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

4

5 **Original Investigation.**

6

7 Matthew Springham^{1,2}

8 Sean Williams³

9 Mark Waldron^{4,5}

10 Chris Mclellan⁶

11 Robert U. Newton²

12

13 ¹ Faculty of Sport, Health and Applied Science, St Marys University, London, UK.

14 ² School of Medical and Health Sciences, Edith Cowan University, Joondalup, AU.

15 ³ Department for Health, University of Bath, Bath, UK.

16 ⁴ College of Engineering, Swansea University, Swansea, UK.

17 ⁵ School of Science and Technology, University of New England, NSW, Armidale, AU

18 ⁶ School of Health and Wellbeing, University of Southern Queensland, Toowoomba,
19 AU.

20

21 Corresponding Author: Springham, M (matt.springham@stmarys.ac.uk)

22

23 **Title**

24

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

28

29 **Running Title**

30

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

33

34 **Abstract**

35

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

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

61

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

63

64 **Introduction**

65

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

73

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

90

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

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

99

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

108

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

121 derived from both sport and non-sport means ²⁻⁴) and the load-recovery relationship
122 in athletes ¹⁵ and football players ^{13,16,20-22,24-26}.

123

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

140

141 **Materials and Methods**

142

143 ***Study design***

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

146 = 181 ± 7.0 cm, body mass = 72.4 ± 5.2 kg) from one English Championship (EC)
147 team across one complete season. Informed consent was obtained from all
148 participants prior to data collection and an ethics declaration was approved for this
149 investigation by the Edith Cowan University (Australia) Human Research Ethics Office.

150

151 ***Training load***

152 Training load was recorded for all pre-season and in-season training sessions.
153 External load was measured using sports GPS and MEMS sensors (Statsports Viper
154 2, Belfast, Northern Ireland, UK), sampling at 10 Hz (GPS) and 100 Hz (tri-axial
155 accelerometer, gyroscope and magnetometer). Typical error for distance and speed
156 for this device are $< 3\%$ and $< 2\%$ ²⁷ respectively. A software application
157 (www.gnssplanning.com) reported previously ⁹, was used to identify a geographical
158 point (ground station) based on the latitude and longitude coordinates of the team
159 training facility. This determined the mean number of satellites and horizontal dilution
160 of precision for GPS data across the sample period, which equated to 8.7 ± 1.0 and
161 $0.66 \pm 0.08\%$ respectively; indicating optimal conditions for satellite transmissions ²⁸.

162

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

171 efforts > 80% of individualised maximal linear running velocity); and high intensity
172 variables: total number of accelerations (ACC), decelerations (DEC) and changes to
173 speed (ACC+DEC) were recorded. Acceleration and DEC efforts were identified
174 according to manufacturer guidelines as a change in player velocity of > 0.5 m/s²
175 maintained for > 0.5 s. Efforts were zone-banded based on the peak magnitude of
176 ACC or DEC with thresholds set at > 3 m/s² and > -3 m/s² respectively. These
177 thresholds are consistent with those reported elsewhere in the football science
178 research literature ^{9,29-32}. Training load data were extracted from GPS devices using
179 manufacturer software (Statsports Viper, Belfast, Northern Ireland, UK). Internal load
180 was recorded using player rating of perceived exertion (RPE) from the CR-10-scale
181 ³³. CR-10 response was collected within 30 min of all training sessions and multiplied
182 by session duration (min) to provide an arbitrary unit (AU) of session load, denoted as
183 sRPE. This method has been validated for use in football previously ³⁴. Data collection
184 and analysis was completed by the same investigator across the entire sample period.

