

1 **Energy expenditure of female international standard soccer players: a doubly labelled**  
2 **water investigation**

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42 **Abstract**

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44 **Purpose:** To quantify total daily energy expenditure (TEE) of international adult female soccer  
45 players. **Methods:** Twenty-four professional players were studied during a twelve-day period  
46 where they participated in an international training camp (also inclusive of two competitive  
47 games) representing the English national team. TEE was assessed via the doubly labelled water  
48 (DLW) method during the full 12 days as well as the initial 4-day period prior to game one.  
49 Energy intake (EI) was also assessed (via weighed food analysis) during the initial 4-day period  
50 to permit estimation of energy availability (EA). **Results:** Mean TEE did not differ ( $P=0.31$ )  
51 between the 12-day ( $2693 \pm 432 \text{ kcal.day}^{-1}$ ; range: 2105-3507;  $54 \pm 6 \text{ kcal.kg}^{-1}$  fat free mass,  
52 FFM) versus the 4-day assessment period ( $2753 \pm 359 \text{ kcal.day}^{-1}$ ; range: 1942-3280;  $56 \pm 8$   
53  $\text{kcal.kg}^{-1}$  FFM). Mean four-day EI was  $1923 \pm 357 \text{ kcal.day}^{-1}$  (range: 1639-2172) and mean  
54 activity energy expenditure was  $1069 \pm 278 \text{ kcal.day}^{-1}$  (range: 155-1549  $\text{kcal.day}^{-1}$ ). When  
55 assessed for estimated EA, 88% of players were categorised with low EA status according to  
56 the threshold of  $<30 \text{ kcal.kg}^{-1}$  FFM. Mean daily carbohydrate intake equated to  $3.3 \pm 0.7 \text{ g.kg}^{-1}$   
57  $^1$  body mass. **Conclusion:** When compared with previously published data from adult male  
58 players, we demonstrate that the relative daily energetic requirements of engaging in  
59 professional soccer training and match play is comparable between sexes. From a practical  
60 perspective, data suggest that practitioners should likely focus education and behaviour change  
61 strategies on “fuelling” for match play and training to optimise both player health and  
62 performance.

63

64 **Keywords:** carbohydrate, energy availability, RED-S

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66

67 **Introduction**

68 In adult male professional soccer players, the physical demands of both match play (1–3) and  
69 training (4–6) are well documented. Such data typically demonstrate that the absolute loads  
70 completed in training are lower than those experienced in match play, as is the case for total  
71 distance (<7 km vs. ~10-13 km), high-speed running distance (<300 m vs. >900 m), sprint  
72 distance (<150 m vs. >200 m), and average speed (<80 m/min vs. ~100-120 m.min<sup>-1</sup>) (7–9).  
73 When assessed during a typical in-season weekly micro-cycle comprising one or two games,  
74 outfield professional players typically expend 3000-4000 kcal.d<sup>-1</sup> (40-60 kcal.kg<sup>-1</sup> fat free mass,  
75 FFM), as quantified using the gold standard doubly labelled water method (9–11).  
76 Accordingly, evidence-based guidelines for the recommended energy and macronutrient intake  
77 to support both daily training and match play have recently been published (12). In this regard,  
78 it is suggested that daily carbohydrate (CHO) intake should equate to 3-8 g.kg<sup>-1</sup> body mass to  
79 allow for flexibility between rest days, training days and match days.

80

81 In contrast to adult male players, the energetic requirements and external training loads  
82 completed by elite female players are not as well understood (13–18). This is of specific interest  
83 given recent reports documenting the prevalence of low energy availability (LEA, defined as  
84 <30 kcal.kg<sup>-1</sup> FFM per day) in female professional players from the English Women’s Super  
85 League (13). Indeed, these researchers observed that between 50-70% of players were  
86 classified with LEA status on both match day and “heavy” training days where daily activity  
87 energy expenditure was >700 kcal.d<sup>-1</sup>, as estimated by global positioning systems (GPS).  
88 Analysis of self-reported energy intakes (EI) also demonstrated that these players consumed a  
89 consistent daily CHO intake of 3-3.5 g.kg<sup>-1</sup> body mass, thereby failing to adjust daily CHO  
90 intake in accordance with alterations to training load or in preparation for match play. Such  
91 data build on previous observations that female players apparently “under-fuel” in relation to

92 daily CHO intake (14–17). Given that 80% and 69% of type 1 and II muscle fibres from elite  
93 female players are classified as empty or almost empty of muscle glycogen immediately post-  
94 match play (18), such relative CHO intakes are likely sub-optimal in relation to promoting  
95 physical performance.

96

97 The reported prevalence of LEA is of particular concern given the potential for players to  
98 develop negative symptoms associated with the Female Athlete Triad (19,20) or Relative  
99 Energy Deficiency in Sport (RED-S) models (21). Nonetheless, despite previous assessments  
100 of activity energy expenditure and energy availability (EA) in such populations (13,15,16,22),  
101 it remains difficult to prescribe evidence based nutritional guidelines owing to the indirect  
102 methodologies employed to quantify daily *total* energy expenditure (TEE) (e.g., activity diaries  
103 and accelerometry which may under- or over-estimate non-exercise activity). In this regard,  
104 the doubly labelled water (DLW) method is the gold standard method of assessing total daily  
105 energy expenditure in free-living conditions *in vivo* (23). Importantly, this non-invasive  
106 method allows for an assessment of energy expenditure over a 7-14 day period (i.e. a typical  
107 in-season micro-cycle) without interfering in day-to-day activities such as soccer training or  
108 match play (23).

109

110 Accordingly, the primary aim of the present study was to therefore assess TEE of female soccer  
111 players via the gold standard DLW method. To this end, we studied 24 English female soccer  
112 players during a twelve-day period where players participated in an international training camp  
113 (also inclusive of two competitive games) representing the English national team. As a  
114 secondary measure, we also assessed energy intake (via weighed food analysis) during the  
115 initial four days of the assessment period to allow for an estimation of energy availability (EA).  
116 Given that this cohort represent players of the highest standard, it is hoped that these data may

117 provide a platform for which to develop evidence based nutritional guidelines that optimise the  
118 health and performance of female players.

