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Possible Hormone Predictors of Physical Performance in Adolescent Team Sport Athletes

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**THE RELATIONSHIP BETWEEN SALIVARY STEROID HORMONE CONCENTRATIONS AND
PHYSICAL PERFORMANCE IN YOUTH TEAM SPORT ATHLETES**

HORMONE-PHYSICAL PERFORMANCE RELATIONSHIPS IN TEAM SPORT ATHLETES

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1 ABSTRACT

2 The research aim of this study was to determine possible hormone predictors of physical performance in
3 adolescent team sport athletes. Saliva samples were collected immediately prior to performance testing sessions
4 from 114 state squad athletes (77 male, 37 female) participating in either Australian football, basketball, hockey,
5 or netball. Participants completed tests of aerobic and anaerobic capacity, agility, power and speed. Samples
6 were collected over 22 months at quarterly, six-monthly and/or yearly intervals depending on the testing
7 schedule of the athlete. Saliva was analysed for testosterone (T), cortisol (C), estradiol (E) and progesterone (P)
8 levels. A strong negative correlation existed between multistage fitness test performance and T:E ratio ($r=-0.76$,
9 $p=0.01$) in females not taking oral contraceptives and a strong positive correlation existed between repeat agility
10 total time and estradiol levels ($r=-0.71$, $p=0.001$) in females taking oral contraceptives. In males, strong negative
11 correlations were evident for individual changes in planned agility time and estradiol levels ($r=0.87$, $p=0.02$),
12 and CMJ height and T:C ($r=-0.88$, $p=0.01$). In females taking oral contraceptives a strong positive correlation
13 was noted between individual change in yo-yo intermittent recovery test performance and T:E ($r=0.74$, $p=0.01$)
14 and a strong negative correlation was noted between 20m speed and T:P ($r=0.73$, $p=0.01$). In females not taking
15 oral contraceptives a strong negative correlation was found between individual change in CMJ height and T:P
16 ($r=-0.72$, $p=0.02$). The findings show that in adolescent team sport athletes the P:E, T:E and the T:P ratios are
17 important predictors of performance in tests of physical capacity. The findings also indicate estradiol and
18 progesterone have a predictive function in the physical performance of adolescent male team sport athletes.

19

20 Keywords: Testosterone; estradiol; progesterone; Cortisol; exercise

21

22 INTRODUCTION

23 Team sports, such as Australian football (AF), basketball, field hockey and netball, require athletes to perform
24 game related skills in combination with various movement patterns. These include rapid accelerations,
25 decelerations and changes of direction, often with jumping or leaping movements, sustaining high levels of
26 intensity with little opportunity for sufficient recovery (19). Talented adolescents (aged 15-19 years) involved in
27 team sports often represent school and club teams at numerous levels, as well as completing training
28 commitments for state teams, and in some cases state institutes or academies (4). Training loads are not often
29 co-ordinated across different commitments potentially exposing athletes to high physical and psychological
30 loads (4). Selection into national squads even as a junior athlete often results in talented adolescent athletes

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31 competing year round without an off-season. Traditionally, monitoring of adolescent athletes to predict
32 performance, injury risk, or overtraining/overreaching has involved training diaries and physical test batteries.
33 These methods add an additional load to athletes who may already be at risk of being overloaded. Saliva
34 analysis of specific hormones related to performance provides another possible tool for monitoring adolescent
35 athletes.

36

37 The use of biochemical markers for both short term and long term monitoring of exercise stress is widespread in
38 adults. Testosterone and cortisol levels and the testosterone to cortisol ratio (T:C ratio) have been identified as
39 markers of training stress (20). A decrease in testosterone levels and an increase in cortisol levels, or a reduction
40 in the T:C ratio, is thought to indicate an increase in catabolic activity, representative of a state of overstrain or
41 insufficient recovery (20). Increases in performance however have been identified in professional soccer players
42 during high intensity training despite a significant decrease in salivary T:C of over 30% (10). The authors
43 suggested that a decrease in T:C ratio may not necessarily lead to a decrease in performance or a state of
44 overtraining, but instead may reflect an optimal state (10). During adolescence steroid hormone secretion is
45 increased to meet the developmental demands of the body (Kerrigan & Rogol 1992). Increased demands on the
46 hormones during adolescence may result in different relationships between changes in hormone levels and
47 changes in performance in adolescents than in adults. In adolescent soccer athletes changes in testosterone levels
48 have positively correlated with changes in countermovement jump height (CMJ) ($r=0.48$), drop jump height
49 ($r=0.40$) and relative VO_{2max} : ($r=0.32$) and a negatively correlated with changes in 30-m sprint speed ($r=-0.34$)
50 (16). These results suggest that testosterone levels may influence performance in adolescent athletes but more
51 research is needed to confirm this result and to identify if other hormones also influence performance.

52

53 In addition to their use as biomarkers in the monitoring of exercise stress, steroid hormones have been identified
54 as predictors of motor performance. Testosterone has been identified as a predictor of countermovement jump
55 (CMJ) performance (33%) and yo-yo intermittent recovery test (yo-yo) performance (21%) in adolescent male
56 soccer athletes (13). Testosterone was also identified as a predictor of CMJ performance (16%) in adolescent
57 male Australian Football athletes however had no relationship with multistage fitness test (MSFT), speed or
58 agility performance (14). In the adolescent male Australian Football athletes progesterone and estradiol were
59 both identified as predictors of speed and agility, though predictive power was greatest when both hormones
60 were factored into the analysis (10m speed: 19.1%, 20m speed: 20.2% and agility: 30.9%) (14). The above

61 studies suggest that pre-exercise hormone levels may be related to motor performance in adolescent team sport
62 athletes however, more information is needed in this area particularly on female participants.