185

186 ***Match load***

187 Match load was recorded for all home and away games. External load variables were
188 measured using 6 fixed semi-automated high definition motion cameras (Chyronhego
189 TRACKAB, London, UK). Following games, raw TRACKAB player position data were
190 converted to equivalent training load variables using manufacturer software
191 (Statsports Viper, Belfast, Northern Ireland, UK). This method has been described
192 previously ³⁵, and is widely used in practice and scientific research literature ^{7,9,35}.
193 Strong relationships are reported between Statsports Viper and TRACKAB for TD (r^2
194 = 0.98) and HSR ($r^2 = 0.98$) ³⁵ and our unpublished data indicate strong relationships
195 for HMLd ($r^2 = 0.93$), ACC ($r^2 = 0.94$), DEC ($r^2 = 0.95$) and number of sprints ($r^2 = 0.97$)

196 using this method during elite-level professional football match play. Internal match
197 load was calculated using the same sRPE method as was used following training.

198

199 **Composite load indices**

200 For each load variable, the pooled (summated training and match derived measures)
201 7 d absolute sum, 28 d absolute sum, exponentially weighted moving average
202 (EWMA) acute load, EWMA chronic load and the EWMA acute : chronic load ratio
203 (A:C) were calculated. EWMA indices were calculated using equations by Williams
204 and colleagues ³⁶:

205

$$206 \quad EWMA_{today} = Load_{today} * \lambda_a + ((1 - \lambda_a) * EMWA_{yesterday})$$

207

208 Where λ_a represents the degree of time decay. Time decay was calculated using:

209

$$210 \quad \lambda_a = 2/(N + 1)$$

211

212 Where N is the chosen time decay constant. Decay factors representing time
213 constants for 7 d (acute) and 28 d (chronic) were used. These equated to 0.25 and
214 0.069 respectively.

215

216 **Saliva Sampling**

217 Saliva samples were collected the morning after rest and / or recovery days across
218 the sample period. Typically, this was two days prior (i.e., match day (MD) -2) and two
219 days after (i.e., MD +2) games during both single and double game weeks. Baseline
220 saliva measures were calculated for individual players as the mean of MD-2 data

221 collected during single game weeks in the first 5-week in-season mesocycle. We
222 reasoned that this best represented when player 'fitness' was high (i.e., following pre-
223 season), when 'fatigue' was low (i.e., early in the competitive season, following a
224 recovery day during single game weeks) and thus when player holistic stress balance
225 was optimal. Players reported to the team training facility between 09:00 and 09:30 on
226 sample collection days. They were asked to abstain from caffeine consumption prior
227 to sample collection and samples were collected prior to breakfast and training. They
228 were asked to sit quietly, swallow existing saliva in the mouth and to then place an
229 oral fluid collector (OFC; SOMA Bioscience, Wallingford, UK) on the tongue. With the
230 mouth closed, 0.5 ml of saliva was collected, as indicated by a volume adequacy
231 indicator on the OFC. The OFC was then placed into 3 ml of buffer solution in a bespoke
232 10 ml container (OFC Buffer; SOMA Bioscience, Wallingford, UK) and mixed gently
233 by hand for 2 min.

234

235 ***Salivary IgA and Cortisol***

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

246 applied football environment. Comparison of the LFD method with the enzyme-linked
247 immunosorbent assay (ELISA) method indicates strong validity for s-IgA ($r = 0.93$; P
248 < 0.001)¹⁰ and s-C ($r^2 = 0.79$)³⁷. Repeated sampling indicates strong reliability for s-
249 IgA (ICC $r = 0.89$, $P < 0.001$ and CV = 9.4%)¹⁰ and s-C (CV = 6.8%)³⁷.

250

251 ***Salivary α -Amylase and Testosterone***

252 The remaining OFC buffer solution was sealed and taken to a private laboratory
253 (SOMA Bioscience, Wallingford, UK) where s-AA ($\mu\text{g/ml}$) and s-T (pg/ml) were
254 measured by ELISA using enzyme immunoassay test kits (EIA; SOMA Bioscience,
255 Wallingford, UK), and an automated analyser (Tecan Nanoquant, Tecan, Männedorf,
256 Switzerland) as per manufacturer guidelines. Following analysis, s-T was converted
257 to its molar value to calculate s-T:C. All analysis was completed by the same
258 laboratory technician. All samples were analysed within 24 h of collection. The intra-
259 and inter- assay CV for s-AA and s-T analysis using this method is 4.71% and 11.4%;
260 and 7.94% and 9.4% respectively; as reported in other applied environments³⁸.

