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1 **ENERGY EXPENDITURE OF INTERNATIONAL FEMALE RUGBY UNION PLAYERS**
2 **DURING A MAJOR INTERNATIONAL TOURNAMENT: A DOUBLY LABELLED WATER**
3 **STUDY**

4
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35 **ABSTRACT**

36 The purpose of this study was to quantify the total energy expenditure (TEE) of international
37 female rugby union players. Fifteen players were assessed over 14-days throughout an
38 international multi-game tournament, which represented two consecutive one-match
39 microcycles. Resting metabolic rate (RMR) and TEE were assessed by indirect calorimetry
40 and doubly labelled water, respectively. Physical activity level (PAL) was estimated
41 (TEE:RMR). Mean RMR, TEE, and PAL were 6.60 ± 0.93 MJ-day⁻¹ (1578 ± 223 kcal-day⁻¹),
42 13.51 ± 2.28 MJ-day⁻¹ (3229 ± 545 kcal-day⁻¹), and 2.0 ± 0.3 AU, respectively. There was no
43 difference in TEE (13.74 ± 2.31 [3284 ± 554 kcal-day⁻¹] vs. 13.92 ± 2.10 MJ-day⁻¹ [3327 ± 502
44 kcal-day⁻¹]; $p = 0.754$), or PAL (2.06 ± 0.26 AU vs. 2.09 ± 0.23 AU; $p = 0.735$) across
45 microcycles, despite substantial decreases in training load (total distance: -8088 m, collisions:
46 -20 n, training duration: -252 min). After correcting for body composition, there was no
47 difference in TEE (13.80 ± 1.74 [3298 ± 416 *adj.* kcal-day⁻¹] vs. 13.16 ± 1.97 [3145 ± 471 *adj.*
48 kcal-day⁻¹] *adj.* MJ-day⁻¹, $p = 0.190$), RMR (6.49 ± 0.81 [1551 ± 194 *adj.* kcal-day⁻¹] vs. $6.73 \pm$
49 0.83 [1609 ± 198 *adj.* kcal-day⁻¹] *adj.* MJ-day⁻¹, $p = 0.633$) or PAL (2.15 ± 0.14 vs 1.87 ± 0.26
50 AU, $p = 0.090$) between forwards and backs. For an injured participant ($n = 1$), TEE reduced
51 by 1.7 MJ-day⁻¹ (-401 kcal-day⁻¹) from pre-injury. For participants with illness ($n = 3$), TEE was
52 similar to pre-illness ($+0.49$ MJ-day⁻¹ [$+117$ kcal-day⁻¹]). The energy requirements of
53 international female rugby players were consistent across one-match microcycles. Forwards
54 and backs had similar *adjusted* energy requirements. These findings are critical to inform the
55 dietary guidance provided to female rugby players.

56 **KEYWORDS**

57 Energy expenditure, female athletes, team sport, sports nutrition, injury, illness

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62 **INTRODUCTION**

63

64 To enhance the health and performance outcomes of female rugby union players, it is critical
65 to accurately determine their energy needs. Rugby union is an intermittent team sport,
66 characterised by periods of high intensity running and collision events, such as tackles,
67 scrums, rucks, and mauls (Nolan et al., 2023; Suarez-Arrones et al., 2014; Woodhouse et al.,
68 2021; Hughes et al., 2017). Players are categorised primarily by two playing positions,
69 forwards, and backs. Forwards typically have greater collision involvement during match-play
70 (Woodhouse et al., 2021; Nolan et al., 2023), and are generally taller (+8.6 cm), heavier (+20.5
71 kg) and possess more fat-free mass (+10.6 kg) (Posthumus et al., 2020). Whereas the backs
72 predominantly perform more running and sprinting efforts (Suarez-Arrones et al., 2014;
73 Woodhouse et al., 2021; Nolan et al., 2023). Collision-based activities during training and
74 match-play are associated with muscle damage (Naughton et al., 2018), muscle soreness
75 (Fletcher et al., 2016), and high energy costs (Costello et al., 2018; Hudson et al., 2019;
76 Naughton et al., 2018). To develop optimal nutritional strategies which promote fuelling and
77 recovery from such demands, it is essential to accurately establish the total energy
78 expenditures (TEE) of female rugby union players. Considering the distinct anthropometric
79 and sport profiles of forwards and backs, investigating whether there are differential energy
80 requirements between these positional groups is also necessary.