63

64 This study had two main aims: firstly to establish whether a predictive relationship exists between salivary
65 hormone (testosterone, cortisol, estradiol and progesterone) levels and physical performance in state level
66 adolescent team sport athletes, and secondly, to assess if individual changes in hormone levels provided stronger
67 predictive markers of changes in physical capacity than baseline values alone. Three hypotheses were
68 developed. 1. Progesterone level would be a predictor of speed, and progesterone, estradiol and the progesterone
69 to estradiol ratio (P:E ratio) would be a predictor of agility. 2. Testosterone levels and the T:C ratio would be
70 predictors of endurance and power. 3. Stronger hormone predictors of performance would be evident when
71 individual changes in hormone levels and motor performance were analysed than when predictors were
72 calculated for the pooled data.

73

74 **METHODS**

75 **Experimental approach to the problem**

76 The aim of this prospective cohort design was to establish hormonal predictors of physical performance in state-
77 level adolescent team sport athletes. We utilized athletes that were undergoing their routine testing by the
78 Tasmanian Institute of Sport (TIS).

79

80 Between April 2013 and February 2015 team sport athletes were evaluated at their pre-scheduled physical
81 testing sessions. Information on the frequency of testing and the tests completed by each sporting group is listed
82 in table 1. The physical performance tests were selected and conducted according to the national protocols for
83 each sport (19) and were administered by the TIS physiologists assigned to that sport by the TIS. Although not
84 included in the national protocol netball athletes also completed a repeat agility test and AF athletes completed
85 an isometric handgrip test for strength.

86

87 Participants arrived at the testing venue at approximately 10am. Upon arrival at the testing venue participants
88 completed anthropometry testing then provided a saliva sample. Participants completed a warm-up of
89 approximately 15 minutes consisting of low-intensity running followed by a series of dynamic warm-up

90 exercises including lunge walks, butt-kicks and leg swings and then short accelerations. Participants then
91 completed the physical tests in the order listed in table 1.

92

93 TABLE 1 ABOUT HERE

94

95 Prior to actual testing, participants were thoroughly familiarized with all testing equipment and procedures. For
96 all testing days, participants were asked to refrain from exhaustive physical activity for 48 hours preceding
97 testing.

98

99 **Subjects**

100 One hundred and fourteen (n=114) adolescent athletes were recruited to participate in this study (mean \pm
101 standard deviation; Male n = 77, age 16.87 ± 1.2 y, height 184.5 ± 6.6 cm, body mass 77.1 ± 6.5 kg; Female n =
102 37, age 16.4 ± 1.1 y, height 175.0 ± 5.9 cm, body mass 68.9 ± 9.8 kg). All participants were members of the TIS
103 or their state sporting organisation representative squads for either AF, basketball, hockey or netball.
104 Participants were included if they were uninjured and free from any medical conditions. Details of hormonal
105 contraceptive use, including the type and brand of contraceptive, was obtained with the participant's permission
106 from the TIS athlete database. The study was approved the Charles Darwin University Human Research Ethics
107 Committee (Approval Number: H12155). The subjects were informed of the benefits and risks of the
108 investigation prior to signing an institutionally approved informed consent document to participate in the
109 study. Parental or guardian signed consent was also obtained when participants were under the age of 18 years
110 of age.

111

112 **Procedures**

113 **Anthropometry:** Body mass and height were measured with standard techniques to the nearest 0.1 cm and 0.1
114 kg, respectively for each participant. To estimate adiposity, skinfold thicknesses were measured at seven sites on
115 the right side of the body (triceps, biceps, subscapular, supraspinale, abdominal, calf and thigh) using a
116 Harpenden skinfold caliper (British Indicators Ltd., Luton). The same International Society for the
117 Advancement of Kinanthropometry (ISAK) accredited investigator took all measurements at each test occasion.

118

119 **Countermovement Jump (CMJ):** CMJ height was used as a test of explosive leg power and was determined
120 with Yardstick© jumping device (Swift, Brisbane, Australia). Participants were required to stand with their feet
121 together side on to the Yardstick©. Keeping their heels on the floor and looking straight ahead, they were
122 required to reach upward with their dominant hand as high as possible, fully elevating the shoulder to displace
123 the Yardstick© vanes. This value was recorded as the reach height. The participant then performed a CMJ with
124 no preliminary steps (except for the basketball athletes who as per the national protocol were permitted one
125 preliminary step) or shuffling with the aim of displacing the highest vane possible. At least three trials were
126 performed with the participant continuing to jump when they felt they were ready until missing three
127 consecutive times. The highest vane displaced was recorded as the jump height. CMJ height was then calculated
128 as the difference between reach height and jump height (cm). TIS reliability scores are high for this test where
129 ICC is $r = 0.89$.

130

131 **Sprints:** The participants were instructed to run as fast as possible in a straight line through electronic light
132 gates (Fusion Sports, Australia, serial number 70019522) spaced at 10, 30 and 40m on a synthetic turf for field
133 hockey athletes and spaced at 5, 10 and 20m on an indoor wooden basketball court for AF, basketball and
134 netball athletes. Participants were instructed to start from a stationary position with their front foot touching the
135 edge of the start line and to ensure that their first movement was in the forward direction (i.e. no rocking).
136 Participants were able to start in their own time once they were informed that the timing system was ready and
137 were instructed not to slow down until they were 5m past the final timing gate. Sprint times began when the
138 participant passed the laser positioned across the start line and concluded when they passed the laser positioned
139 across the final marker. The fastest 5, 10 and 20m time for the AF basketball and netball athletes and the fastest
140 10 and 40m time for the hockey athletes was used for analysis. TIS reliability scores are high for this test where
141 ICC is $r = 0.97$.