119

## 120 **Methods**

### 121 **Participants**

122 Twenty-four female professional international soccer players volunteered to take part in the  
123 study. Cohort participant characteristics (also categorised according to playing position) are  
124 presented in Table 1. All players remained injury free for the duration of the study. All  
125 experimental procedures and associated risks were explained to players and written informed  
126 consent was obtained. The study was conducted according to the Declaration of Helsinki and  
127 was approved by the University Ethics Committee of Liverpool John Moores University.

128

### 129 **Overview of study design**

130 An overview of the experimental protocol is shown in Figure 1. All players completed a 9-day  
131 international training camp in November 2019 comprising 4 training days, 1 rest day, 2 travel  
132 days and 2 match days. Players completed the training prescribed by the national team's  
133 coaching staff and were available for team selection to play in 2 competitive international  
134 matches on days 5 (home game) and 8 (away game) during the study period. Three players did  
135 not play in either match and where appropriate, these players' data are not reported (indicated  
136 accordingly). TEE was assessed during a 12-day (9-day camp followed by 3-days at home)  
137 and 4-day assessment period using the DLW method whilst energy intake was also assessed  
138 during the 4 days prior to match one. TEE was assessed over 12 days (as opposed to 9-days)  
139 due to logistical challenges of urine collection on days 9 to 11 of the study. Players completed  
140 the second international football match abroad in Croatia on day 8. On day 9, players travelled  
141 back to the UK and were then driven from the airport to their homes. This resulted in no

142 opportunity to collect urine samples on this day. It was decided between international staff and  
143 domestic club staff that players were to rest at home on day 10 and 11 without any interruptions.  
144 On day 12, players arrived back at their respective clubs for duty, allowing a final urine sample  
145 to be collected. External loading was quantified from all pitch-based training sessions and  
146 games. To compare data across time, days are expressed in proximity to the match e.g., one  
147 day before the game is referred to as match day (MD) minus one (i.e., MD-1) whereas the day  
148 after the game is referred to as MD+1 etc.

149

### 150 **Baseline measures**

151 Due to logistical issues associated with player availability, body composition was assessed for  
152 18 players only, occurring 2-4 weeks prior to the training camp via whole-body dual-energy  
153 X-ray absorptiometry (DXA) (Hologic QDR Series, Discovery A, Bedford, MA, USA), where  
154 the effective radiation dose was 0.01 mSv per person. All scans were performed and analysed  
155 by the same trained operator in accordance with best practice procedures (24). Resting  
156 metabolic rate (RMR) was estimated for each player using a recent female athlete specific  
157 predictive equation (25). This equation ( $RMR = 120.81 + (4.88 \times \text{Stature}[\text{cm}]) + 8.24 \times \text{FFM}[\text{kg}]$   
158  $+ (5.71 \times \text{Age}[\text{years}])$ ) was selected as it was developed using healthy female athletes of a similar  
159 age-range and FFM to those in the present study. On the morning of day 1 of the training camp,  
160 all players (i.e. n=24) were assessed for body mass and stature. Under standardised conditions  
161 (>8 hours overnight fast), measurement of stature (SECA, model-217, Hamburg Germany) and  
162 body mass (SECA, model-875, Hamburg, Germany) were measured to the nearest 0.1 cm and  
163 0.1 kg, respectively according to the International Society for the Advancement of  
164 Kinanthropometry (ISAK) guidelines (26) by an ISAK Level-1 practitioner.

165

### 166 **Quantification of external training and match load**

167 The decision to wear GPS units during training was left to the players (goalkeepers do not wear  
168 these units). As such, thirteen outfield players who completed all training sessions and matches  
169 wore the same portable global GPS units (Apex, STATSports, Newry, Northern Ireland) for  
170 all pitch-based training sessions and both matches. Pitch-based sessions were monitored using  
171 the GPS units as previously described in professional soccer players (4,27,28). The GPS unit  
172 was placed inside a custom-made manufacturer provided vest (Apex, STATSports, Newry,  
173 Northern Ireland) that held the unit on the upper back between both scapulae, allowing clear  
174 exposure of the GPS antennae to acquire a clear satellite connection. External load variables  
175 selected for analysis from the training and match data were duration of activity (min), total  
176 distance covered (km) and high-speed running (defined as  $>5.30$  to  $6.30 \text{ m}\cdot\text{s}^{-1}$ ,  $>19.08$  to  $22.68$   
177  $\text{km}\cdot\text{h}^{-1}$ ).

178

#### 179 **Measurement of energy expenditure using the DLW method**

180 Twenty-four players were available for assessment of TEE. Energy expenditure was  
181 determined via the DLW method (the gold standard method of measuring energy expenditure  
182 in free-living conditions) which we have previously used in professional team sport athletes  
183 (9,11,29). During the evening of day zero, between the hours of 18:00-20:00, players provided  
184 a background urine sample. Players then consumed a single bolus oral dose weighed to four  
185 d.p. of deuterium ( $^2\text{H}$ ) and oxygen ( $^{18}\text{O}$ ) stable isotopes in the form of water ( $^2\text{H}_2^{18}\text{O}$ ), with a  
186 desired enrichment of 10%  $^{18}\text{O}$  and 5%  $^2\text{H}_2$  using the calculation:

187

$$188 \text{ Dose (mL)} = 0.65(\text{body mass, g}) \times \text{DIE} / \text{IE},$$

189

190 Where 0.65 is the approximate proportion of the body comprised of water, DIE is the desired  
191 initial enrichment ( $\text{DIE} = 618.923 \times \text{body mass (kg)}^{-0.305}$ ) and IE is the initial enrichment (10%)

192 100,000 parts per million (30) dosed according to body weight two-to-three weeks prior to the  
193 national camp. To ensure the whole dose was administered, participants were observed  
194 consuming each bolus dose and each glass vial was refilled with additional water which players  
195 were asked to consume. Time of dosing was recorded. Isotopes were purchased from Sercon  
196 (Cheshire, UK).

197         During the morning of day one (07:00-10:00), body mass was assessed (SECA, model-  
198 875, Hamburg, Germany), and participants were asked to provide a urine sample, collected in  
199 a 50 ml tube. This allowed initial isotope enrichment to be determined following total body  
200 water equilibrium (30). Thereafter, body mass was collected during the morning of day two,  
201 three, four, five, six and 12 and urine samples (second pass of the day) were collected on day  
202 two, three, four, five, six, seven, eleven and twelve (in line with logistical constraints), to  
203 determine elimination rates of both isotopes via the multi-point method (23).