81 Despite the availability of high-quality research on the energy needs of male rugby players,
82 there remains a critical knowledge gap concerning female rugby players in the literature.
83 Recent studies have quantified TEE among female university rugby players (9.56 ± 0.7
84 MJ.day⁻¹ [2286 ± 168 kcal.day⁻¹]) (Traversa et al., 2022) and international seven's players

85 (14.6 ± 1.6 MJ.day⁻¹ [3490 ± 382 kcal.day⁻¹]) (Curtis et al., 2023). However, these studies are
86 limited by the use of indirect assessment methods, which could not be worn during
87 competition, and may not account for non-training activities and the energy cost of muscle
88 recovery (Costello et al., 2018; Hudson et al., 2019; Costello et al., 2022). In contrast, in-
89 season energy expenditures measured using the gold standard doubly labelled water (DLW)
90 method, have been reported for senior male rugby league players (22.5 ± 2.7 MJ.day⁻¹ [5378
91 ± 645 kcal.day⁻¹]) (Morehen et al., 2016), as well as for young male rugby league (18.28 ± 4.1
92 MJ.day⁻¹ [4369 ± 979 kcal.day⁻¹]) and union (18.26 ± 4.69 MJ.day⁻¹ [4365 ± 1122 kcal.day-
93 1]) players (Smith et al., 2018). Additionally, pre-season TEE data is available for young male
94 rugby league players (18.36 ± 3.05 MJ.day⁻¹ [4388 ± 729 kcal.day⁻¹]) (Costello et al., 2019).
95 However, the application of these findings is limited by the anthropometric and physiological
96 differences between the sexes (e.g., with regard to reproductive endocrinology) (Brazier et al.,
97 2020; Yao et al., 2021; Hackney et al., 2019; Wohlgemuth et al., 2021), along with differences
98 in match demands (Woodhouse et al., 2021) and training schedules (Hackney et al., 2019;
99 Wohlgemuth et al., 2021). Thus, specific research is required to accurately determine the
100 energy requirements of female rugby players, including during match-play.

101 The expansion of female rugby participation and the rise in professionalism (World Rugby,
102 2022) underscores the importance of quantifying TEE to establish a robust evidence-base
103 for informing dietary recommendations (Holtzman and Ackerman, 2021). This is of particular
104 interest given recent reports documenting a high prevalence (47%) of female rugby players
105 are at risk of low energy availability (LEA; <30 Kcal·kg⁻¹ fat free mass (FFM) per day) (O'Neill
106 et al., 2022). High incidence (23-88%) of LEA among other male and female team sport
107 athletes is also well documented (Dobrowolski and Wlodarek, 2020; Magee et al., 2020;
108 Moss et al., 2021; Morehen et al., 2021; Tokuyama et al., 2021). A common explanation for
109 LEA is the unintentional mismatch of energy intakes to expenditure, which may highlight a lack
110 of knowledge regarding individual energy requirements (Mountjoy et al., 2018). Energy deficits
111 of -47% and -50% have been observed throughout training and competition periods in

112 international female rugby seven players, with accompanying decreases in body mass (Curtis
113 et al., 2023). Unchecked energy deficits can lead to problematic LEA, adversely affecting
114 training and performance, and may result in health-related consequences associated with
115 relative energy deficiency in sport (REDs), including menstrual dysfunction, decreased bone
116 density, endocrine and metabolic disturbances, and increased illness and injury prevalence
117 (Mountjoy et al., 2018; Areta et al., 2021; Mountjoy et al., 2023). These findings reaffirm the
118 importance of accurately quantifying energy requirements in female players.

119

120 Accordingly, the aim of this study was to establish the TEE of international female rugby union
121 players by DLW, during an in-season period inclusive of competitive match-play. A secondary
122 aim was to establish players resting metabolic rate (RMR) and physical activity levels (PAL),
123 while investigating any differences between forwards and backs.

124

125 **MATERIALS AND METHODS**

126 **Study design and participants**

127 Fifteen international female RU players, from the same team, were purposefully recruited from
128 each playing position, to participate in a 14-day cross-sectional study during the Women's Six
129 Nations Championship, 2022. Eligibility criteria included internationally capped, free from
130 illness and injury and > 18 years. The assessment period was split into two 7-day one-match
131 microcycles, and included eight training days, three rest days, one travel day, and two match
132 days (Table 1). All participants were selected to play in international matches on days 3 (home
133 game) and 10 (away game). Participants completed coach prescribed training. On day three
134 of the assessment period, one participant sustained an anterior cruciate ligament (ACL) injury.
135 On day four, three participants tested positive for Covid-19. Participants with injury and illness
136 completed all research testing procedures and prescribed protocols. Specifically, the injured
137 participant completed off-feet conditioning and upper body gym-based training. Participants
138 with Covid-19, isolated at home, and did not return to camp until returning a negative
139 polymerase chain reaction (PCR) test on days 9 and 12. Whilst isolating, these participants

140 completed home-based loads inclusive of walking, gentle jogging, and circuit training. Table 1
141 provides an overview of the training and match schedule and details home-based loads
142 completed away from the training environment by each participant.

143 Total energy expenditure was measured by DLW. Resting metabolic rate was measured by
144 indirect calorimetry. Body composition and water turnover was determined by the deuterium
145 isotope dilution approach. Internal training load, including home-based loads, was assessed
146 by sessional ratings of perceived exertion (sRPE). External load was assessed by global
147 position system (GPS) and notational analysis. Training loads were quantified for all pitch-
148 based training sessions and matches. All testing took place within the team's accommodation
149 and training facilities. Ethics approval was provided by Leeds Beckett University (100577).
150 Participants provided informed consent before participating in the study and consented to the
151 sharing of individual data. Participants baseline characteristics are presented in Table 2.