261

262 ***Statistical analysis***

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

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

273

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

282

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

296 random effects term of the mixed model. Thresholds for ES were: 0.0-0.2 = *Trivial*;
297 0.2-0.6 = *Small*; 0.6-1.2 = *Moderate*; 1.2-2 = *Large*; >2 = *Very Large*. The conditional
298 R² value (which considers both fixed and random effects in the model) is also provided
299 as a goodness-of-fit measure for these relationships. Data for non-linear relationships
300 is presented as means and 95% CI with estimated salivary variable responses at
301 typically very low (-2 SD), low (-1 SD), mean, high (+ 1 SD) and very high (+2 SD)
302 values of each training and match load predictor variable .

303

304 **Results**

305

306 ***Predictors of Salivary Proteins***

307 *s-IgA*

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

310

311 *s-AA*

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

314

315 ***Predictors of Salivary Hormones***

316 *s-T*

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

321 A), chronic HSR ($P = 0.001$, $ES = Trivial$) (Figure 1, Panel B) and chronic HMLd ($P =$
322 0.001 , $ES = Small$) (Figure 1, Panel C). For TD, s-T was highest at very high chronic
323 load (+2 SD). For HSR, s-T was highest at very low (-2 SD) and very high (+2 SD)
324 chronic load. For HMLd, s-T was highest at squad mean chronic load.

325

326 ***Insert Table 1 Here***

327

328 ***Insert Figure 1 Here***

329

330 s-C

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

336

337 ***Insert Table 2 Here***

338

339 s-T:C

340 Four variables were retained from the variable selection process for s-T:C (Table 3).
341 Linear effects were identified for phase of season ($P = < 0.001$, $ES = Small \uparrow$) and
342 chronic HSR ($P = 0.554$, $ES = Trivial \uparrow$). Non-linear effects were identified for chronic
343 DEC ($P = 0.039$, $ES = small$) (Figure 1, Panel E) and chronic ACC+DEC ($P = 0.039$,
344 $ES = Small$) (Figure 1, Panel F). For chronic DEC, s-T:C was highest at squad mean

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

347

348 ****Insert Table 3 Here****

349

350 **Discussion**

351

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

363

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

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

389

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

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

409

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

420 the threshold employed herein ($> -3 \text{ m/s}^2$)²⁹. Thus, these relationships also implicate
421 chronic high-intensity training volume as an important moderating factor for T and T:C
422 profile changes in football players. Moreover, these findings indicate merit in ensuring
423 that players are exposed to appropriate chronic HMLd and DEC loads to optimise T
424 and T:C responses, but to avoid *excessive* chronic HMLd and DEC loads (i.e., +1 and
425 +2 SD of chronic HMLd and DEC training and match load herein), since these
426 scenarios might compromise the hormonal response.

427

428 The notion that chronic high-intensity training volume can exert an important influence
429 on hormonal profile is also supported somewhat by the significant, albeit *trivial*, 'U'-
430 shaped relationship identified between s-C and chronic HSR (Table 2 and Figure 1,
431 Panel D). For this relationship, the s-C response was highest at *very low* and *very high*
432 chronic HSR load and was lowest at approximately the squad mean HSR load. Cortisol
433 is secreted in response to HPA axis activation and is used as a quantitative stress
434 biomarker in athletes¹⁷. Accordingly, this suggests that periods of low 'fitness' (i.e.,
435 when chronic HSR is *very low*) and high 'fatigue' (i.e., when chronic HSR is *very high*)
436 exert compromising effects on C concentration in football players. Interestingly, this
437 finding is consistent with previous research, reporting increases in s-C during periods
438 of increased training intensity¹² and load²¹ in football players. Practically, the nature
439 of this relationship indicates merit in exposing players to an appropriate chronic HSR
440 load (to optimise the C response) but to avoid excessively low (i.e., -2 and -1 SD) and
441 high (i.e., +1 and +2 SD) chronic HSR loads, since this might compromise the C
442 response.