204         For the DLW analysis, urine was encapsulated into capillaries, which were then vacuum  
205 distilled (31), and water from the resulting distillate was used. This water was analysed using  
206 a liquid water analyser (Los Gatos Research; (32)). Samples were run alongside three  
207 laboratory standards for each isotope and three International standards (Standard Light Artic  
208 Precipitate, Standard Mean Ocean Water and Greenland Ice Sheet Precipitation; (30,33)) to  
209 account for machine day to day variation and correct delta values to parts per million. Isotope  
210 elimination rates were converted to EE using an updated two-pool model equation (34) and a  
211 mean calculated food quotient of  $0.85 \pm 0.2$ . The results from the energy expenditure data are  
212 expressed as a daily average from the 12-day data collection period and also the initial 4-day  
213 collection period. Physical activity level (PAL) was also calculated for each player by dividing  
214 TEE by RMR. PAL data is provided for 18 players only, given that 6 players were not available  
215 for DXA assessment (hence predicted RMR was not calculated for these players).

216



217 **Assessment of energy and macronutrient intake**

218 All twenty-four players on camp completed assessment of dietary and energy intake. Dietary  
219 intake was assessed for the first four days of the study via weighed food inventory. A four-day  
220 assessment period was chosen due to logistical issues with overseas travel for the rest of the  
221 study. This method of energy intake assessment has previously been used alongside DLW with  
222 athletes (35). All main meals were consumed (i.e., breakfast, lunch, and dinner) in the presence  
223 of the research team. Any snacks consumed outside of these meals was reported to the research  
224 team via the remote food photography method, as described previously (9,11,36). All players  
225 were free to self-select food choices and had received no prior education on nutrition strategies  
226 for training days. As such, players were asked to continue with their habitual nutritional  
227 practices through the study period. The information gained from this study was then used to  
228 produce individualised education and behaviour change strategies. Weighed food intake was  
229 assessed using an identified weighing station for main meals only, which included four separate  
230 calibrated weighing scales (Salter 1160 BKDR, Tonbridge, Kent, UK) placed on top of four  
231 separate A3 1cm cubed template place mats. The members of research team operating the  
232 stations during breakfast, lunch and dinner included three Sport and Exercise Register (SENr)  
233 registered performance nutritionists. Once participants had selected their first item of food,  
234 they arrived at the weighing station, placed their plate on the scale and informed the registered  
235 nutritionist the weight of the plate. This number was then populated into a pre-designed  
236 spreadsheet with a description of the food item underneath their name. For example, the  
237 participant would tell the member of staff the weight of their food item i.e., 762 g of white  
238 pasta, to inform both the weight and item of food. The participant would then place their second  
239 chosen item of food on the plate, for example chicken, and would return to the weighing station  
240 to re-weigh their plate, by calling out the weight and food item to the member of staff.  
241 Participants would follow the same process of calling out the new total weight and food item

242 to one of the three nutritionists who again would populate the spreadsheet. The spreadsheet  
243 was pre-designed to subtract the weight of the plate from the initial food item to allow  
244 quantification of food item number 1. Subsequently, as each food item was then added to the  
245 participant plate, the spreadsheet would automatically subtract the previous food item away  
246 from the measured food item so quantification of each food item could be calculated  
247 independently. This process was repeated until all participants had completed their total meal  
248 choice, at which point a photographic picture was captured of the complete final meal and  
249 weight and stored for later analysis. If players had finished eating and still had food left on  
250 their plate, they were asked to return to the weighing station to see a member of the research  
251 team who would subtract any food items left off the original completed meal total via the  
252 spreadsheet. In addition to weighing food, the remote food photographic method (RFPM) was  
253 used (11), to understand and retrieve information on what players consumed away from the  
254 three main mealtimes. This included EI consumed during “snack windows” provided on camp  
255 and EI consumed in hotel rooms. Players were asked to provide a photograph of the food or  
256 drink that they consumed and were sent to the research team on a smart phone via WhatsApp  
257 messaging service, as described previously (36). Thirdly, to further enhance reliability and  
258 ensure that participants missed no food or drink consumption, six random 24-hr food recalls  
259 were also performed by two members of the research team to cross check methods one and  
260 two. To obtain energy and macronutrient composition, professional dietary analysis software  
261 (Nutritics Ltd, Ireland) was used by a Sport and Exercise Nutrition register accredited  
262 practitioner with experience working with Nutritics Ltd. All energy intake is reported in  
263 kilocalories (kcal) and kilocalories per kilogram of total body mass ( $\text{kcal}\cdot\text{kg}^{-1}$ ). Macronutrient  
264 intakes were also analysed and reported in grams (g) and grams per kilogram of body mass  
265 ( $\text{g}\cdot\text{kg}^{-1}$ ).

266 Menu construction and the preparation of meals and snacks were undertaken by the  
267 national team's professional chef and performance nutrition team and developed in line with  
268 the demands of the training camp and consideration of proximity to each game. Throughout  
269 the duration of energy intake assessment, meals were consumed at the base camp hotel for the  
270 squad with menus provided on a buffet style basis. Breakfast options available daily included:  
271 eggs, beans, toast, porridge, muesli, fruits and yoghurts. Lunch and dinner had different options  
272 that included one red meat option, one poultry option, one fish option, three-to-four  
273 carbohydrate options (e.g., pasta, rice, potatoes, quinoa), three vegetable options alongside a  
274 salad bar and snacks such as yoghurts, nuts, cereal bars and condiments. During training  
275 sessions, players were provided with low calorie isotonic sports drinks (Lucozade Lite), water  
276 and upon request, isotonic energy gels (Science in Sport, GO Isotonic Gels, UK). Protein drinks  
277 (Science in Sport, Whey Protein, UK) were provided after training sessions. All carbohydrate  
278 provided during training were optional and consumed *ad libitum* as opposed to individualised  
279 prescription to players.