142

143 **Multistage Fitness Test (MSFT):** The MSFT was used as a measure of aerobic endurance in the AF and
144 hockey athletes. Participants were required to run back and forth on a measured 20m synthetic turf hockey field
145 track keeping in time with a series of audio signals from an mp3 provided by the Australian Institute of Sport
146 and validated against the MSFT compact disc (Australian Coaching Council, Belconnen, ACT, 1998). Each end
147 on the 20m track was marked with cones. A test administrator was positioned at each line to ensure that the
148 athlete placed a foot on or over the line before the audio signal and to ensure that the participant did not attempt

149 the next shuttle until the audio signal had sounded. Participants received a warning the first time they failed to
150 reach the line before the audio signal and were eliminated the second consecutive time. The total distance
151 covered in metres that was reached in time with the audio signal was recorded as their final score. TIS reliability
152 scores are high for this test where ICC is $r = 0.97$.

153

154 **Yo-yo Intermittent Recovery Test (yo-yo):** The yo-yo test was used as a measure of aerobic endurance in the
155 basketball and netball athletes. Participants were required to run 40m on an out and back course (20 m each way
156 turning around at a line marked by cones) in time with an audio recording. Upon reaching the start point, the
157 participants were to walk around a set of cones 5m away before returning to the start line to await the next audio
158 cue indicating to begin the next shuttle. The time between the audio cues gradually decreased. Participants
159 received a warning the first time they failed to reach the start line before the audio signal and were eliminated
160 the second time. The total distance covered in metres was recorded as their final score. TIS reliability scores for
161 this test are $r = 0.75$.

162

163 **Sit and Reach:** The sit and reach test was used as a measure of combined hamstring and spinal flexibility in
164 the basketball athletes. Participants were required to sit on the floor with their legs extended out in front of them
165 placing their bare feet against the edge of the sit and reach box (Flextester, Novel Products, Illinois USA).
166 Keeping one hand directly on top of each other participant was then required to slide their hands along the
167 top of the sit and reach box and hold the end-stretch for two seconds whilst maintaining straight legs. The
168 distance of the fingers past the toes as marked on the sit and reach box was recorded to the nearest 0.5cm. TIS
169 reliability scores are high for this test where ICC is $r = 0.98$.

170

171 **Basketball Agility (Agility Left and Right):** A schematic diagram of the agility test is presented in figure 1.
172 The agility test involved maximal effort trials of a predetermined course consisting of four 30cmx30cm taped
173 'pivot boxes' and was timed using electronic timing lights (Fusion sports, Australia). Basketball athletes
174 completed three maximal trials beginning first with the front left box and then three trials beginning with the
175 front right box with at least 30 seconds recovery between trials. The fastest of the three trials for each direction
176 was recorded. TIS reliability scores are high for this test where ICC is $r = 0.85$.

177

178

179 FIGURE 1 ABOUT HERE

180

181 **Line Drill:** The line drill was used as a test of anaerobic capacity in the basketball athletes. Participants were
182 required to run as fast as possible to the closest free throw line (5.8m), back to the base line (5.8m), to the centre
183 line (14m), back to the base line (14m), to the distant free throw line (22.2m), back to the base line (22.2m), to
184 the opposite base line (28m) and finally to the base line where the test commenced (28m). Electronic light gates
185 (Fusion sports, Australia) positioned on the base line recorded the start and finish time (seconds). Each
186 participant completed one trial, with the time taken to complete the trial recorded as the final score. TIS
187 reliability scores for this test are $r = 0.78$.

188

189 **Planned Agility:** The planned agility test was completed by the netball and AF athletes. It involved maximal
190 effort trials around a pre-determined course with poles comprising three left and two right 90° turns and was
191 timed using electronic timing lights (Fusion sports, Australia) placed at the start and finish lines. Athletes
192 completed three maximal trials with at least 2 minutes recovery between trials. The fastest of the three trials was
193 recorded. Figure 2 is a schematic of the planned agility test. TIS reliability scores are high for this test where
194 ICC is $r = 0.94$.

195

196 FIGURE 2 ABOUT HERE

197

198 **Repeat Agility Test:** The repeat agility test (4 x planned agility circuit on a 20 s cycle), which measures
199 anaerobic capacity, was also completed by the netball participants. The total time in seconds of the four agility
200 trials was used as the repeat agility score.

201

202 **Handgrip Strength:** The handgrip strength of the AF athletes was measured bilaterally using a hand-held
203 dynamometer (Original Smedley Dynamo Meter 100kg; TTM, Tokyo, Japan). The participants performed three
204 maximal effort contractions with a rest period of 15 to 20s between each effort. The maximum value from the 3
205 trials was recorded (ICC $r=0.98$) (11).