443

444 Surprisingly, no training and match load variables related to s-IgA or s-AA profile
445 changes. This contrasts previous research, indicating that s-IgA and s-AA measures
446 are sensitive to changes in load in football players ^{13,14,16} and professional ¹⁷ and
447 Paralympic ^{18,19} swimmers. Indeed, existing data typically indicate reductions in s-IgA
448 in response to acute ¹⁴ and chronic ^{13,16} periods of increased load in football players.
449 We propose several explanations for this finding. First, consistent with previous
450 recommendations, ³⁶ we quantified 'acute' load using an EWMA 7 d decay factor,
451 spanning 168 hr of training and competition time. Though equivalent data are
452 unavailable for s-AA, s-IgA is reported to normalise in ~ 18 to 60 h following training
453 and match-play ⁴³ in football players, respectively. Thus, it is possible that s-IgA
454 measures are not sensitive to load accrued > 60 h preceding sample collection.
455 Indeed, s-IgA measures might indicate short-term (i.e., 1-3 d), but not long-term (i.e.,
456 4-7 d) stress balance within-microcycles in football players. It is also possible that our
457 finding is explained somewhat by the effect of non-training related stress on SNS
458 activation. Indeed, lifestyle factors and other sources of psychophysiological stress
459 that were not quantified in the current investigation could have 'masked' load-induced
460 secretory changes to s-IgA and s-AA. For example, it is known that both s-IgA and s-
461 AA are sensitive to changes in psychological stress ¹⁵. Importantly, since acute load
462 variables did not relate to any of the salivary biomarkers, it is evident that EWMA 7 d
463 'acute' and A:C measures should not be used as surrogate measures of player
464 'fatigue' status in isolation.

465

466 **Strengths and Limitations**

467

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

487

488 **Practical Applications**

489

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

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

502

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

509

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

516

517 **Conclusion**

518

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

523

524 **Disclosure of Interest**

525

526 The authors report no conflict of interest.

527

528 **References**

529

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661

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

s-IgA					
<i>Predictors</i>	<i>Estimates</i>	<i>ES</i>	<i>CI</i>	<i>Standardized CI</i>	<i>P</i>
(Intercept)	89.71		43.8 – 135.7		<0.001
Phase of Season	34.8	<i>Trivial</i> ↑	8.1 – 61.6	0.02 – 0.13	0.011
Random Effects					
σ^2	18274				
T00 Player_ID	1357				
ICC	0.07				
N Player_ID	18				
Observations	1154				
Marginal R ²	0.123				
Conditional R ²	0.184				

664

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

666

s-AA					
<i>Predictors</i>	<i>Estimates</i>	<i>ES</i>	<i>CI</i>	<i>Standardized CI</i>	<i>P</i>
(Intercept)	169.8		111.4 – 228.4		<0.001
Phase of Season	-163.0	<i>Small</i> ↓	-197.5 – -128.6	-0.30 - -0.20	<0.001
Random Effects					
σ^2	29408				
T00 Player_ID	3711				
ICC	0.11				
N Player_ID	18				
Observations	1136				
Marginal R ²	0.212				
Conditional R ²	0.300				

667

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

671

s-T					
<i>Predictors</i>	<i>Estimates</i>	<i>ES</i>	<i>CI</i>	<i>Standardized CI</i>	<i>P</i>
(Intercept)	208.2		5.58 – 410.8		0.044
Phase of Season	-73.9	<i>Trivial</i> ↓	-124.8 – -23.0	-0.12 - 0.01	0.004
EWMA chronic TD ²	0.00	<i>Large</i>	0.00 – 0.00	0.03 – 0.28	0.015
EWMA chronic HSR ²	0.02	<i>Trivial</i>	0.01 – 0.03	0.08 – 0.30	0.001
EWMA chronic HMLd ²	0.00	<i>Small</i>	-0.01 – -0.00	-0.53 – -0.14	0.001
EWMA chronic sRPE	-1.32	<i>Moderate</i> ↓	-2.13 – -0.50	-0.61 – -0.14	0.002
EWMA A:C HSR	16.9	<i>Trivial</i> ↑	3.87 – 29.98	0.03 – 0.20	0.011
Random Effects					
σ^2	40663				
T00 Player_ID	3396				
ICC	0.08				
N _{Player_ID}	18				
Observations	1093				
Marginal R ²	0.087				
Conditional R ²	0.157				