280

### 281 **Estimation of energy availability**

282 Given that FFM was known for 18 players only (due to completion of DXA assessment), EA  
283 was initially estimated for this cohort. However, due to a sample error with the urine sample  
284 provided by one player on day 4, this player's 4-day analysis of TEE was not completed, hence  
285 EA is estimated for 17 players. The thermic effect of food (TEF) was assumed to be 10% of EI  
286 for all individuals (37), subsequently enabling estimations of activity energy expenditure (AEE  
287 = TEE – [RMR + TEF]) and energy availability (EA = EI – [AEE/FFM]) (38) during the initial  
288 four days of the training camp. Energy availability was defined using the following thresholds:  
289 optimal (> 45 kcal.kg FFM<sup>-1</sup>.day<sup>-1</sup>), reduced (30-45 kcal.kg FFM<sup>-1</sup>.day<sup>-1</sup>) and low (<30 kcal.kg  
290 FFM<sup>-1</sup>.day<sup>-1</sup>) (20).

291

## 292 **Statistical Analysis**

293 All data were initially assessed for normality of distribution using Shapiro-Wilk's test.  
294 Differences in training load, match load and energy intake across days were analysed using a  
295 one-way repeated measures ANOVA. Where significant main effects were present, Tukey  
296 post-hoc analysis was conducted to locate specific differences. Comparisons between energy  
297 intake and expenditure were analysed using a paired t-test. Ninety-five percent confidence  
298 intervals (95% CI) for the differences are also presented. Relationships between TEE and body  
299 mass, fat-free mass, stature, RMR and four-day AEE were assessed using Pearson's  
300 correlation. All statistical analysis were completed using SPSS (version 27, SPSS, Chicago,  
301 IL) where  $P < 0.05$  is indicative of statistical significance. Data are presented as mean  $\pm$  SD.

302

303

## 304 **Results**

### 305 *Baseline characteristics*

306 Player characteristics including stature, body mass, fat-free mass, fat mass, percent  
307 body fat, bone mineral content and bone mineral density are presented in Table 1. Data are  
308 presented for the full cohort as well as mean data from positional groups.

309

### 310 *Training and match load*

311 External loading variables are presented for  $n=13$  in accordance with those players who wore  
312 GPS monitors across all training sessions and games. Training duration (Figure 2A) was longer  
313 on MD-4 ( $89 \pm 4$  min) compared to MD-1 for match one ( $61 \pm 2$  min; 95% CI = 22 to 32 min;  
314  $P < 0.01$ ) and MD-1 for match two ( $63 \pm 7$  min; 95% CI = 17 to 34 min;  $P < 0.01$ ). Similarly,  
315 MD-3 training duration ( $89 \pm 5$  min) was also longer than MD-1 training duration for match

316 one (95% CI = 21 to 33 min;  $P < 0.01$ ) and match two (95% CI = 18 to 33 min;  $P < 0.01$ ). In  
317 contrast, no difference was apparent for the duration of match one ( $64 \pm 33$  min) and match  
318 two ( $73 \pm 31$  min) compared to the remaining training days ( $P > 0.05$ ).

319 In accordance with exercise duration, more distance (Figure 2B) was covered on MD-  
320 4 ( $6020 \pm 620$  m) compared to MD-1 for match one ( $2927 \pm 862$  m; 95% CI = 2090 to 4095  
321 m;  $P < 0.01$ ) and MD-1 for match two ( $4063 \pm 540$  m; 95% CI = 1177 to 2736 m;  $P < 0.01$ ).  
322 Similarly, MD-3 distance covered ( $6340 \pm 537$  m) was greater than MD-1 distance covered for  
323 match one (95% CI = 2264 to 4562 m;  $P < 0.01$ ) and match two (95% CI = 1721 to 2833 m;  
324  $P < 0.01$ ). The distance covered on MD-1 for match one was significantly lower than both the  
325 distance covered on MD-1 for match two ( $P = 0.012$ ) and the distance covered in match two  
326 ( $7430 \pm 3237$  m; 95% CI = -7734 to -1272 m;  $P = 0.004$ ). There was no significant difference  
327 in distance covered between match day one ( $6243 \pm 340$  m) and all other days ( $P > 0.05$ ).

328 High-speed running distance (Figure 2C) was significantly greater during match one  
329 ( $361 \pm 183$  m) compared to MD-4 ( $126 \pm 85$  m; 95% CI = 73 to 395 m;  $P < 0.01$ ), MD-1 for  
330 match one ( $85 \pm 79$  m; 95% CI = 102 to 450 m;  $P < 0.01$ ) and MD-1 for match two ( $77 \pm 41$  m;  
331 95% CI = 107 to 460 m;  $P < 0.01$ ). High-speed running distance was significantly greater during  
332 match two ( $337 \pm 197$  m) when compared to MD-1 for both match one ( $P < 0.01$ ) and match  
333 two ( $P = 0.013$ ), although no significant difference was apparent with other training days or  
334 match one ( $P > 0.05$ ). There was no significant difference in high-speed running distance  
335 between other training days ( $P > 0.05$ ).

336

### 337 *Energy expenditure*

338 Mean TEE for the whole cohort ( $n = 24$ ) across the full 12-day period was  $2693 \pm 432$  kcal.day<sup>-1</sup>  
339 (range: 2105-3507 kcal.day<sup>-1</sup>),  $43 \pm 6$  kcal.kg<sup>-1</sup> (range 33-55 kcal.kg<sup>-1</sup>) and  $54 \pm 6$  kcal.kg<sup>-1</sup>  
340 FFM (range: 45-68 kcal.kg<sup>-1</sup> FFM). Mean four-day TEE ( $n = 23$ ) was  $2753 \pm 359$  kcal.day<sup>-1</sup>

341 (range: 1942-3280 kcal.day<sup>-1</sup>), 44 ± 7 kcal.kg<sup>-1</sup> (range 29-55 kcal.kg<sup>-1</sup>) and 56 ± 8 kcal.kg<sup>-1</sup>  
342 FFM (range: 37-68 kcal.kg<sup>-1</sup> FFM). There was no significant difference between 12-day TEE  
343 and 4-day absolute TEE (P=0.307). Mean four-day AEE (n=23) was 1058 ± 352 kcal.day<sup>-1</sup>  
344 (range: 155-1549 kcal.day<sup>-1</sup>) and mean PAL values (n=18) was 1.79 ± 0.24 (range: 1.4-2.2).  
345 For illustrative purposes, individual data points (where players are represented within their  
346 positional groups) are displayed in Figure 3 A-D.

