, i.e., when two games are played in quick succession.

Our unpublished findings indicate equivocal post-match changes to s-IgA during periods of normal player loading, and an increased post-match s-IgA response during high player loading (Figure 1, Panel A). We measured s-IgA in 10 professional male outfield players around two league games. Game 1, during a single game week (i.e., when one game was played in seven days) and game 2, the second game during a double game week (i.e., when two games were played in five days). The same players played between 75 and 90 min in game 1 and in both games during the double game week. For game 1 we observed a *moderate* pre-match anticipatory rise in s-IgA at - 1 hr, which returned to pre-match (-24 h) levels at 1 hr and 72 h post-match. For the double game week, we observed *small* and *moderate* increases to s-IgA at 1 h and 72 h post-match, respectively. These findings might be explained by the additional psychophysiological stress associated with playing two games in five days. This is supported somewhat by a concurrent increase in salivary cortisol (s-C) observed at the same time points (Figure 1, Panel C). The response might also be explained by the effect of non-training 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.

*** INSERT FIGURE 1 HERE***

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 and colleagues, [26] reported a reduction to s-IgA in English Premier League (EPL) players across a condensed winter fixture period (seven games in 30 d), which normalised ten days after players returned to regular game density. Similarly, Owen and colleagues [32] reported an ~ 50% reduction to s-IgA during a seven-day period of intensified training. More recently, a reduction to s-IgA was also reported following four 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 and colleagues [25] and Dunbar and colleagues [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 and colleagues [33] reported *large* inverse correlations for measures of training volume (i.e., training duration and total distance) and training intensity (i.e., number of accelerations) with s-IgA responses across three consecutive days of training in elite level players. Since other research indicates that s-IgA normalises in <3 d following match play, [23] we proposed [14] that s-IgA might not be sensitive to training and match loads quantified using time windows > 3 d. Thus, on balance, it appears that s-IgA might be sensitive to recent (i.e., < 3 d) but not longer-term (i.e., > 3 d) changes to training and match volume and intensity in football players.

To date, only two studies have examined the cross-season s-IgA response in football players. [15,34] The researchers collected bi-weekly [15] and weekly [34] saliva samples across English Championship (EC) [15] and EPL [34] seasons. We [15] reported a *small* cross-season reduction to s-IgA and that s-IgA was lower in mesocycles characterised by high player load and higher in mesocycles characterised by low player load. Conversely, Dunbar and colleagues [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 EC has a substantially greater fixture density than the EPL. [5] Consequently, the s-IgA response observed in the EC [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 and colleagues, [25] who reported that a two-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-weeks) alleviations to player load facilitate immunological recovery.

In summary, ~~we consider that~~ there is ~~some~~ evidence of short-term reductions to s-IgA following high isolated match loads and ~~that there is good evidence of~~ chronic reductions to s-IgA during sustained periods of high load in football players. Furthermore, ~~there is also~~ some research indicates that s-IgA relates to URTI, URTS and perceived fatigue status in football players; ~~which~~ 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 vs. Haematological 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 characterise 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 synthesised from cholesterol in the Leydig cells of the testes under the intermediary control of several other hormones, including progesterone, dehydroepiandrosterone (DHEA) and androstenedione. [65] To a smaller extent, it is synthesised in the zona reticularis of the adrenal cortex. The principle role of testosterone is to exert anabolic and anti-catabolic 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-axis (HPG) 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 (GnRH). This, in-turn, stimulates the secretion of luteinizing hormone (LH) 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 and colleagues [66] reported equivocal pre-to post match changes to T. [66] More recently, Thorpe and Sunderland [29] reported a 44% increase to s-T immediately post-match, [29] and Rowell and colleagues [68] reported post-match increases to s-T for ~ 18 h. Match-induced increases to CNS activity, increased haemoconcentration, 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 post-match 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 (i.e., 'acute' relative to 'chronic' load [A:C]) for high-speed running distance (HSR) was retained as a predictor of the s-T response, exerting only a *trivial* effect. Conversely, Rowell and colleagues 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 h post-match during normal game density (game 1), (Figure 1, panel B). 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-post match increases to s-T, and an overall suppression of s-T at - 1 h (*large*), 1 h (*large*) and 72 h (*small*) compared to game 1. This suggests a downregulation of the HPG axis during periods of increased player loading, signalling a fatigued or otherwise maladaptive training state. [10-12] Importantly, we also observed disparity in individual player responses for s-T (Figure 2, Panel B), supporting the need for individualised monitoring in practice.