152

153 *****INSERT TABLE 1 NEAR HERE*****

154 *****INSERT TABLE 2 NEAR HERE*****

155

156 **Body mass & stature**

157 Body mass (BM) and stature (SECA, Birmingham, United Kingdom) were measured on the
158 morning of day 0, following an ≥ 8 hour overnight fast and the removal of heavy clothing
159 (nearest 0.1 cm and 0.1 kg) by an ISAK (the International Society for the Advancement of
160 Kinanthropometry) Level-1 accredited practitioner (Marfell-Jones et al., 2006). Thereafter, BM
161 was collected on days 1, 2, 3, 6, 7, 8, 9, 10 and 14, under the same conditions, between 07:00
162 – 09:00 (Table 1).

163

164 **Resting metabolic rate**

165 Resting metabolic rate was measured between 08:00 – 10:00 on day 0 by indirect calorimetry
166 (Cortex 3B-R3 MetaLyzer, CORTEX Biophysik GmbH, Leipzig, Germany) under a ventilated

167 hood system. Participants were assessed under standardised conditions (i.e., >8-hour
168 overnight fast, >12-hour abstention from alcohol, nicotine, and caffeine) (Compher et al.,
169 2006). Up to four assessments simultaneously took place in a quiet, dimly lit, thermoneutral
170 (20°C – 25°C) room within the teams accommodation (Compher et al., 2006). Prior to each
171 assessment, the calorimeter was calibrated to within 0.02% of two known gas concentrations
172 (15% O₂ and 5% CO₂, and ambient air (20.93% and 0.04%)). Participants laid in a comfortable
173 supine position and were instructed to stay awake (Compher et al., 2006).

174 Data were collected over a 20-minute period. The first 10-minutes of data were discarded
175 and the second 10 minutes was used to calculate RMR (Iraki et al., 2021). Ventilatory oxygen
176 (VO₂) and carbon dioxide consumption (VCO₂) were measured continuously by an online gas
177 analyser (Metalyzer 3BR3, Cortex, Leipzig, Germany), and averaged every 30 seconds to
178 remove artefacts (e.g., changes in breathing patterns). All participants had a coefficient of
179 variation of ≤ 10% for VO₂ and VCO₂ (7.0% ± 2.0% and 7.3% ± 1.6%), respectively, during the
180 10-minute assessment period (*Supplementary material, resource 1*). Data were exported into
181 Microsoft Excel (2019, Seattle, USA) for substrate oxidation rate calculations (Frayn, 1983).
182 Subsequently, energy expenditure was estimated using the Frayn equation (Southgate and
183 Durnin, 1970).

184

185 **Doubly labelled water**

186 *Total energy expenditure*

187 Total energy expenditure was measured using the DLW technique (Speakman, 1997). A
188 single bolus dose consisting of deuterium (²H) and oxygen (¹⁸O) stable isotopes was prepared
189 for each participant and weighed to four decimal places. Doses were calculated relative to the
190 participants BM (Schoeller et al., 1980), which were recorded two weeks prior to the
191 assessment period. Doses were approximately 5% deuterium and 10% oxygen¹⁸ and were
192 characterised to calculate the exact enrichment of the dose provided.

193 Dose administrations occurred on day 0. A baseline urine sample was provided before oral
194 DLW consumption (07:30 – 09:30). Time of dosing was recorded to the nearest minute. A
195 second urine sample was collected after 6.75 ± 1.1 hours, allowing for total body water
196 equilibrium of the isotopes (Schoeller et al., 1980). During the equilibrium period, participants
197 were rested and consumed food and fluids *ad libitum*. Thereafter, participants provided daily
198 morning urine samples (second pass of the day) for the duration of the study to determine
199 elimination rates of both isotopes via the multipoint method. Samples were collected in 35 mL
200 sample tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany) and placed in time, date, and
201 participant ID labelled zip-lock bags. On days 4, 5, 11, 12 and 13, urine samples were collected
202 remotely and stored in the home-fridges of participants, until returning to camp with their
203 samples. Urine samples were filtered in compliance with the Human Tissue Act 2004, at Leeds
204 Beckett University, England, UK. Once filtered, samples were stored at -80°C in 2 mL micro
205 tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany) until later analysis.

206 Analysis was performed using a Liquid Isotope Water Analyser (Los Gatos Research,
207 USA)(Berman et al., 2013), at the University of Aberdeen, Scotland, UK. Urine was
208 encapsulated in capillaries, then vacuum distilled (Nagy, 1983), and water from the resulting
209 distillate was used. Samples were run alongside five laboratory standards and three
210 international standards for each isotope to adjust for day-to-day variation and correction from
211 delta values to ppm. Carbon dioxide production was calculated from the isotope elimination
212 rates (k_d and k_o) and the isotope dilution spaces (N_o and N_d) using the Speakman et al. (2021)
213 two-pool equation (Speakman et al., 2021), and converted to energy expenditure using the
214 Weir equation and an estimated respiratory quotient of 0.85 (RQ) (Speakman et al., 2021),
215 given all participants consumed a mixed diet (Ainslie et al., 2003; Westerterp, 1999).