347

### 348 *Energy intake and macronutrient intake*

349 Mean energy intake (n=24) during the 4-day assessment period was 1923 ± 232 kcal.day<sup>-1</sup>  
350 (range: 1639-2172 kcal.day<sup>-1</sup>). Both absolute (P<0.01) and relative (P<0.01) mean energy  
351 intake (Figure 4A and B) was significantly different between training days. In absolute terms,  
352 players consumed less energy on MD-3 (1639 ± 285 kcal.day<sup>-1</sup>) compared to MD-4 (2172 ±  
353 373 kcal.day<sup>-1</sup>, 95% CI -807 to -259 kcal.day<sup>-1</sup>, P<0.01), MD-2 (1919 ± 319 kcal.day<sup>-1</sup>, 95%  
354 CI -554 to -5 kcal.day<sup>-1</sup>, P=0.04) and MD-1 (1962 ± 452 kcal.day<sup>-1</sup>, 95% CI -597 to -48  
355 kcal.day<sup>-1</sup>, P=0.01). In contrast, there was no difference between the MD-4 and MD-2 (P=0.80)  
356 or MD-1 (P=0.19) and between MD-2 and MD-1 (P=0.97). In relative terms, players consumed  
357 less energy on MD-3 (26 ± 5 kcal.kg<sup>-1</sup>.day<sup>-1</sup>) compared with MD-4 (34 ± 6 kcal.kg<sup>-1</sup>.day<sup>-1</sup>, 95%  
358 CI 34 to 13 kcal.kg<sup>-1</sup>.day<sup>-1</sup>, P<0.01) and MD-1 (31 ± 8 kcal.kg<sup>-1</sup>.day<sup>-1</sup>, 95% CI -10 to 1 kcal.kg<sup>-1</sup>  
359 .day<sup>-1</sup>, P=0.02). In contrast, no difference was apparent between MD-3 and MD-2 (30 ± 6  
360 kcal.kg<sup>-1</sup>.day<sup>-1</sup>, P=0.07), MD-4 and MD-2 (P=0.11) or MD-1 (P=0.25) and between MD-2 and  
361 MD-1 (P=0.97).

362 Mean absolute CHO intake (Figure 4C) was similar (P=0.37) between MD-4 (218 ± 56  
363 g.day<sup>-1</sup>), MD-3 (203 ± 57 g.day<sup>-1</sup>), MD-2 (192 ± 45 g.day<sup>-1</sup>) and MD-1 (203 ± 71 g.day<sup>-1</sup>).  
364 Similarly, mean relative CHO intake (Figure 4D) was similar (P=0.38) between MD-4 (3.5 ±

365 0.9 g.kg<sup>-1</sup>.day<sup>-1</sup>), MD-3 (3.2 ± 1.0 g.kg<sup>-1</sup>.day<sup>-1</sup>), MD-2 (3.0 ± 0.7 g.kg<sup>-1</sup>.day<sup>-1</sup>) and MD-1 (3.2 ±  
366 1.1 g.kg<sup>-1</sup>.day<sup>-1</sup>).

367 Mean absolute protein intake was significantly different (P<0.01; Figure 4E) between  
368 training days such that on MD-4 (123 ± 21 g.day<sup>-1</sup>), MD-3 (120 ± 33 g.day<sup>-1</sup>) and MD-1 (135  
369 ± 24 g.day<sup>-1</sup>) more protein was consumed than on MD-2 (100 ± 23 g.day<sup>-1</sup>; 95% CI = 5 to 41  
370 g.day<sup>-1</sup>; P<0.01, 95% CI = 2 to 39 g.day<sup>-1</sup>; P=0.02 and 95% CI = 18 to 52 g.day<sup>-1</sup>; P<0.01,  
371 respectively). No difference was observed between MD-4, MD-3, and MD-1 (P>0.05). Mean  
372 relative protein intake was significantly different (P<0.01; Figure 4F) between training days  
373 such that on MD-4 (1.9 ± 0.2 g.kg<sup>-1</sup>.day<sup>-1</sup>), MD-3 (1.9 ± 0.4 g.kg<sup>-1</sup>.day<sup>-1</sup>) and MD-1 (2.1 ± 0.4  
374 g.kg<sup>-1</sup>.day<sup>-1</sup>) more protein was consumed than on MD-2 (1.6 ± 0.4 g.kg<sup>-1</sup>.day<sup>-1</sup>; 95% CI = 0.0  
375 to 0.6 g.kg<sup>-1</sup>.day<sup>-1</sup>; P<0.01, 95% CI = 0.0 to 0.5 g.kg<sup>-1</sup>.day<sup>-1</sup>; P=0.03 and 95% CI = 0.3 to 0.8  
376 g.kg<sup>-1</sup>.day<sup>-1</sup>; P<0.01, respectively).

377 Mean absolute fat intake was significantly different (P<0.01; Figure 4G) between  
378 training days such that on MD-4 (90 ± 21 g.day<sup>-1</sup>), more fat was consumed than on MD-3 (38  
379 ± 14 g.day<sup>-1</sup>; 95% CI = 37 to 66 g.day<sup>-1</sup>; P<0.01) and MD-1 (67 ± 24 g.day<sup>-1</sup>; 95% CI = 3 to 42  
380 g.day<sup>-1</sup>; P<0.01). Similarly, more fat was consumed on MD-2 (87 ± 33 g.day<sup>-1</sup>; 95% CI = 28  
381 to 69 g.day<sup>-1</sup>; P<0.01) than MD-3 and MD-1 (67 ± 24 g.day<sup>-1</sup>; 95% CI = 15 to 43 g.day<sup>-1</sup>;  
382 P<0.01) compared to MD-3. Mean relative fat intake was significantly different (P<0.01;  
383 Figure 4H) between training days such that on MD-4 (1.4 ± 0.3 g.kg<sup>-1</sup>.day<sup>-1</sup>), more fat was  
384 consumed compared to MD-3 (0.6 ± 0.2 g.kg<sup>-1</sup>.day<sup>-1</sup>; 95% CI = 0.5 to 1.0 g.kg<sup>-1</sup>.day<sup>-1</sup>; P<0.01)  
385 and MD-1 (1.0 ± 0.4 g.kg<sup>-1</sup>.day<sup>-1</sup>; 95% CI = 0.0 to 0.6 g.kg<sup>-1</sup>.day<sup>-1</sup>; P<0.01). Similarly, more  
386 fat was consumed on MD-2 (1.3 ± 0.5 g.kg<sup>-1</sup>.day<sup>-1</sup>) when compared to MD-3 (95% CI = 0.4 to  
387 1.1 g.kg<sup>-1</sup>.day<sup>-1</sup>; P<0.01) and on MD-1 when compared to MD-3 (95% CI = 0.2 to 0.6 g.kg<sup>-1</sup>.  
388 day<sup>-1</sup>; P<0.01).