206

207

208

209 **Saliva Analysis:** The participants were instructed to refrain from eating, drinking fluids other than water, or
210 brushing their teeth for one hour prior to saliva collection to avoid sample contamination. Approximately 2 ml
211 of saliva was collected by the passive drool method in sterile containers immediately prior to the participants'
212 warm-up. All samples were coded and kept at -80°C until analysis. After thawing, the samples were centrifuged
213 at 4000 rpm for 5 minutes. The samples were then analysed in duplicate for testosterone, cortisol, progesterone
214 and estradiol by commercially available kits as per manufacturer's instructions (Salimetrics LLC, USA). The
215 minimum detection limit was 1.0 pg/mL for testosterone (mean 4.6% CV), 0.003 $\mu\text{g}/\text{dL}$ for cortisol (5.2% CV),
216 0.1 pg/mL for estradiol (5.2% CV) and 5 pg/mL for progesterone (6.1% CV).

217

218 **Statistical Analyses**

219 All variables were tested for normality using the Shapiro-Wilk test prior to analysis. Non-normally distributed
220 data was log-transformed prior to analysis to create a normal distribution when possible. Due to well-known
221 gender differences in human physiology (see (3) for a comprehensive review) data for males and females were
222 analysed separately. During analysis the female participants' results were grouped according to whether the
223 subject was taking hormonal contraceptives at the time of testing (NOC = not taking oral contraceptives, OC =
224 taking oral contraceptives) as the exogenous hormones found in oral contraceptives are able to bind to other
225 steroid receptors and either inhibit the receptors activation or induce transactivation.(18) Associations between
226 baseline and relative changes in hormone levels (change (Δ) in absolute values from session 1 to each
227 subsequent test session) and physical performance were determined using Pearson's correlation for normal data
228 and Spearman's correlation for non-normal data. Correlations were considered very strong if $r=0.90$ to 1.0 or $-$
229 0.90 to 1.0 , strong if $r=0.7$ to 0.9 or -0.7 to -0.9 , moderate if $r=0.5$ to 0.7 or -0.5 to -0.7 and weak if $r=0.3$ to 0.5
230 or -0.3 to -0.5 .(17) Statistical analysis was performed using SPSS software (version 16; SPSS Inc., Chicago, IL,
231 USA), with significance set at $p \leq 0.05$.

232

233 **RESULTS**

234 Over 22 months 161 samples (Male, $n=90$; Female NOC, $n=39$; Female OC, $n=32$) were collected. Mean saliva
235 hormone values and physical test results for the male and female participants (including NOC and OC groups)
236 are presented in table 2. In males, agility ($n=56$) was weakly correlated with estradiol levels ($r=0.47$, $p<0.001$),
237 and the P:E ($r=-0.43$, $p=0.001$), and T:E ratios ($r=-0.35$, $p=0.01$). Weak correlations were evident between 10m
238 ($n=78$) and 20m speed ($n=64$) and the P:E (10m $r=-0.36$, $p=0.002$; 20m $r=-0.34$, $p=0.002$) and T:P ratios (10m

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239 $r=0.33$, $p=0.003$; 20m $r=0.37$, $p=0.001$). Handgrip strength ($n=34$) was weakly correlated with estradiol levels
240 ($r=-0.48$, $p=0.04$) and the T:E ratio ($r=0.35$, $p=0.04$), and running left VJ height ($n=56$) was weakly correlated
241 with the T:E ratio ($r=-0.35$, $p=0.01$). In the NOC group, distance covered in the MSFT was strongly correlated
242 with the T:E ratio ($r=-0.76$, $p=0.01$) and moderately correlated with the T:P ratio ($r=-0.68$, $p=0.02$). In the NOC
243 group, 1 step CMJ height ($n=10$) was correlated with estradiol ($r=0.81$, $p=0.01$), agility left ($n=10$) was
244 correlated with estradiol ($r=-0.71$, $p=0.01$) and the T:E ($r=-0.82$, $p=0.01$) and T:P ratios ($r=0.71$, $p=0.03$), and
245 the line drill performance ($n=10$) correlated with estradiol ($r=-0.86$, $p=0.001$), progesterone $r=-0.79$, ($p=0.01$)
246 and the T:P ($r=0.70$, $p=0.04$) and T:E ratios ($r=0.78$, $p=0.01$). In the OC group, repeat agility total time ($n=20$)
247 was strongly correlated with estradiol levels ($r=-0.71$, $p=0.001$) and moderately correlated with progesterone
248 levels ($r=-0.51$, $p=0.01$) and the T:E ratio ($r=0.63$, $p=0.004$).

249

250 TABLE 2 ABOUT HERE

251

252 Mean individual changes in saliva values and physical test results over time for the male and female participants
253 (including NOC and OC groups) are displayed in table 3. Correlations between individual changes in physical
254 performance and individual changes in salivary hormone parameters are presented in table 4. In males, strong
255 correlations were evident between a Δ in planned agility time and a Δ in estradiol levels ($p=0.02$), and a Δ in
256 CMJ height and a Δ in the T:C ratio ($p=0.01$). In the NOC group, a strong correlation was evident between a Δ
257 in CMJ height and a Δ in the T:P ratio ($p=0.02$) and a moderate correlation was evident between a Δ in CMJ
258 height and a Δ in the T:E ratio ($p=0.03$). A moderate correlation was also evident between a Δ in 10 m speed and
259 a Δ in testosterone levels ($p=0.003$). In the OC group, a Δ in the distance covered in the yo-yo test was strongly
260 related to a Δ in the T:E ratio ($p=0.01$) and moderately related to a Δ in estradiol levels ($p=0.02$) and the P:E
261 ratio ($p=0.05$). A strong correlation was evident between a Δ in 20 m speed and a Δ in the T:P ratio ($p=0.01$) and
262 moderate correlations were evident between a Δ in 20 m speed and a Δ in the T:C ratio ($p=0.03$), and a Δ in 5 m
263 speed and a Δ in the T:P ratio ($p=0.04$).

