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Article

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

James C. Morehen[1,2], Christopher Rosimus[2], Bryce P. Cavanagh[2], Catherine Hambly[3], John R. Speakman[3], Kirsty J. Elliot-Sale[4], Marcus P. Hannon[1] and James P. Morton[1]

[1]Research Institute for Sport and Exercise Sciences (RISES)
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF
UK

[2]The Football Association,
London
UK

[3]Institute of Biological and Environmental Sciences
University of Aberdeen
Aberdeen
UK

[4]Musculoskeletal Physiology Research Group,
Sport Health and Performance Enhancement Research Centre,
Nottingham Trent University,
Nottingham, UK

Address for correspondence:
Dr James C Morehen
Research Institute for Sport and Exercise Sciences (RISES)
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF
Email: j.c.morehen@ljmu.ac.uk

Abstract

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

Keywords: carbohydrate, energy availability, RED-S

Introduction

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

In contrast to adult male players, the energetic requirements and external training loads completed by elite female players are not as well understood (13–18). This is of specific interest given recent reports documenting the prevalence of low energy availability (LEA, defined as <30 kcal.kg^{-1} FFM per day) in female professional players from the English Women's Super League (13). Indeed, these researchers observed that between 50-70% of players were classified with LEA status on both match day and "heavy" training days where daily activity energy expenditure was >700 kcal.d^{-1}, as estimated by global positioning systems (GPS). Analysis of self-reported energy intakes (EI) also demonstrated that these players consumed a consistent daily CHO intake of 3-3.5 g.kg^{-1} body mass, thereby failing to adjust daily CHO intake in accordance with alterations to training load or in preparation for match play. Such 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 accelerometery 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 + (4.88xStature[cm]) + 8.24xFFM[kg])

158 + (5.71xAge[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 $m.s^{-1}$, >19.08 to 22.68

177 $km.h^{-1}$).

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 (^2H) and oxygen (^{18}O) stable isotopes in the form of water ($^2H_2^{18}O$), with a

186 desired enrichment of 10% ^{18}O and 5% $^2H_2$ 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 (DIE = 618.923 x 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 ± 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

Assessment of energy and macronutrient intake

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

264   intakes were also analysed and reported in grams (g) and grams per kilogram of body mass

265   ($g.kg^{-1}$).

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^{-1}.day^{-1}), reduced (30-45 kcal.kg FFM^{-1}.day^{-1}) and low (<30 kcal.kg

290 FFM^{-1}.day^{-1}) (20).

291

Statistical Analysis

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

Results

Baseline characteristics

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

Training and match load

External loading variables are presented for n=13 in accordance with those players who wore GPS monitors across all training sessions and games. Training duration (Figure 2A) was longer on MD-4 (89 $\pm$ 4 min) compared to MD-1 for match one (61 $\pm$ 2 min; 95% CI = 22 to 32 min; $P<0.01$) and MD-1 for match two (63 $\pm$ 7 min; 95% CI = 17 to 34 min; $P<0.01$). Similarly, 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 ± 33 min) and match

318 two (73 ± 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 ± 620 m) compared to MD-1 for match one (2927 ± 862 km; 95% CI = 2090 to 4095

321 km; P<0.01) and MD-1 for match two (4063 ± 540 m; 95% CI = 1177 to 2736 m; P<0.01).

322 Similarly, MD-3 distance covered (6340 ± 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 ± 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 ± 340 m) and all other days (P>0.05).

328   High-speed running distance (Figure 2C) was significantly greater during match one

329 (361 ± 183 m) compared to MD-4 (126 ± 85 m; 95% CI = 73 to 395 m; P<0.01), MD-1 for

330 match one (85 ± 79 m; 95% CI = 102 to 450 m; P<0.01) and MD-1 for match two (77 ± 41 m;

331 95% CI = 107 to 460 m; P<0.01). High-speed running distance was significantly greater during

332 match two (337 ± 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 ± 432 kcal.day^{-1}

339 (range: 2105-3507 kcal.day^{-1}), 43 ± 6 kcal.kg^{-1} (range 33-55 kcal.kg^{-1}) and 54 ± 6 kcal.kg^{-1}

340 FFM (range: 45-68 kcal.kg^{-1} FFM). Mean four-day TEE (n=23) was 2753 ± 359 kcal.day^{-1}

341 (range: 1942-3280 kcal.day^{-1}), 44 ± 7 kcal.kg^{-1} (range 29-55 kcal.kg^{-1}) and 56 ± 8 kcal.kg^{-1}

342 FFM (range: 37-68 kcal.kg^{-1} 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^{-1}

344 (range: 155-1549 kcal.day^{-1}) 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^{-1}

350 (range: 1639-2172 kcal.day^{-1}). 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^{-1}) compared to MD-4 (2172 ±

353 373 kcal.day^{-1}, 95% CI -807 to -259 kcal.day^{-1}, P<0.01), MD-2 (1919 ± 319 kcal.day^{-1}, 95%

354 CI -554 to -5 kcal.day^{-1}, P=0.04) and MD-1 (1962 ± 452 kcal.day^{-1}, 95% CI -597 to -48

355 kcal.day^{-1}, 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^{-1}.day^{-1}) compared with MD-4 (34 ± 6 kcal.kg^{-1}.day^{-1}, 95%

358 CI 34 to 13 kcal.kg^{-1}.day^{-1}, P<0.01) and MD-1 (31 ± 8 kcal.kg^{-1}.day^{-1}, 95% CI -10 to 1 kcal.kg^{-1}

359 .day^{-1}, P=0.02). In contrast, no difference was apparent between MD-3 and MD-2 (30 ± 6

360 kcal.kg^{-1}.day^{-1}, 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^{-1}), MD-3 (203 ± 57 g.day^{-1}), MD-2 (192 ± 45 g.day^{-1}) and MD-1 (203 ± 71 g.day^{-1}).