389

390 ***Energy intake versus energy expenditure (n = 24) and energy availability (n = 17)***

391

392 In relation to the initial 4-day assessment period, there was a significant difference between EI  
393 and TEE ( $-825 \pm 419 \text{ kcal}\cdot\text{day}^{-1}$ ; 95% CI  $-1006$  to  $-643 \text{ kcal}\cdot\text{day}^{-1}$ ;  $P < 0.01$ ) (see Figure 5A).  
394 However, despite significant differences in EI and TEE, body mass did not change across this  
395 time period (see Figure 5B) ( $0.01 \pm 1.16 \text{ kg}$ ; 95% CI  $-0.48$  to  $0.51 \text{ kg}$ ;  $P = 0.95$ ). Mean daily (n  
396 = 17) estimated energy availability was  $18 \pm 9 \text{ kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$  (range:  $2$ - $36 \text{ kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$ ).  
397 Overall, 88% of players assessed for EA represented with  $< 30 \text{ kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$   
398 (see Figure 5C).

399

400 ***Factors affecting TEE and AEE***

401 There was a significant positive relationship between 12-day TEE and body mass ( $r^2 = 0.56$ ;  
402  $P < 0.01$ ), fat-free mass ( $r^2 = 0.65$ ;  $P < 0.01$ ) and predicted RMR ( $r^2 = 0.51$ ;  $P < 0.01$ ). There was  
403 also a significant positive relationship between four-day TEE and four-day AEE ( $r^2 = 0.97$ ;  
404  $P < 0.01$ ). There was no significant relationship between TEE and stature ( $r^2 = 0.15$ ;  $P > 0.05$ ).  
405 Data are presented in Figure 6.

406



## 407 **Discussion**

408 In using the DLW method, we provide the first direct assessment of total daily energy  
409 expenditure of adult female professional soccer players. Our measurements were obtained from  
410 players of the highest standard and were collected over a 12-day period when players were  
411 representing their national team. When compared with previously published data from adult  
412 male players, we demonstrate that the relative daily energetic requirements of engaging in  
413 professional soccer training and match play is comparable between sexes. As such, these data  
414 now provide a platform for which to develop evidence based nutritional guidelines for this  
415 population. From a practical perspective, our data suggest that practitioners should likely focus  
416 education and behaviour change strategies (at least for the present cohort) on “fuelling” for  
417 match play and training to optimise both player health and performance.

418

419 Previous assessments of daily TEE and AEE in female soccer players have been quantified  
420 using a combination of indirect methods such as accelerometers, heart rate monitors, activity  
421 logs and prediction equations (19, 24, 25, 44, 46). In absolute terms, such studies report that  
422 the TEE of female soccer players ranges from ~2400-2700 kcal.day<sup>-1</sup> (22,39,40). In using the  
423 DLW method, we observed comparable mean four-day (three training days, one rest day) TEE  
424 of 2753 ± 359 kcal.day<sup>-1</sup> (range: 1942-3280 kcal.day<sup>-1</sup>) whilst mean TEE from the full 12-day  
425 assessment period was 2693 ± 423 kcal.day<sup>-1</sup> (range: 2105-3507 kcal.day<sup>-1</sup>). In absolute terms,  
426 our data demonstrate a lower TEE to that previously observed in adult male professional  
427 players where mean expenditure was approximately 3500 kcal.d<sup>-1</sup> (9–11). Nonetheless, when  
428 expressed in relative terms (alongside comparable PAL values of 1.4-2.2), it is therefore  
429 apparent that the daily energetic requirements of both males and females engaging in  
430 professional soccer training and match play typically equates to 40-60 kcal.kg<sup>-1</sup> FFM.

431

432 Notwithstanding the limitations of comparing indirect and direct assessment methods, the  
433 present data also suggest that the energy requirements of competing and training at an  
434 “international” level may be higher than that associated with the players’ respective domestic  
435 level competition. For example, when compared with players from the English Women’s Super  
436 League (WSL), assessments of the AEE of the goalkeepers ( $924 \pm 133 \text{ kcal.day}^{-1}$ ), defenders  
437 ( $964 \pm 436 \text{ kcal.day}^{-1}$ ), midfielders ( $1318 \pm 195 \text{ kcal.day}^{-1}$ ) and attackers ( $1073 \pm 348 \text{ kcal.day}^{-1}$ )  
438 studied here is greater than the mean AEE ( $418 \text{ kcal.day}^{-1}$ ) of those players training within  
439 the domestic WSL (13). It is noteworthy, however, that the DLW derived assessment of AEE  
440 documented here is inclusive of all activity “outside” of pitch-based training such as strength-  
441 based sessions undertaken in the gym, recovery swimming pool sessions, as well as non-  
442 training related activity such as walking to and from the training centre and hotel and walking  
443 up and down stairs etc. In contrast, the AEE quantified by Moss et al. (19) is derived from a  
444 combination of metabolic equivalents and/or accelerometers worn during training, matches and  
445 strength and conditioning sessions only. Additionally, the training loads completed by Moss et  
446 al. (19) was completed in the final month of the season (May), a time when training loads are  
447 typically reduced in comparison to other phases of the season.