216 Total energy expenditure ($\text{MJ}\cdot\text{day}^{-1}$ [$\text{kcal}\cdot\text{day}^{-1}$]) is reported as a 14-day and two 7-day
217 averages. Physical activity level (PAL) was calculated by dividing TEE by measured RMR
218 ($\text{MJ}\cdot\text{day}^{-1}$ [$\text{kcal}\cdot\text{day}^{-1}$]).

219

220 *Body composition and water turnover*

221 Body composition and water turnover were measured by deuterium isotope dilution. Total body
222 water was calculated from the stable isotope dilution spaces based on the intercept of the
223 elimination plot of deuterium (Speakman et al., 2021).

224

$$225 \quad N = [(N_o/1.007) + (N_d/1.043)]/2 \quad [\text{eq. 1}]$$

226

227 Whereby, N_o is the oxygen dilution space and N_d is the deuterium dilution space (Speakman
228 et al., 2021).

229

230 Body composition was determined using a two-compartmental model of fat-mass (FM) and
231 fat-free mass (FFM) (Krumbiegel, 2010; *Introduction to body composition assessment using*
232 *the deuterium dilution technique with analysis of urine samples by isotope ratio mass*
233 *spectrometry*, 2011; Westerterp, 2018). Fat-free mass (kg) was determined by dividing total
234 body water (kg) by 0.732. Fat mass (kg) was calculated by subtracting FFM (kg) from BM (kg)
235 (Fomon et al., 1982). Body fat percentage (%) was calculated by dividing FM (kg) by BM (kg)
236 and multiplying by 100 (Withers et al., 1998).

237

238 Water turnover was calculated by multiplying the rate constant of the post-dose decline in
239 deuterium enrichment by the total water pool (Lifson and McClintock, 1966), and is reported
240 as a 14-day average.

241

242 **Quantification of training, match, and home-based loads**

243 External loads were quantified by GPS, triaxial accelerometers, and notional analysis.
244 Participants were assigned their own GPS units (Vector S7, Catapult Innovations, Melbourne
245 Australia) which were placed in their shirts on the upper back between both scapulae.
246 Variables selected for analysis were training and match duration (min), total distance (m),

247 average speed ($\text{m}\cdot\text{min}^{-1}$), high speed running (m ; $>5\text{m/s}$), and player load (AU) (Bridgeman
248 and Gill, 2021; Roe et al., 2016). Notational analysis was used to quantify collision-based
249 events. Expert analysts filmed (7 years' experience; Sony CX625 Hancam) and coded (5
250 years' experience; Hudl Sportscode) the sum of collision events (i.e., rucks, mauls, scrums,
251 tackles, and ball carries). Internal loads were assessed by sRPE. Participants reported their
252 RPE, in isolation, 30 minutes after each training session and match using a modified Borg
253 scale (Foster et al., 2001), which was multiplied by session duration to calculate the load in
254 arbitrary units (AU) (Foster et al., 2001). Participants also reported their RPE and the duration
255 of any non-prescribed activities and home-based loads, such as walking and swimming, using
256 an online form.

257

258 **Statistical analysis**

259 Statistics were conducted in SPSS (version 29; SPSS, Chicago, USA). Data are reported as
260 mean \pm standard deviation (SD). Statistical significance was set at $p < 0.05$. Participants 3, 8,
261 12 and 14 were excluded from statistical analysis due to injury and illness, respectively. Data
262 for RMR is presented as $n = 15$ as this was collected pre-injury and illness.

263

264 General linear models were used to evaluate the effect of positional group and microcycle on
265 TEE. To control for the effects of body composition, adjusted TEE (*adj.* $\text{MJ}\cdot\text{day}^{-1}$ [$\text{kcal}\cdot\text{day}^{-1}$])
266 and RMR (*adj.* $\text{MJ}\cdot\text{day}^{-1}$ [$\text{kcal}\cdot\text{day}^{-1}$]) was calculated by including FFM and FM as covariates
267 (Ravussin and Bogardus, 1989). Participants were included as a random effect and playing
268 position (i.e., forwards and backs) was included as a fixed effect. Separate models were
269 specified with microcycle as a fixed effect, with no covariates included. The normality of
270 residuals was checked through visual inspection of Q-Q plots. Tukey pairwise comparisons
271 were performed to identify significant differences ($p < 0.05$). A paired t-test was used to assess
272 changes in BM across microcycles, where BM was an average of days 0-2 and 14-15,
273 respectively. Relationships between energy expenditure, anthropometric, and load variables
274 were assessed using Pearson's correlation.

275

276 To avoid mathematical bias from composite variables (i.e., TEE relative to FFM), only absolute
277 values of TEE and FFM have been compared to the literature (Ravussin and Bogardus, 1989).