364 Similarly, mean relative CHO intake (Figure 4D) was similar (P=0.38) between MD-4 (3.5 ±

365 0.9 g.kg^{-1}.day^{-1}), MD-3 (3.2 ± 1.0 g.kg^{-1}.day^{-1}), MD-2 (3.0 ± 0.7 g.kg^{-1}.day^{-1}) and MD-1 (3.2 ±

366 1.1 g.kg^{-1}.day^{-1}).

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^{-1}), MD-3 (120 ± 33 g.day^{-1}) and MD-1 (135

369 ± 24 g.day^{-1}) more protein was consumed than on MD-2 (100 ± 23 g.day^{-1}; 95% CI = 5 to 41

370 g.day^{-1}; P<0.01, 95% CI = 2 to 39 g.day^{-1}; P=0.02 and 95% CI = 18 to 52 g.day^{-1}; 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^{-1}.day^{-1}), MD-3 (1.9 ± 0.4 g.kg^{-1}.day^{-1}) and MD-1 (2.1 ± 0.4

374 g.kg^{-1}.day^{-1}) more protein was consumed than on MD-2 (1.6 ± 0.4 g.kg^{-1}.day^{-1}; 95% CI = 0.0

375 to 0.6 g.kg^{-1}.day^{-1}; P<0.01, 95% CI = 0.0 to 0.5 g.kg^{-1}.day^{-1}; P=0.03 and 95% CI = 0.3 to 0.8

376 g.kg^{-1}.day^{-1}; 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^{-1}), more fat was consumed than on MD-3 (38

379 ± 14 g.day^{-1}; 95% CI = 37 to 66 g.day^{-1}; P<0.01) and MD-1 (67 ± 24 g.day^{-1}; 95% CI = 3 to 42

380 g.day^{-1}; P<0.01). Similarly, more fat was consumed on MD-2 (87 ± 33 g.day^{-1}; 95% CI = 28

381 to 69 g.day^{-1}; P<0.01) than MD-3 and MD-1 (67 ± 24 g.day^{-1}; 95% CI = 15 to 43 g.day^{-1};

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^{-1}.day^{-1}), more fat was

384 consumed compared to MD-3 (0.6 ± 0.2 g.kg^{-1}.day^{-1}; 95% CI = 0.5 to 1.0 g.kg^{-1}.day^{-1}; P<0.01)

385 and MD-1 (1.0 ± 0.4 g.kg^{-1}.day^{-1}; 95% CI = 0.0 to 0.6 g.kg^{-1}.day^{-1}; P<0.01). Similarly, more

386 fat was consumed on MD-2 (1.3 ± 0.5 g.kg^{-1}.day^{-1}) when compared to MD-3 (95% CI = 0.4 to

387 1.1 g.kg^{-1}.day^{-1}; P<0.01) and on MD-1 when compared to MD-3 (95% CI = 0.2 to 0.6 g.kg^{-1}

388 $^{-1}$.day^{-1}; 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 ± 419 kcal.day^{-1}; 95% CI -1006 to -643 kcal.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 ± 1.16 kg; 95% CI -0.48 to 0.51 kg; P=0.95). Mean daily (n

396   = 17) estimated energy availability was 18 ± 9 kcal.kg FFM^{-1}.day^{-1} (range: 2-36 kcal.kg FFM^{-1}

397   ^{1}.day^{-1}). Overall, 88% of players assessed for EA represented with <30 kcal.kg FFM^{-1}.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

Discussion

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

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

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 ± 133 kcal.day^{-1}), defenders

437   (964 ± 436 kcal.day^{-1}), midfielders (1318 ± 195 kcal.day^{-1}) and attackers (1073 ± 348 kcal.day^{-}

438   ^{1}) studied here is greater than the mean AEE (418 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 ± 1.4 km and 0.35 ± 0.18 km respectively) compared with other international

453   (9.9 ± 1.8 km and 1.5 ± 0.1 km respectively) and domestic (9.7 ± 1.4 km and 1.3 ± 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 km.h^{-1}) is

458   lower than this study (>19 km.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 kcal.day^{-1} (13), 2226 $\pm$ 368 kcal.day^{-1} (39) and 2387 $\pm$ 177

464   kcal.day^{-1} (16). In contrast, we report estimated energy intakes that are approximately 200-300

465   kcal.d^{-1} lower (mean of four-days: 1923 $\pm$ 357 kcal.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 g.kg^{-1}. Notably, only one

471   player consumed the recommended range of 6-8 g.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 g.kg^{-1}.day^{-1}; range 1.6

474   to 2.1 g.kg^{-1}.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 $g \cdot kg^{-1} \cdot day^{-1}$ 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 $kcal.kg^{-1}$ 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 $kcal.kg^{-1}$ 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

526

527　**Conflict of interest**

528　The authors report no potential conflict of interest. The results of the present study do not

529　constitute endorsement by ACSM. All results presented here are done so clearly, honestly,

530　and without fabrication, falsification, or inappropriate data manipulation.

531

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

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

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

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

Figure 4. (**A**) Absolute and (**B**) relative energy intake, (**C**) absolute and (**D**) relative carbohydrate intake, (**E**) absolute and (**F**) relative protein intake and (**G**) absolute and (**H**) relative fat intake across the initial 4-day assessment period (n=24 for all variables). Black circles represent individual players. [a] denotes significant difference from MD-4, [b] denotes significant difference from MD-3, [c] denotes significant difference from MD-2, [d] denotes 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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