672

673 **Table 2.** Predictors of Salivary Cortisol (s-C). EWMA, exponentially weighted moving average; HSR,
 674 high-speed running; ², denotes a non-linear relationship

675

s-C					
<i>Predictors</i>	<i>Estimates</i>	<i>ES</i>	<i>CI</i>	<i>Standardized CI</i>	<i>P</i>
(Intercept)	12.79		8.47 – 17.12		<0.001
Phase of Season	-8.53	<i>Small</i> ↓	-10.5 – -6.6	-0.34 – -0.22	<0.001
EWMA chronic HSR ²	0.00	<i>Trivial</i>	0.00 – 0.00	0.04 – 0.15	0.001
Random Effects					
σ^2	74.24				
T00 Player_ID	5.29				
ICC	0.07				
N _{Player_ID}	18				
Observations	1083				
Marginal R ²	0.138				
Conditional R ²	0.195				

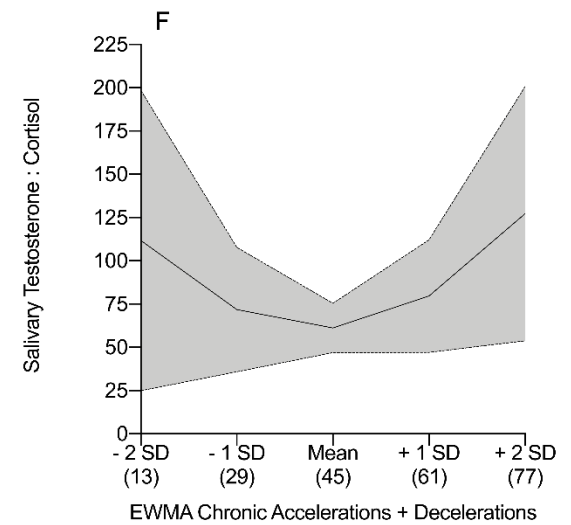
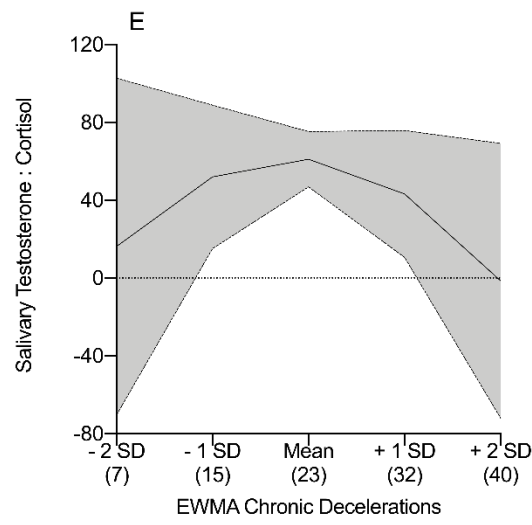
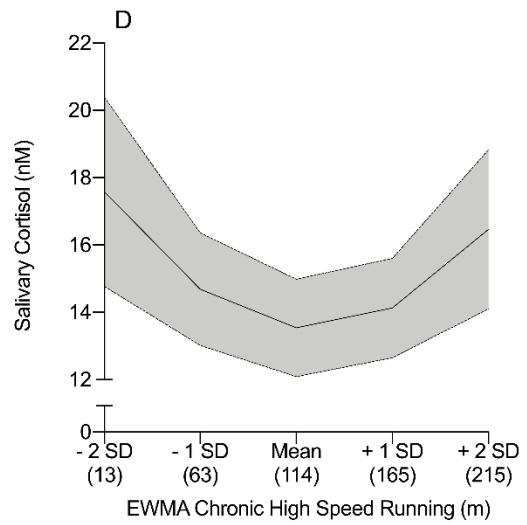
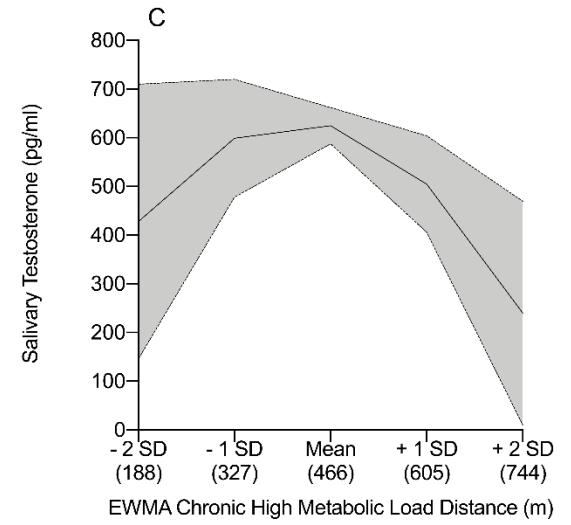
