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Morehen, JC, Rosimus, C, Cavanagh, BP, Hambly, C, Speakman, JR, Elliott-Sale, KJ, Hannon, MP and Morton, JP (2022) Energy Expenditure of Female International Standard Soccer Players: A Doubly Labeled Water Investigation. Medicine and Science in Sports and Exercise, 54 (5). pp. 769-779. ISSN 0195-9131

DOI: https://doi.org/10.1249/MSS.0000000000002850

Publisher: Lippincott, Williams & Wilkins

Version: Accepted Version

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Additional Information: This is an Author Accepted Manuscript of an article published in Medicine and Science in Sports and Exercise, by Lippincott, Williams & Wilkins.

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

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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**

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

Measurement of energy expenditure using the DLW method

Twenty-four players were available for assessment of TEE. Energy expenditure was determined via the DLW method (the gold standard method of measuring energy expenditure in free-living conditions) which we have previously used in professional team sport athletes (9,11,29). During the evening of day zero, between the hours of 18:00-20:00, players provided a background urine sample. Players then consumed a single bolus oral dose weighed to four d.p. of deuterium (^{2}H) and oxygen (^{18}O) stable isotopes in the form of water ($^{2}H_2{}^{18}O$), with a desired enrichment of 10% ^{18}O and 5% $^{2}H_2$ using the calculation:

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

Where 0.65 is the approximate proportion of the body comprised of water, DIE is the desired 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

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

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).

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 ± 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 ± 4 min) compared to MD-1 for match one (61 ± 2 min; 95% CI = 22 to 32 min; $P<0.01$) and MD-1 for match two (63 ± 7 min; 95% CI = 17 to 34 min; $P<0.01$). Similarly, MD-3 training duration (89 ± 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^{-}
339 ^{1} (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^{-}
359 ^{1}.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 $g.kg^{-}$
388 $^{1}.day^{-1}$; P<0.01).

389

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

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

Factors affecting TEE and AEE

There was a significant positive relationship between 12-day TEE and body mass (r^2 = 0.56; 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 also a significant positive relationship between four-day TEE and four-day AEE (r^2 = 0.97; P<0.01). There was no significant relationship between TEE and stature (r^2 = 0.15; P>0.05). Data are presented in Figure 6.

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 ± 444 $kcal.day^{-1}$ (13), 2226 ± 368 $kcal.day^{-1}$ (39) and 2387 ± 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 ± 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 ± 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

target education and behaviour change strategies on "fuelling" for match play and training to optimise both player health and performance. Based on our assessment of TEE, it is suggested that relative intakes of CHO, fat and protein corresponding to 4-8 (to account for rest-days, 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 provide a reasonable starting point for which to meet the daily energy requirements of female soccer players of professional standard.

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

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

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

Acknowledgements

This research was funded by The Football Association. The authors would like to thank all of the players for their commitment during this study.

Conflict of interest

The authors report no potential conflict of interest. The results of the present study do not constitute endorsement by ACSM. All results presented here are done so clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Table 1. Baseline player characteristics of elite English female soccer players competing at international level. Stature, body mass, fat-free mass, fat mass and percent body fat values are presented according to playing position. Stature and body mass n=24. Fat-free mass, fat mass, percent body fat, bone mineral content, bone mineral density, pelvis bone mineral density, Z-score derived from DXA n=18. Predicted resting metabolic rate (RMR) n=18. Predicted RMR = 120.81 + (4.88xStature[cm]) + 8.24xFFM[kg]) + (5.71xAge[years]) (25).

Position	**Goalkeepers**	**Defenders**	**Midfielders**	**Attackers**	**Squad**
Stature (cm)	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)
Body Mass (kg)	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)
Fat-Free Mass (kg)	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)
Fat Mass (kg)	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)
Percent Body Fat (%)	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)
Whole Body Bone Mineral Content (g)	2808 ± 361 (n=3)	2837 ± 158 (n=6)	2803 ± 236 (n=4)	2637 ± 165 (n=5)	2766 ± 213 (n=18)
Whole Body Bone Mineral Density (g/cm^2)	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)
Pelvis Bone Mineral Density (g/cm^2)	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)
Whole Body Z-score	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)
Predicted RMR (kcal.day^{-1})	1549 ± 56 (n=3)	1515 ± 71 (n=6)	1494 ± 95 (n=4)	1449 ± 46 (n=5)	1486 ± 66 (n=18)

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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716
717
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. [a] denotes
722 significant difference from MD-4, P<0.05. [b] denotes significant difference from MD-3, P<0.05.
723 [c] denotes significant difference from MD-1 prior to match 1, P<0.05. [d] denotes significant
724 difference from MD one, P<0.05. [f] 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. [a] denotes significant difference from MD-4, [b] denotes
737 significant difference from MD-3, [c] denotes significant difference from MD-2, [d] denotes
738 significance difference from MD-1

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

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