278

279 **RESULTS**

280 **Training and match load**

281 Training and match load variables for participants who were free from injury or illness (n=11)
282 are presented by microcycle and position in Figure 1A-H. Training duration (Figure 1A;
283 forwards: -222 min, $P = 0.028$; backs: -289 min, $P = 0.043$), total distance (Figure 1C; forwards:
284 -7132 m, $P = 0.001$; backs: -9235 m, $P = 0.001$), player load (Figure 1F; forwards: -602 AU,
285 $P = 0.001$; backs: -791 AU, $P = 0.001$), and sRPE (Figure 1H; forwards: -961 AU, $P = 0.001$;
286 backs: -980 AU, $P = 0.001$) were significantly lower in microcycle 2 compared to microcycle
287 1. Average speed was significantly higher in microcycle 2 compared to microcycle 1, for backs
288 (Figure 1D; +9 m/min, $P = 0.022$). All other differences across weekly microcycles were non-
289 significant ($P > 0.061$).

290 Training duration (Figure 1A; microcycle 1: +78 min, $P = 0.017$), total distance (Figure 1C;
291 microcycle 1: +5665 m, $P = 0.017$), average speed (Figure 1D; microcycle 1: 10 m/min, $P =$
292 0.001 ; microcycle 2: 14 m/min, $P = 0.005$), high-speed running (Figure 1E; microcycle 1: 1082
293 m, $P = 0.001$; microcycle 2: 593.9, $P = 0.044$), player load (Figure 1F; microcycle 1; 467 AU,
294 $P = 0.010$), was significantly greater in backs than forwards. Contact count (Figure 1G;
295 microcycle 1: 33 n, $P = 0.021$, microcycle 2; 53 n, $P = 0.019$) was significantly greater in
296 forwards than backs. All other differences between forwards and backs were non-significant
297 ($P > 0.087$).

298

299 The training load for the injured participant (n=1) was lower in all variables in microcycle 2
300 compared to microcycle 1 (training duration: -278 min, match duration: -10 min, total distance:
301 -6975 m, average speed: - 145 m/min, high-speed running: - 205 m, player load: -675 AU,
302 collisions: -30 n, and sRPE: -259 AU).

303

304 The training load for participants experiencing illness (n=3) was lower in microcycle 2
305 compared to microcycle 1 (training duration: -365 ± 100 min, match duration: -78 ± 3 min, total
306 distance: -9586 ± 1939 m, high-speed running: -367 ± 299 m, player load: -909 ± 149 AU,
307 collisions: -63 ± 16 n, and sRPE: -657 ± 373 AU). Average speed was higher in microcycle 2
308 (30 ± 7 m/min).

309

310

*****INSERT FIGURE 1 NEAR HERE*****

311

312

313 **Resting and total energy expenditure**

314 **Participants free from injury and illness**

315 Mean 14-day TEE, RMR and PAL for participants who were free from injury or illness (n=11)
316 was 13.51 ± 2.28 MJ-day⁻¹ (range, 9.10 – 16.12 MJ-day⁻¹ [3229 ± 545 kcal-day⁻¹]), 6.60 ± 0.93
317 MJ-day⁻¹ (range, 5.27-7.72 MJ-day⁻¹ [1578 ± 223 kcal-day⁻¹]), and 2.0 ± 0.2 AU (range, 1.6 –
318 2.3). Mean 14-day water turnover (n=11) was 4.1 ± 0.8 L-day⁻¹ (range; 2.7 – 5.2 L-day⁻¹).

319

320 There was no significant difference in TEE (13.74 ± 2.32 MJ-day⁻¹ [3284 ± 554 kcal-day⁻¹] vs.
321 13.92 ± 2.10 MJ-day⁻¹ [3327 ± 502 kcal-day⁻¹]; $p = 0.754$), or PAL (2.06 ± 0.26 AU vs. $2.09 \pm$
322 0.23 AU; $p = 0.735$) across weekly microcycles. There was no significant change in BM across
323 the 14-days (0.4 ± 0.7 kg; $p = 0.101$).

324

325 Forwards had a significantly greater 14-day TEE than backs (14.89 ± 1.45 MJ-day⁻¹ [$3560 \pm$
326 346 kcal-day⁻¹] vs. 11.85 ± 2.02 MJ-day⁻¹ [2832 ± 483 kcal-day⁻¹]; $p = 0.025$). There was no
327 significant difference in RMR (6.90 ± 0.56 MJ-day⁻¹ [1660 ± 155 kcal-day⁻¹] vs. 6.26 ± 1.20
328 MJ-day⁻¹ [1496 ± 287 kcal-day⁻¹]; $p = 0.233$) or PAL (2.15 ± 0.14 AU vs 1.87 ± 0.26 AU; $p =$
329 0.090) between forwards and backs. When adjusted for body composition (FFM and FM),
330 there was no significant difference in TEE (13.80 ± 1.74 *adj.* MJ-day⁻¹ [3298 ± 416 *adj.* kcal-day⁻¹]

331 1] vs. 13.16 ± 1.97 *adj.* MJ·day⁻¹ [3145 ± 471 *adj.* kcal·day⁻¹]; $p = 0.628$) or RMR (6.49 ± 0.81
332 *adj.* MJ·day⁻¹ [1551 ± 194 *adj.* kcal·day⁻¹] vs. 6.73 ± 0.83 *adj.* MJ·day⁻¹ [1609 ± 198 *adj.* kcal·day⁻¹];
333 $p = 0.633$) between forwards and backs. Figure 2A-B shows TEE by positional group for
334 each microcycle.