264

265 TABLE 3 AND 4 ABOUT HERE

266

267

268

269 **DISCUSSION**

270 The aim of this study was to determine possible hormone predictors of physical performance in adolescent team
271 sport athletes and to assess if individual changes in hormone levels provided stronger predictive markers of
272 changes in physical capacity than discrete values. Our results identified some significant hormonal predictors of
273 physical performance. Individual changes in estradiol levels were a stronger predictor of planned agility
274 performance than pooled estradiol levels in the male athletes.

275

276 Published research on adolescent Australian football athletes identified estradiol and progesterone as predictors
277 of 10 and 20m sprint speed (14). Given this, we hypothesised that salivary progesterone levels would be
278 positively associated with speed and power test performance in our sample of adolescent athletes. This is the
279 first study of its kind to identify a positive association between the P:E ratio and speed and agility performance,
280 and a negative association between the T:P ratio and speed in males. It is proposed that this finding may be due
281 to progesterone's antagonistic effects of estradiol at a receptor level. Progesterone functions as an inhibitor of
282 estrogen (21), and estrogen receptors (12). Progesterone represses estrogen receptor transcriptional activity,
283 antagonizes estrogen action at the molecular level and prevents estrogen induction by down-regulating estrogen
284 protein concentration (12). Progesterone has also been found to affect estrogen stimulated responses, though the
285 mechanisms in which this occurs is unclear (5-7). In the female OC and NOC groups, progesterone levels were
286 not associated with speed and agility, however, moderate to strong negative associations between changes in
287 speed and salivary testosterone levels, T:C and T:P ratios were identified in both groups. To the best of our
288 knowledge, no study has directly investigated possible associations between speed or agility and testosterone
289 levels in females, though a study on male Rugby Union athletes did identify a positive relationship between both
290 salivary testosterone levels and T:C ratio and speed (8). This result in male Rugby Union athletes contrasts with
291 our finding in female team sport athletes. Recent research suggests that testosterone's responses during exercise
292 may be mediated by cortisol levels however the mechanisms are yet to be elucidated (9). The finding that
293 cortisol levels may mediate testosterone's responses compliment findings in behavioural studies. Behavioural
294 studies have reported testosterone relationships that are mostly positive when cortisol levels are low and
295 negative when cortisol levels are high (15). Female participants in this study had notably higher cortisol levels
296 (NOC = 12.8 ± 7.6 nmol/L, OC = 11.2 ± 4.6 nmol/L) than the male Rugby Union athletes (Backs = 5.1 ± 2.4
297 nmol/L, Forwards = 7.3 ± 3.5 nmol/L) in the study by Crewther, Lowe, Weatherby, Gill and Keogh (8) which

298 may explain why a the T:C ratio in our study had a negative relationship with speed whereas the relationship
299 was positive in the male Rugby Union athletes.

300

301 In contrast to previous findings in male adolescent athletes (1), individual changes in the T:C ratio were
302 negatively associated with individual changes in CMJ height in male participants. A decrease in the bioavailable
303 T:C ratio has been previously linked to overtraining (20), however increases in performance have been
304 identified in professional soccer players during high intensity training despite a significant decrease in salivary
305 T:C of over 30% (10). It has been suggested that a decrease in T:C ratio may not necessarily lead to a decrease
306 in performance or a state of overtraining, but instead may reflect an optimal performance state (10). Rather than
307 a measure of overtraining, the T:C ratio may be indicative of training stress (20). Training stress is necessary to
308 induce positive changes in response to training, however the optimal level of stress required, and how it is
309 reflected in the T:C ratio, remains unknown.

310

311 This study is not without limitations. Interpretation of our results is limited by the small sample size for some of
312 the physical performance tests. The results of this study are restricted to the population of athletes tested and
313 may not be indicative of the general population or other athlete groups. For research outcomes to be adopted and
314 utilised effectively within sports they must be proven to be feasible and effective in real world settings (2). As a
315 consequence of this concept, the researchers were interested in identifying hormone predictors of performance
316 that were independent of other factors such as training phase, diet, clothing and sleep patterns. As a result
317 training phase, diet, clothing and sleep patterns were not reported or considered in the analysis of this study..

318

319 In conclusion, the current results showed that in adolescent team sport athletes the P:E, T:E and the T:P ratios
320 are important predictors of motor performance in tests of physical capacity. The current findings also allow us
321 indicate estradiol and progesterone have a role in the physical performance of adolescent male team sport
322 athletes. Contrary to the initial hypothesis, in most cases analysis of individual changes in hormone levels did
323 not act as stronger predictors of performance than discrete values.

324

325

326

327

328 **PRACTICAL APPLICATIONS**

329 This study provides insights into possible hormone predictors of performance in adolescent team sport athletes.
330 Coaches and support staff should keep in mind when using hormone levels to predict athlete performance that
331 monitoring changes in hormone levels and performance, and looking at discrete variables of hormones and
332 performance do not yield identical results. In adolescent female team sport athletes, the preferred method of
333 monitoring should be individual changes in hormone levels (testosterone, P:E, T:P and T:E ratios) and
334 performance. It is also important to note whether female athletes are taking hormonal contraceptives at the time
335 of testing, as the exogenous hormone contained in hormonal contraceptives may affect the results. In adolescent
336 male team sport athletes, the method of prediction should depend on the variable being predicted. The results of
337 this study indicate that changes in estradiol levels may provide a useful biomarker of agility, and support the use
338 of the T:C ratio as a marker of training stress in adolescent male team sport athletes. Additionally discrete
339 measurements of the P:E ratio may be useful in predicting speed and agility in adolescent male team sport
340 athletes. This has possible implications as an initial screening tool for talent identification models applied in
341 different sports.