INSERT FIGURE 2 HERE

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 three, [42] four, [38] and six [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 and colleagues [71] reported increases to s-T following a 28 d period of high load in football players, and Gleeson and colleagues [40] reported an increase to s-T following a 21 d 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* non-linear 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, signalling a fatigued or maladaptive training state.

In summary, ~~we consider that~~ there is ~~good~~ evidence of short-term increases to s-T following football match play, and ~~some evidence to indicate~~ that this effect might be compromised during periods of high player training or match load. There is also ~~some~~ 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

298 the translocation of glucose receptors to the cell membrane. Importantly, cortisol inhibits
299 components of inflammatory and immunological function, [31,72] and as such, is a widely used
300 biomarker of recovery status in athletes. [29,31,36-38,40,42,44,46,52,53,58,72-75]
301
302 Cortisol synthesis and secretion are governed by the hypothalamic-pituitary-adrenal (HPA)
303 axis, under ANS control. Psychological or physiological stress stimulate corticotropin-
304 releasing-hormone (CRH) secretion from the paraventricular nucleus of the hypothalamus.
305 This, in-turn stimulates the secretion of adrenocorticotropic hormone (ACTH) from the anterior
306 pituitary gland, which increases cholesterol concentration and the cellular activity of desmolase
307 in the inner mitochondrial membrane of the adrenal gland. Cholesterol is then converted to
308 pregnenolone and progesterone, which converts to 17-A-hydroxyprogesterone, 11-
309 deoxycortisol, and then cortisol; which is secreted into the systemic circulation. Regulation of
310 cortisol secretion is mediated by a negative feedback mechanism governed by
311 mineralocorticoid (MR) and glucocorticoid (GR) receptors in the hypothalamus, which reduce
312 secretion of CRH, and ACTH and, therefore, cortisol. Owing to the reactivity of the HPA axis
313 to psychophysiological stress, cortisol is considered to indicate holistic stress balance in
314 athletes. [46,73]
315
316 Cortisol exerts its cellular effects by binding to MR and GR. Since MR have a ~ 10-fold higher
317 affinity for C than GR, MR are considered to govern baseline homeostatic actions, whereas GR
318 only become occupied by C during phasic peaks. [76] Thus, *moderate* C concentrations are
319 considered to 'prime' the immune system in anticipation of a threat via the MR, whereas *high*
320 concentrations dampen inflammation via the GR. [77] The GR regulate homeostatic corrections
321 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 h post-match. For example, Ispirlidis and colleagues, [66] Carli and colleagues, [80] and Silva and colleagues [81] reported post-match increases to C, that returned to pre-match levels at 45 min, [80] 24 h, [66] and 72 h [81] post-match. More recently, Rowell and colleagues [68] reported increases to s-C at 30 min post-match in players with 'low', 'medium' and 'high' match loads. Interestingly, s-C reduced to below pre-match levels at 42 h post-match 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, two of these investigations also reported lower C at 36 h [47] and 96 h [46] post-match, relative to pre-match. Cunniffe and colleagues [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 post-match 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 1, panel C). Interestingly, s-C recovered to below pre-match levels at + 72 h 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 two games in five 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 and colleagues, [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 and colleagues [38] reported a mid-season peak in C when match load was high and Handziski and colleagues [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 non-linearly related to EWMA chronic HSR 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' (i.e., when chronic load is very low) and high player 'fatigue' (i.e., when chronic load is very high), giving rise to increased s-C. Similarly, Rowell and colleagues [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, ~~we consider that~~ there is ~~good~~ evidence that s-C is sensitive to football match play and ~~some evidence that s-C is sensitive to~~ 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 particular 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 h. Thorpe and Sunderland [29] reported a similar T:C 1 h 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 DHEA into T, which is secreted in response to the same adrenocorticotropic hormone as C (pregnenolone). [29,67] Indeed, Edwards and colleagues [67] attributed similar findings to the same mechanism. Notwithstanding, Rowell and colleagues [68] reported an immediate reduction to s-T:C following match play, driven by increases to s-C, which normalised in ~ 18 h. 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 and colleagues [81] reported a post-match reduction to T:C for ~ 48 h, owing to post-match increases to C. Findings are broadly consistent with reports from rugby [47,82,84] and AFL [46] cohorts, where ~ 14 to 72 h post-match 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 h post-match during normal (game 1) and high (game 2) game density scenarios, respectively, (Figure 1, Panel D). Consistent with previous research, [68,81] this response was driven by post-match increases to s-C in both scenarios (Figure 1, Panel C), and to the additional effect of suppressed s-T during game 2 (Figure 1, Panel B). Importantly, for game 1, s-T:C recovered to pre match (- 1 h) levels at 72 h post-match but remained supressed at 72 h post-match following game 2 (*moderate*). This likely reflects the greater psychophysiological stress of playing two games in five 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 and colleagues [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 and colleagues [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 and colleagues [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 pre-season 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, ~~we consider that~~ there is ~~good~~ evidence that s-T:C measures are sensitive to football match play and longer term (~ 10 d to 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 and colleagues, [71] reported *small* to *large* reductions to in-season T:C measures when 10 d to 14 d 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 post-match 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 non-invasive 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 non-sport related stress. Consequently, a multivariate approach to individualised player monitoring is advised, whereby measures of player load and non-sport stress (i.e., perceived wellbeing reviews) are used to contextualise immunoendocrine measures. Since data indicates 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 post-match immunoendocrine responses necessitate ~ 48 h and ~ 72 h to normalise during periods of normal and high game density, respectively. In cases where sustained compromised s-IgA or hormonal responses are observed, two- to five- 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 - 10% for s-IgA, s-T and s-C when measured using lateral flow or the enzyme-linked immunosorbent assay