448

449 The external training and match loads observed here are lower than the respective loads  
450 associated with other international and domestic level soccer match play (41–43). For  
451 example, total distance and high speed running distance covered by outfield players is lower  
452 in our study ( $8.8 \pm 1.4 \text{ km}$  and  $0.35 \pm 0.18 \text{ km}$  respectively) compared with other international  
453 ( $9.9 \pm 1.8 \text{ km}$  and  $1.5 \pm 0.1 \text{ km}$  respectively) and domestic ( $9.7 \pm 1.4 \text{ km}$  and  $1.3 \pm 0.9$   
454 respectively) soccer matches (42). Difference between studies are most likely due to variation  
455 in methods used to collect match load data, where in previous studies, distance covered and  
456 high speed running was estimated from time motion analysis as opposed to GPS adopted here.

457 Additionally, the thresholds used for high-speed running in previous studies ( $>18 \text{ km}\cdot\text{h}^{-1}$ ) is  
458 lower than this study ( $>19 \text{ km}\cdot\text{h}^{-1}$ ) and makes it difficult to compare between studies. Such  
459 challenges in the lack of a definitive approach to identify high-intensity actions and the  
460 subsequent ambiguity in this area has recently been documented (44).

461

462 In relation to energy intake, previous studies in female soccer players have reported estimated  
463 energy intakes of  $2124 \pm 444 \text{ kcal}\cdot\text{day}^{-1}$  (13),  $2226 \pm 368 \text{ kcal}\cdot\text{day}^{-1}$  (39) and  $2387 \pm 177$   
464  $\text{kcal}\cdot\text{day}^{-1}$  (16). In contrast, we report estimated energy intakes that are approximately 200-300  
465  $\text{kcal}\cdot\text{d}^{-1}$  lower (mean of four-days:  $1923 \pm 357 \text{ kcal}\cdot\text{day}^{-1}$ ), a finding that may be due, in part,  
466 to the differing methods employed (e.g., self-reported food diaries versus researcher supervised  
467 weighed food intakes, the latter which may have influenced player food choices towards under-  
468 consumption of foods). In agreement with recent observations from players from the English  
469 Women's Super League (13), we also observed minimal CHO periodisation with players  
470 reporting comparable and consistent daily CHO intakes of 3.0 to 3.5  $\text{g}\cdot\text{kg}^{-1}$ . Notably, only one  
471 player consumed the recommended range of 6-8  $\text{g}\cdot\text{kg}^{-1}$  on the day before the match (12), thus  
472 it is likely that players commenced the first game with sub-optimal muscle glycogen stores  
473 (18). In contrast, mean protein intake across all training days ( $1.8 \pm 0.4 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ; range 1.6  
474 to 2.1  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) was aligned to supporting training adaptations (45) and in accordance with  
475 recommendations for professional soccer players (12). When taken together, it therefore  
476 appears that female soccer players may not consume (or periodise) sufficient CHO intake to  
477 meet the demands of training and competition, a factor that could lead to chronically low  
478 energy availability and symptoms associated with the female athlete triad (21) or RED-S  
479 models (19). Unfortunately, we are limited in that we do not currently provide any data  
480 assessing the impacts of the energy intake reported here on health and performance outcomes.  
481 Nonetheless, from a practical perspective, our data suggest that practitioners should likely

482 target education and behaviour change strategies on “fuelling” for match play and training to  
483 optimise both player health and performance. Based on our assessment of TEE, it is suggested  
484 that relative intakes of CHO, fat and protein corresponding to 4-8 (to account for rest-days,  
485 training days, match day minus 1, match day etc), 1.5-2 and 1.6-2 g·kg<sup>-1</sup>·day<sup>-1</sup> body mass would  
486 provide a reasonable starting point for which to meet the daily energy requirements of female  
487 soccer players of professional standard.

488

489 Although we readily acknowledge the difficulties in assessing energy availability (46) as well  
490 as the limitation of our four-day assessment period via weighed food inventory (i.e. players  
491 may alter food intake because of researcher presence), it is noteworthy that the estimated  
492 prevalence of LEA observed here (i.e., 88%, 15 out of 17, players presented with LEA <30  
493 kcal.kg<sup>-1</sup> FFM) is greater than previous reports where 70, 24 and 65% of players presented with  
494 LEA in English (13), American (14) and Polish national leagues (40) respectively. The lower  
495 absolute energy intakes reported here coupled with the potentially increased physical demands  
496 associated with competing at international level (when compared to domestic level  
497 competition) may be a contributing factor. Whilst we also acknowledge the limitations (35,36)  
498 associated with dietary assessment and potential under-reporting (as evidenced by the lack of  
499 statistical change in body mass), further work is required to ascertain whether players’ chosen  
500 dietary choices were an unconscious or conscious decision that is based upon beliefs  
501 surrounding optimal nutritional practices. We also acknowledge that the classification of LEA  
502 status as <30 kcal.kg<sup>-1</sup> FFM is based upon laboratory studies that typically adopt short-term  
503 periods of “consistent” daily EI, EE and therefore EA. For example, studies which established  
504 EA concepts did so over short (four-to-seven days) periods where careful but artificial control  
505 of diet and exercise was prescribed (20). The application of such a threshold to real world  
506 situations is likely limited by the fact that daily energy expenditure fluctuates day-to-day in

507 accordance with alterations to eating schedules, training load, and competitive demands.  
508 Accordingly, the prevalence of LEA status in the present study (and associated long term  
509 physiological implications) may be over-estimated. Further studies are required to evaluate the  
510 prevalence of LEA using longer assessment timeframes. Furthermore, assessment of within-  
511 day and between-day EA combined with screening tools (21,47,48) and clinical markers would  
512 help gain greater accuracy with current assessments of EA in female athletes in the applied  
513 field.

514

515 In summary, we provide the first report to directly assess total daily energy expenditure in a  
516 cohort of adult female professional soccer players of international standard. Our data suggest  
517 that the relative daily energetic requirements of engaging in professional soccer training and  
518 match play is comparable in males and females. From a practical perspective, our data suggest  
519 that individualised education and behaviour change strategies should focus on “fuelling” (i.e.  
520 increasing daily CHO intake) for match play and training to optimise health and performance.

521

522

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526

### 527 **Conflict of interest**

528 The authors report no potential conflict of interest. The results of the present study do not  
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530 and without fabrication, falsification, or inappropriate data manipulation.