342

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Table 1. Performance testing frequency, time and tests performed by sport

Sport	Test occasions	Testing frequency	Testing time	Performance tests
AF	2	yearly	10:00	handgrip strength, CMJ, running left VJ, running right VJ, 5 m, 10 m and 20 m sprint, planned agility, MSFT.
Basketball	2	quarterly	10:00	sit and reach, 1 step CMJ, 5m, 10m and 20 m sprint, left and right sided agility, line drill, yoyo.
Hockey	2	half yearly	10:00	CMJ, 10 m, 40 m sprint, MSFT.
Netball	6	quarterly	10:00	CMJ, 5 m, 10 m and 20 m sprint, planned agility, repeat agility, yoyo.

Abbreviations: AF = Australian Football; CMJ = countermovement jump; MSFT = multi stage fitness test; TIS = Tasmanian Institute of Sport; VJ = vertical jump; yoyo = yoyo intermittent recovery test level 1.

Table 2. Hormone levels and ratios by test group (mean ± standard deviation)

	Male	NOC	OC
HORMONE VALUES			
Estradiol (pg/mL)	2.1 ± 1.1	2.4 ± 1.0	3.1 ± 3.6
Progesterone (pg/mL)	106.3 ± 70.7	119.4 ± 56.0	123.5 ± 65.5
Testosterone (pg/mL)	142.5 ± 44.3	90.9 ± 35.5	81.1 ± 31.1
Cortisol (nmol/L)	9.4 ± 5.7	12.8 ± 7.6	11.2 ± 4.6
P:E ratio	54.6 ± 34.4	52.1 ± 118.9	52.8 ± 25.9
T:C ratio	0.7 ± 0.5	0.3 ± 0.2	0.3 ± 0.2
T:P ratio	1.9 ± 1.3	0.9 ± 0.4	0.8 ± 0.5
T:E ratio	79.8 ± 35.6	42.2 ± 18.9	37.6 ± 20.9
PHYSICAL TESTS			
Handgrip Strength (kg)	49.4 ± 6.5	-	-
5m Sprint (s)	1.11 ± 0.07	1.22 ± 0.07	1.20 ± 0.07
10m Sprint (s)	1.84 ± 0.09	2.05 ± 0.09	2.00 ± 0.09
20m Sprint (s)	3.12 ± 0.12	3.55 ± 0.17	3.43 ± 0.14
40m Sprint (s)	5.59 ± 0.19	6.33 ± 0.21	-
Planned Agility (s)	8.31 ± 0.28	9.07 ± 0.39	8.77 ± 0.26
CMJ (cm)	60 ± 7	42 ± 5	47 ± 4
Running Left VJ (cm)	74 ± 12	-	-
Running Right VJ (cm)	71 ± 10	-	-
1 step CMJ (cm)	69 ± 7	45 ± 5	-
Line Drill (cm)	27.46 ± 0.75	31.13 ± 1.19	-
Sit and Reach (cm)	12 ± 4	11 ± 4	-
Agility Left (s)	5.36 ± 0.28	6.08 ± 0.17	-
Agility Right (s)	5.39 ± 0.25	6.02 ± 0.20	-
Repeat Agility Total Time (s)	-	38.52 ± 1.59	37.71 ± 1.90
MSFT Distance (m)	2542 ± 229	1871 ± 209	-
Yoyo Distance (m)	2507 ± 413	1097 ± 441	1093 ± 299

Abbreviations: NOC = females not taking oral contraceptives; OC = females taking oral contraceptives; P:E ratio = progesterone to estradiol ratio; T:C ratio = testosterone to cortisol ratio; T:E ratio = testosterone to progesterone ratio; T:P ratio = testosterone to progesterone ratio; CMJ = countermovement jump; MSFT = multi stage fitness test; VJ = vertical jump; yoyo = yoyo intermittent recovery test level 1, - = not applicable

Table 3. Individual change in hormone levels and physical test performance by test group (mean \pm standard deviation)

	Male	NOC	OC
HORMONE VALUES			
Estradiol (pg/mL)	-1.1 \pm 2.0	0.3 \pm 1.2	2.4 \pm 5.0
Progesterone (pg/mL)	-14.2 \pm 67.0	12.1 \pm 57.1	25.1 \pm 96.4
Testosterone (pg/mL)	-14.2 \pm 67.4	-11.5 \pm 33.0	5.5 \pm 30.6
Cortisol (nmol/L)	-3.8 \pm 6.4	-0.5 \pm 7.1	1.4 \pm 5.1
P:E ratio	53.4 \pm 51.6	5.9 \pm 25.9	-5.6 \pm 32.7
T:C ratio	0.1 \pm 0.9	-0.05 \pm 0.1	0.003 \pm 0.2
T:P ratio	-1.5 \pm 3.0	-0.5 \pm 0.7	0.06 \pm 0.6
T:E ratio	27.8 \pm 37.8	-17.4 \pm 18.9	-6.0 \pm 18.2
PHYSICAL TESTS			
5m Sprint (s)	-0.04 \pm 0.08	-0.01 \pm 0.04	0.05 \pm 0.08
10m Sprint (s)	-0.06 \pm 0.06	-0.0006 \pm 0.05	0.06 \pm 0.09
20m Sprint (s)	-0.11 \pm 0.08	0.04 \pm 0.08	0.08 \pm 0.11
Planned Agility (s)	-0.34 \pm 0.31	0.15 \pm 0.10	0.16 \pm 0.17
CMJ (cm)	-1 \pm 2	0 \pm 3	2 \pm 4
MSFT Distance (m)	42 \pm 122	-	-
Yoyo Distance (m)	-	-60 \pm 153	-147 \pm 273