335

336 *****INSERT FIGURE 2 NEAR HERE*****

337

338 **Participants who had injury and illness**

339 The 14-day TEE of the injured participant was 13.61 MJ·day⁻¹ (3253 kcal·day⁻¹). Total energy
340 expenditure was 14.55 MJ·day⁻¹ (3478 kcal·day⁻¹) and 12.85 MJ·day⁻¹ (3071 kcal·day⁻¹) during
341 microcycle 1 (when the participant sustained the injury) vs. microcycle 2 (when they were
342 injured). The corresponding PAL was 2.14 and 1.89 AU. Mean 14-day water turnover was 4.3
343 L·day⁻¹.

344 The 14-day TEE of participants with COVID-19 was 13.0 ± 2.74 MJ·day⁻¹ (3107 ± 655 kcal·day⁻¹).
345 Total energy expenditure was 13.17 ± 2.28 MJ·day⁻¹ (3148 ± 545 kcal·day⁻¹) and $13.66 \pm$
346 3.13 MJ·day⁻¹ (3265 ± 748 kcal·day⁻¹) during microcycle 1 (when participants tested positive
347 for COVID-19) vs. microcycle 2 (when participants were isolating), respectively. The
348 corresponding PAL was 2.15 ± 0.22 and 2.21 ± 0.09 AU. Mean 14-day water turnover was
349 3.6 ± 1.1 L·day⁻¹ (range; $2.7 - 4.9$ L·day⁻¹).

350

351 **Factors affecting energy expenditure**

352 A correlation matrix has been provided in *supplementary material, resource 2*. Individual data
353 are reported in Figure 3.

354

355 *****INSERT FIGURE 3 NEAR HERE*****

356

357 **DISCUSSION**

358 Practitioners working with female rugby union players require a high-quality evidence-base to
359 support athlete health and performance. Therefore, this study utilised gold-standard methods
360 to measure the resting and total energy expenditures of international female rugby union
361 players across an in-season period inclusive of competitive match-play. Female rugby union
362 players have energy requirements representative of a vigorously active lifestyle. For
363 participants who were free from injury and illness, there was no difference in TEE across the
364 two one-match microcycles, despite a substantial decrease in load across the second
365 microcycle. Despite insignificant differences in RMR and PAL between forwards and backs,
366 forwards had significantly greater TEEs. However, when adjusted for differences in FM and
367 FFM, TEEs were non-significant between positional groups. For participants who had an injury
368 or illness, there was a decrease and no change in TEE across microcycles, respectively.
369 These findings are critical to ensuring the provision of evidence-based dietary guidance to
370 female rugby players.

371

372 In this study, female rugby union players exhibited energy needs indicative of high activity
373 levels (>2.0 AU) (Westerterp, 2013), with a RMR similar to that of sub-elite and elite
374 counterparts ($6.91 \pm 0.7 \text{ MJ}\cdot\text{day}^{-1}$ [$1651 \pm 167 \text{ kcal}\cdot\text{day}^{-1}$]) (O'Neill et al., 2022), but less than
375 adolescent males (Smith et al., 2018; Costello et al., 2019), likely due to males' greater BM
376 and FFM. We report higher reported TEEs than that of university female players ($+3.9 \text{ MJ}\cdot\text{day}^{-1}$
377 [$932 \text{ kcal}\cdot\text{day}^{-1}$]) (Traversa et al., 2022), but lower than female rugby seven's players ($-1.0 -$
378 $2.0 \text{ MJ}\cdot\text{day}^{-1}$ [$237 - 476 \text{ kcal}\cdot\text{day}^{-1}$]) (Curtis et al., 2023), potentially reflecting differences in
379 training intensity and frequency. However, the indirect assessment methods used by Traversa
380 et al. (Traversa et al., 2022) and Curtis et al. (Curtis et al., 2023) may have resulted in
381 underestimated TEEs. When compared to male adolescents (pre-season; $-4.9 \text{ MJ}\cdot\text{day}^{-1}$ [$-$
382 $1171 \text{ kcal}\cdot\text{day}^{-1}$])(Costello et al., 2019), (in-season; $-4.8 \text{ MJ}\cdot\text{day}^{-1}$ [$-1147 \text{ kcal}\cdot\text{day}^{-1}$])(Smith et
383 al., 2018), and male senior (in-season; $-9 \text{ MJ}\cdot\text{day}^{-1}$ [$-2151 \text{ kcal}\cdot\text{day}^{-1}$])(Morehen et al., 2016)
384 rugby players, female players have lower TEEs but similar physical activity levels (1.4 – 2.0

385 AU). These findings suggest that the higher energy expenditures in males could be attributed
386 to their greater FFM and RMR.