531

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702 **Table 1.** Baseline player characteristics of elite English female soccer players competing at  
703 international level. Stature, body mass, fat-free mass, fat mass and percent body fat values are  
704 presented according to playing position. Stature and body mass n=24. Fat-free mass, fat mass,  
705 percent body fat, bone mineral content, bone mineral density, pelvis bone mineral density, Z-  
706 score derived from DXA n=18. Predicted resting metabolic rate (RMR) n=18. Predicted RMR  
707 =  $120.81 + (4.88 \times \text{Stature}[\text{cm}]) + 8.24 \times \text{FFM}[\text{kg}] + (5.71 \times \text{Age}[\text{years}])$  (25).  
708

<b>Position</b>	<b>Goalkeepers</b>	<b>Defenders</b>	<b>Midfielders</b>	<b>Attackers</b>	<b>Squad</b>
<b>Stature (cm)</b>	174.3 ± 0.5 (n=3)	169.7 ± 2.4 (n=9)	168.2 ± 9.2 (n=4)	163.0 ± 3.5 (n=8)	168.1 ± 5.9 (n=24)
<b>Body Mass (kg)</b>	67.0 ± 8.7 (n=3)	62.4 ± 3.2 (n=9)	60.4 ± 5.0 (n=4)	60.1 ± 1.1 (n=8)	62.1 ± 4.7 (n=24)
<b>Fat-Free Mass (kg)</b>	45.5 ± 3.5 (n=3)	44.1 ± 3.6 (n=6)	42.8 ± 3.9 (n=4)	41.6 ± 2.1 (n=5)	43.2 ± 3.4 (n=18)
<b>Fat Mass (kg)</b>	14.4 ± 5.1 (n=3)	11.1 ± 1.3 (n=6)	10.3 ± 3.0 (n=4)	12.2 ± 1.4 (n=5)	11.8 ± 2.7 (n=18)
<b>Percent Body Fat (%)</b>	22.9 ± 5.2 (n=3)	19.5 ± 2.6 (n=6)	18.6 ± 4.6 (n=4)	20 ± 2.7 (n=5)	20.6 ± 3.7 (n=18)
<b>Whole Body Bone Mineral Content (g)</b>	2808 ± 361 (n=3)	2837 ± 158 (n=6)	2803 ± 236 (n=4)	2637 ± 165 (n=5)	2766 ± 213 (n=18)
<b>Whole Body Bone Mineral Density (g/cm<sup>2</sup>)</b>	1.26 ± 0.12 (n=3)	1.33 ± 0.06 (n=6)	1.35 ± 0.11 (n=4)	1.26 ± 0.10 (n=5)	1.31 ± 0.10 (n=18)
<b>Pelvis Bone Mineral Density (g/cm<sup>2</sup>)</b>	1.37 ± 0.19 (n=3)	1.28 ± 0.11 (n=6)	1.35 ± 0.19 (n=4)	1.42 ± 0.11 (n=5)	1.38 ± 0.13 (n=18)
<b>Whole Body Z-score</b>	2.7 ± 1.0 (n=3)	2.4 ± 0.5 (n=6)	2.7 ± 1.2 (n=4)	2.1 ± 0.5 (n=5)	2.4 ± 0.7 (n=18)
<b>Predicted RMR (kcal.day<sup>-1</sup>)</b>	1549 ± 56 (n=3)	1515 ± 71 (n=6)	1494 ± 95 (n=4)	1449 ± 46 (n=5)	1486 ± 66 (n=18)

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711 **Figure 1.** Schematic overview of the 12-day study period including the 9-day national training  
712 camp. TEE was assessed over 12 days and 4-days (as opposed to 9-days) due to logistical challenges  
713 of urine collection on day 9 to 11 of the study. Day 6 and days 9-12 represented rest days during which  
714 no scheduled training took place.

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718 **Figure 2.** (A) Training and match-play duration, (B) total distance, and (C) high speed running  
719 distance in during an international training camp from female soccer players. White bars  
720 represent training days, denoted as days away from match day (MD), i.e., MD-5, etc., and grey  
721 bars represent match day. No training was completed on days with no data bars. <sup>a</sup> denotes  
722 significant difference from MD-4,  $P < 0.05$ . <sup>b</sup> denotes significant difference from MD-3,  $P < 0.05$ .  
723 <sup>c</sup> denotes significant difference from MD-1 prior to match 1,  $P < 0.05$ . <sup>d</sup> denotes significant  
724 difference from MD one,  $P < 0.05$ . <sup>f</sup> denotes significant difference from MD two,  $P < 0.05$ . Black  
725 circles represent individual players. All data are representative of  $n=13$  in accordance with  
726 players who wore GPS monitors.

727  
728 **Figure 3.** (A) Mean twelve daily total energy expenditure ( $n=24$ ), (B) mean four-day total  
729 energy expenditure ( $n=23$ ), (C) mean four-day activity energy expenditure ( $n=23$ ), (D)  
730 physical activity level ( $n=18$ ) within each positional group. Black circles represent individual  
731 players.

732  
733 **Figure 4.** (A) Absolute and (B) relative energy intake, (C) absolute and (D) relative  
734 carbohydrate intake, (E) absolute and (F) relative protein intake and (G) absolute and (H)  
735 relative fat intake across the initial 4-day assessment period ( $n=24$  for all variables). Black  
736 circles represent individual players. <sup>a</sup> denotes significant difference from MD-4, <sup>b</sup> denotes  
737 significant difference from MD-3, <sup>c</sup> denotes significant difference from MD-2, <sup>d</sup> denotes  
738 significance difference from MD-1

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740 **Figure 5.** (A) Difference between TEE and EI (n=23), (B) changes in body mass (n=24) and  
741 (C) mean estimated daily energy availability (n=17) when assessed across the initial 4-day  
742 assessment period. Black circles represent individual players.

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744 **Figure 6.** The relationship between mean 12-day total energy expenditure (TEE) and (A) body  
745 mass (P<0.01), (B) fat free-mass (P<0.01), (C) stature (P>0.05), predicted resting metabolic  
746 rate (RMR; P<0.01) and (E) 4-day TEE versus 4-day activity energy expenditure (AEE;  
747 P<0.01). Black circles represent individual players.

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