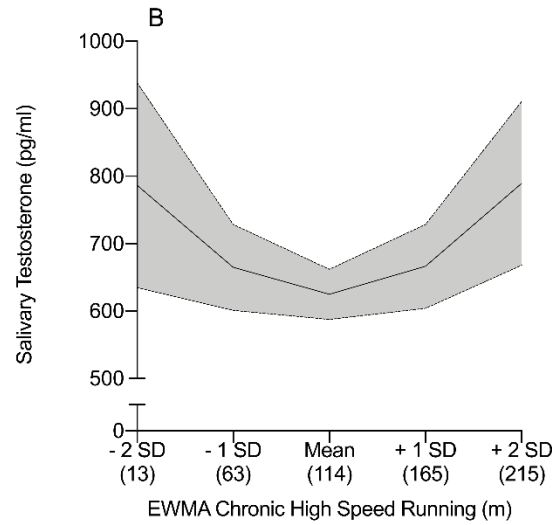
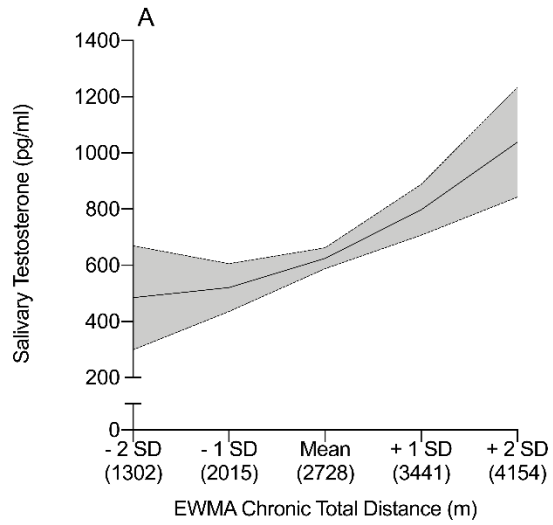
676

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

680

s-T:C					
<i>Predictors</i>	<i>Estimates</i>	<i>ES</i>	<i>CI</i>	<i>Standardized CI</i>	<i>P</i>
(Intercept)	59.4		30.1 – 88.7		<0.001
Phase of Season	48.9	<i>Small</i> ↑	34.7 – 63.0	0.17 – 0.30	<0.001
EWMA chronic DEC ²	-0.20	<i>Small</i>	-0.39– -0.01	-0.41 – -0.01	0.039
EWMA chronic ACC+DEC ²	0.06	<i>Small</i>	0.00 – 0.11	0.01 – 0.44	0.039
EWMA chronic HSR	0.05	<i>Trivial</i> ↑	-0.12 – 0.23	-0.10 – 0.18	0.554
Random Effects					
σ^2	3447				
T00 Player_ID	669				
ICC	0.16				
N Player_ID	18				
Observations	1064				
Marginal R ² /	0.066				
Conditional R ²	0.218				

681



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

695