Abbreviations: NOC = females not taking oral contraceptives; OC = females taking oral contraceptives; P:E ratio = progesterone to estradiol ratio; T:C ratio = testosterone to cortisol ratio; T:E ratio = testosterone to progesterone ratio; T:P ratio = testosterone to progesterone ratio; CMJ = countermovement jump; MSFT = multi stage fitness test; NOC = females not taking oral contraceptives; OC = females taking oral contraceptives; VJ = vertical jump; yoyo = yoyo intermittent recovery test level 1, - = not applicable

Table 4. Correlations between individual changes in physical performance and individual changes in salivary hormone parameters

Test	Estradiol (pg/mL)	Progesterone (pg/mL)	Cortisol (nmol/L)	Testosterone (pg/mL)	P:E	T:C	T:P	T:E
MALES								
ΔPlanned Agility (n=7)	0.87*	0.58	-0.08	0.48	0.34	0.32	0.14	-0.35
Δ5m (n=8)	<0.01	-0.02	-0.21	0.02	0.06	0.15	-0.12	-0.44
Δ10m (n=12)	0.10	-0.10	<-0.01	0.14	0.02	0.14	0.33	-0.23
Δ20m (n=8)	0.15	-0.23	-0.42	0.15	<-0.01	0.45	0.31	0.04
ΔMSFT (n=11)	0.27	0.57	-0.28	0.18	0.41	-0.20	0.04	-0.35
CMJ (n=7)	-0.71	-0.25	0.42	-0.47	-0.22	-0.88*	-0.51	0.20
OC								
ΔPlanned Agility (n=10)	0.46	-0.08	0.10	0.31	-0.48	-0.18	-0.06	-0.66
Δ5m (n=12)	<0.01	0.42	-0.22	-0.07	-0.12	0.37	0.63*	0.02
Δ10m (n=13)	0.46	0.50	-0.31	0.26	<-0.01	0.46	0.31	-0.28
Δ20m (n=12)	0.44	0.21	-0.55	-0.15	-0.35	0.69*	0.73*	-0.14
ΔYoyo (n=12)	-0.68*	0.36	-0.09	-0.25	0.58*	0.38	-0.34	0.74**
ΔCMJ (n=11)	-0.36	0.39	0.65	0.02	0.28	-0.56	-0.37	0.31
NOC								
ΔPlanned Agility (n=10)	-0.29	0.37	0.37	-0.19	0.43	-0.29	-0.03	0.17
Δ5m (n=13)	0.04	0.08	-0.07	0.38	0.12	0.03	-0.04	-0.21
Δ10m (n=16)	-0.05	0.22	0.02	0.68*	0.34	0.14	-0.04	0.21
Δ20m (n=13)	-0.18	0.17	-0.04	0.60	0.53	-0.15	-0.22	0.06
ΔYoyo (n=13)	0.06	-0.10	-0.08	0.51	-0.51	0.49	0.46	0.36
ΔCMJ (n=10)	0.44	0.23	0.07	-0.40	0.29	-0.46	-0.72*	-0.69*

*P≤0.05, **P≤0.01, Abbreviations: CMJ = countermovement jump; MSFT = multi stage fitness test; yoyo = yoyo intermittent recovery test level 1