387

388 Compared to other female team-sport athletes, we report higher (~ 2.2 MJ \cdot day $^{-1}$ [526 kcal \cdot day $^{-1}$])
389 TEEs than in-season values for international female soccer players measured by DLW
390 (Morehen et al., 2021). Despite comparable PALs (1.4–2.2), differences in absolute TEEs may
391 be explained by the greater levels of FFM possessed by female rugby players (+15.7 kg),
392 alongside the recovery costs of a collision-based sport. On the contrary, reported TEEs are
393 lower (~ 1.12 MJ \cdot day $^{-1}$ [268 kcal \cdot day $^{-1}$]) than those observed in elite junior female basketball
394 players (Silva et al., 2013). Although female rugby players have a greater BM, FFM, and RMR
395 (+12.2 kg, 8 kg and 286 kcal \cdot day $^{-1}$, respectively), female basketball players had a more
396 condensed training schedule, reflected by a high PAL (2.6 AU), which may account for their
397 greater overall TEEs.

398

399 Mean TEEs were similar across the two one-match microcycles, suggesting that player energy
400 requirements may not differ, despite differences in training load (Figure 1). The collision-
401 induced muscle damage sustained on MD (microcycle 1), could have accounted for the similar
402 TEEs observed in microcycle two ($+0.18$ MJ \cdot day $^{-1}$ [+43 kcal \cdot day $^{-1}$]) (Costello et al., 2018;
403 Hudson et al., 2019), despite participants completing less overall load (total distance: -8088
404 m, collisions: -20 n, training duration: -252 min, and sRPE: -969 AU). Muscle damage has
405 been shown to disrupt homeostasis by initiating biochemical, endocrine (McLellan et al.,
406 2011), and neuromuscular responses (McLellan and Lovell, 2012), which can remain elevated
407 for 2-5 days following collision activity (Smart et al., 2008; Cunniffe et al., 2010). Such
408 responses may have large energy costs due to the associated requirements of recovery (i.e.,
409 increased protein turnover) (Peake et al., 2017). Match-day collisions have been associated
410 with increases in RMR (0.97 MJ \cdot day $^{-1}$ [231 kcal \cdot day $^{-1}$]) (Hudson et al., 2019). As such, when
411 players train less to recover from collision-based damage (as evidenced by the removal of a

412 training session in microcycle two by the coaching team (Table 1)), it appears that their TEEs
413 may remain elevated due to the energy cost of recovery from such damage. This is supported
414 by comparable PALs observed across microcycles (2.06 ± 0.26 vs. 2.09 ± 0.23 AU), which
415 may indicate increases in the players RMR, although this was not re-assessed. Accordingly,
416 female rugby players (and other collision-based athletes) should consider fuelling for the
417 “muscle damage caused” alongside the kinematic “work required” (Costello et al., 2018;
418 Hudson et al., 2019).

419

420 Forwards had a greater TEE than backs, potentially due to their greater FFM and collision
421 involvement. On average, forwards expended $3.04 \text{ MJ}\cdot\text{day}^{-1}$ ($727 \text{ kcal}\cdot\text{day}^{-1}$) more than backs,
422 however, this was non-significant when adjusting for differences in body composition (FM and
423 FFM). Individuals with greater levels of FFM have been shown to have an increased capacity
424 for energy expenditure due to an increase in metabolically active tissue (Pontzer et al., 2021;
425 Gallagher et al., 1996). Moreover, training-based collisions have been shown to increase TEE
426 by $4.96 \pm 0.97 \text{ MJ}$ ($1186 \pm 232 \text{ kcal}\cdot\text{day}^{-1}$) over a five-day period (Costello et al., 2018), whilst
427 collisions during match-play are followed by increased RMR (Hudson et al., 2019). Despite
428 this, forwards have reduced running demands than backs. Therefore, the increased energy
429 cost of collisions in forwards, is potentially off set by the reduced locomotor demands
430 associated with their tactical role. Consequently, practitioners should consider individualising
431 player fuelling requirements by differences in body composition (FM and FFM) rather than
432 position.

433

434 This study provides DLW assessed TEE for a female rugby player during injury. Participant 3
435 sustained a non-collision related anterior cruciate ligament (ACL) injury on day 3 (MD) of the
436 assessment period. The participant was not immobilised, but was non-weight bearing on her
437 injured limb, resulting in reduced training loads (total distance: -6975 m, collisions: -30, training
438 duration: -278 min, and sRPE: -259 AU). Reduced load corresponded with a decrease of -
439 $1.69 \text{ MJ}\cdot\text{day}^{-1}$ ($407 \text{ kcal}\cdot\text{day}^{-1}$; -11.6%) in TEE from microcycle one. Similar absolute energy

440 requirements have also been observed in a female Super League netball player during week
441 1 of a medial collateral ligament injury ($13.82 \text{ MJ}\cdot\text{day}^{-1}$ [$3303 \text{ kcal}\cdot\text{day}^{-1}$]). However, there was
442 no accompanying decrease in TEE from pre-injury ($+0.85 \text{ MJ}\cdot\text{day}^{-1}$ [$203 \text{ kcal}\cdot\text{day}^{-1}$]) (Costello
443 et al., *unpublished observations*). Until further data is available, injured players should be
444 supported in line with their specific level of immobilization and rehabilitation, with a focus on
445 lean mass maintenance (Rollo et al., 2021). Further research is required to support
446 practitioners working with female athletes through injury.