(ELISA) method. Importantly, random error can be introduced by a researcher or practitioner- (i.e., standardisation of sample collection and analysis methods), and the measured player- (i.e., compliance with standardised pre-sample and sample provision guidelines) related factors. Accordingly, practitioners should be appropriately trained, and sample collection and analysis methods should be strictly standardised. The latter should afford particular consideration for sample collection location in the mouth (i.e., under the tongue, where saliva naturally pools), player dietary habits (i.e., abstaining from caffeine consumption) prior to sampling and time of day (i.e., 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 standardised for longitudinal monitoring purposes. In practice, and applied research studies alike, samples are most commonly collected before training (i.e., ~ 09:00 – 10:00), under resting conditions, thus permitting time for analysis prior to training, whichoffers 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 behaviours that might induce this (i.e., tooth brushing), 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 (i.e., ~ 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 (i.e., swab-based systems that collect ~ 0.5 ml of oral fluid in ~ 20 s) are more commonly utilised in practice. However, readers are advised that further research is required to examine how flow rate affects-IgA concentration in low

volume (i.e., 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, ~~we consider that~~ there is s~~ufficient~~ evidence to support the use of these measures as part of an individualised multivariate player monitoring system in elite-level professional football players.

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Figure Captions

Figure 1. Panel A: Salivary immunoglobulin-A (s-IgA); Panel B: salivary testosterone (s-T), Panel C: salivary cortisol (s-C) and Panel D: salivary testosterone:cortisol ratio (s-T:C) responses to professional football match play during single- (black line) and double- (grey line) game weeks. Error bars denote SD. Symbols denote the clinical significance of biomarker changes using Cohen's *d* effect sizes and thresholds proposed by Hopkins and colleagues [85]: *, 0.0-0.2 = *trivial*; **, 0.2-0.6 = *small*; ***, 0.6-1.2 = *moderate*; ****, 1.2-2 = *large*; *****, >2 = *very large*. Note: unpublished data.

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