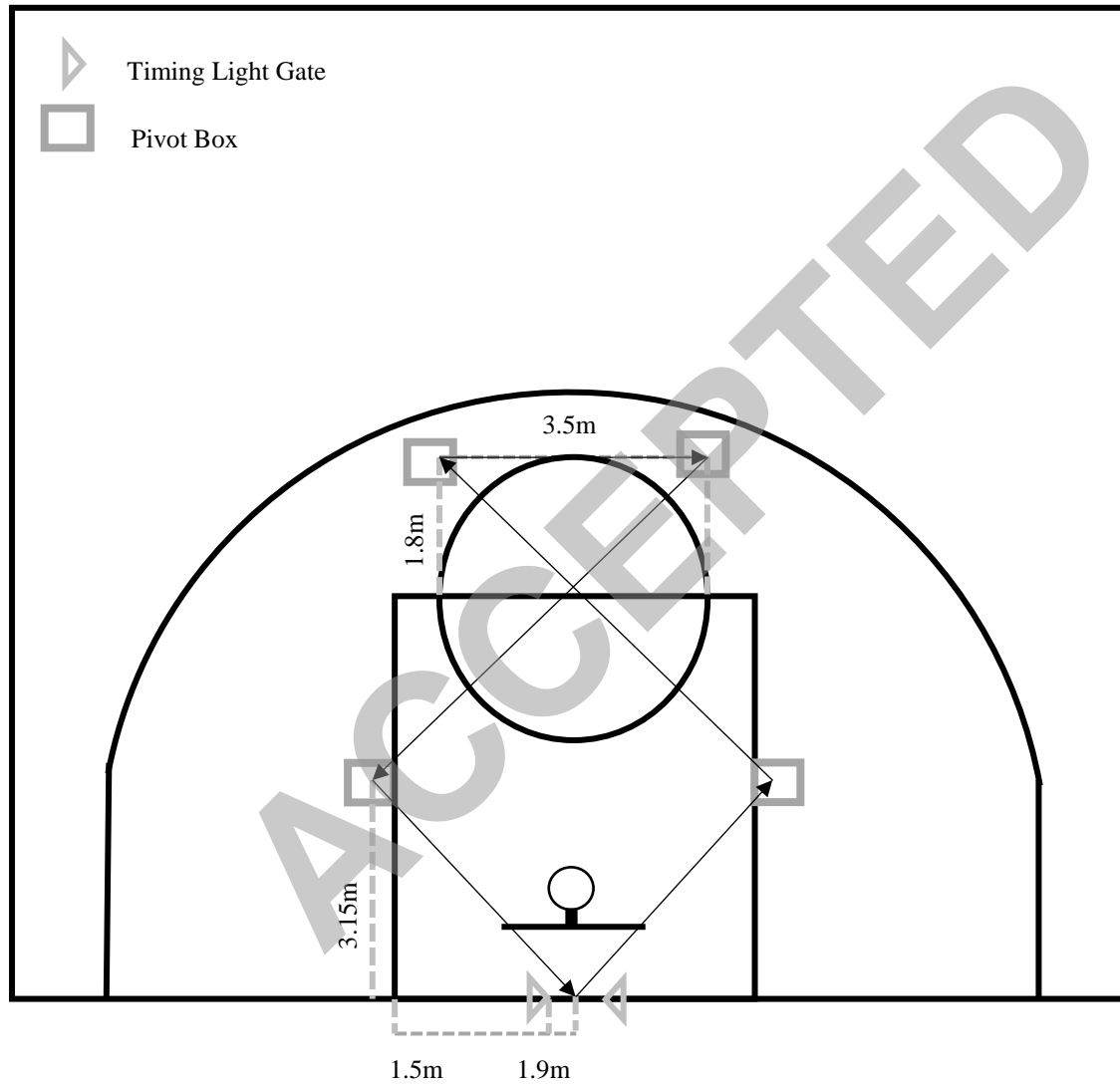


Figure 1. Schematic diagram of the basketball agility test

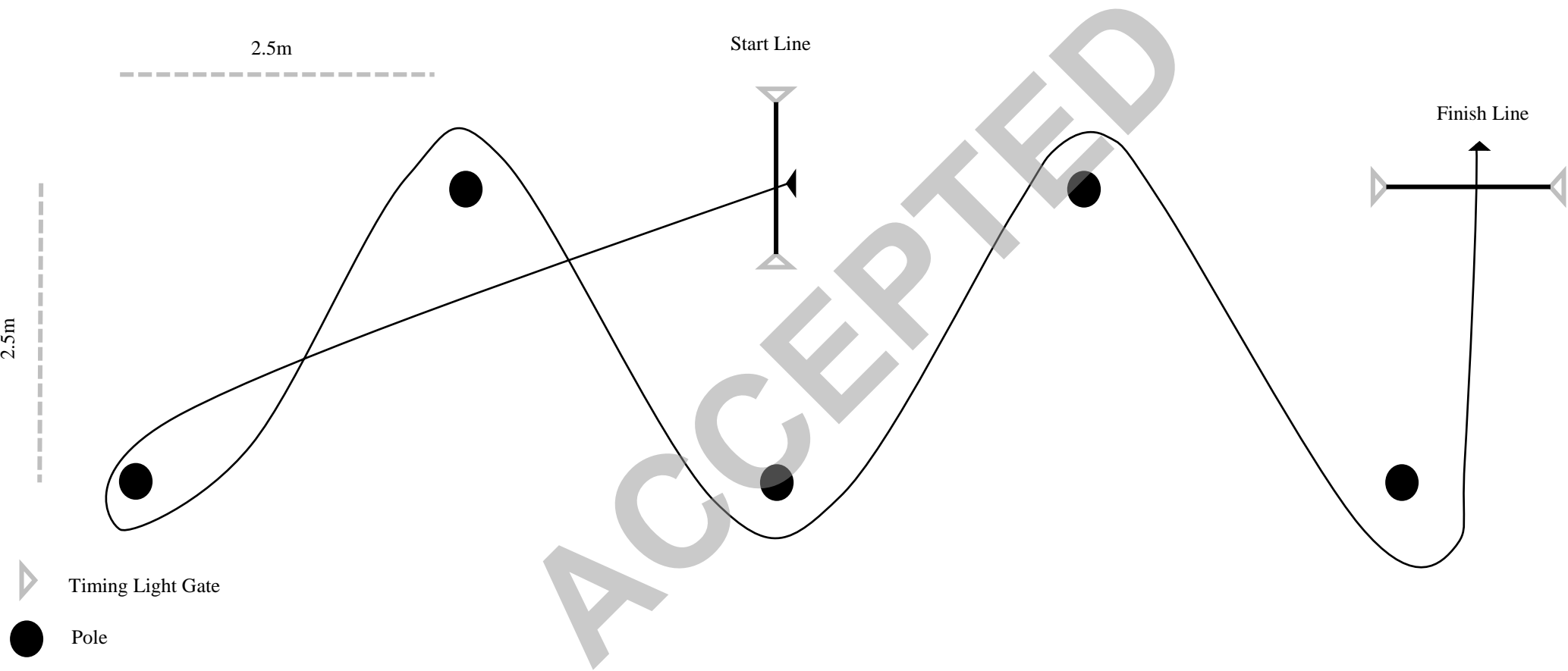


Figure 2. Schematic diagram of the planned agility test