447

448 Total energy expenditure is also presented for three players during a period of illness.
449 Participants 8, 12 and 14 tested positive for Covid-19 on day 4 (MD+1) and were required to
450 isolate until returning a negative PCR test on days 13, 10, and 13, respectively. Despite
451 reduced loads, mean TEE was similar across microcycles for these participants. These
452 findings may reflect increased metabolic demands associated with illness (Cicchella et al.,
453 2021). For example, prolonged and progressive increases in RMR have been observed via
454 indirect calorimetry in patients with Covid-19 ($+0.03 \text{ MJ}\cdot\text{kg}\cdot\text{BM}\cdot\text{day}^{-1}$ [$7.2 \text{ kcal}\cdot\text{kg}\cdot\text{BM}\cdot\text{day}^{-1}$])
455 (Niederer et al., 2021). However, these data were collected on critically ill patients in the
456 intensive care unit. An alternative hypothesis is that the comparable TEEs observed across
457 microcycles are a consequence of the elevated energy cost of recovery from damage accrued
458 during MD, as all participants played the full 80 minutes. Consequently, players should
459 maintain energy intakes during bouts of illness, especially when recovering from previously
460 high training and match loads.

461

462 **Study limitations**

463 This study is limited by the inability of the DLW technique to report day-to-day variations in
464 TEE. Therefore, recommendations for daily energy intake could not be presented. In addition,
465 data are collected from a relatively small sample of players from one international team,
466 reducing the generalisability to the wider population, although this sample is similar to other
467 studies. Case examples are presented for specific episodes of illness and injury, however

468 metabolic responses may be different depending on each presentation of injury and illness.
469 This study is strengthened by employing high-quality methods in an under-represented
470 sample of female athletes taking part in international competition. Future research should
471 investigate how energy requirements change day-to-day, alongside periods of reduced load
472 (e.g., substitution, injury, and illness).

473

474 **CONCLUSION**

475 This study provides the first gold standard assessed TEE data for female rugby union players.
476 Energy requirements were repeatable across one match microcycles, despite large reductions
477 in training load. Forwards have significantly greater energy requirements compared to backs,
478 however, when adjusted for differences in FFM and FM, TEEs were comparable. These
479 findings suggest that fuelling strategies should be consistent across one-match microcycles
480 and individualised by player body composition as opposed to positional groups. Practitioners
481 should consider the increased energy demands associated with recovery from the collision
482 nature of the sport. Injury resulted in a decrease in energy expenditure, in line with reduced
483 training loads. There was no change in energy requirements in players with illness. These data
484 now provide a much-needed foundation to develop strategies which serve to protect female
485 rugby players health and optimise their performance.

486

487 **Practical Implications**

- 488 • Mean TEE was similar across two one-match microcycles, despite a substantial
489 reduction in training load across the second microcycle. Accordingly, female rugby
490 players should consider fuelling for the “muscle damage caused” alongside the
491 kinematic “work required”. Meanwhile, practitioners can plan for a consistent nutrition
492 service delivery and coaching of player fuelling and recovery behaviours, across one-
493 match microcycles.

- 494 • Forwards and backs had comparable TEEs when adjusted for differences in body
495 composition (FM and FFM). Consequently, practitioners should consider
496 individualising player fuelling requirements by differences in body composition (e.g.,
497 how much FFM and FM individuals have), rather than by position.
- 498 • These findings provide a foundation from which practitioners can develop evidence
499 based nutritional strategies to support female rugby players with training and match
500 demands.

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525 Author Contributions

526 LW, BJ, SB, AB and NC conceptualised the study. Data were collected and analysed by LW,
527 CH, FM, BS, JRS and SR. Data interpretation and manuscript preparation was undertaken by
528 LW, BJ and NC. All authors approved the final version of the manuscript.

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531 Competing Interests

532 LW is responsible for nutrition service delivery to the National Women's Team at the Scottish
533 Rugby Union (SRU). AB is head of athletic development at the SRU. CR and FM provide
534 sports science support to the SRU.

535 Patient Consent for Publication

536 Not applicable.

537 Patient and Public Involvement

538 Patients and/or the public were not involved in the design, conduct, reporting, of dissemination
539 of this research.

540 Ethics Approval

541 This study involved human participants and was approved by the Local Ethics Committee,
542 Leeds Beckett University (100577).

543 Provenance and Peer Review

544 Not commissioned, externally peer reviewed.

545 Equity, Diversity, and Inclusion Statement

546 To address the underrepresentation of females in sport science research, this study
547 deliberately recruited female rugby players, some of whom are members of the LGBTQIA2S+
548 community. The research team included four females and seven males, who are early career

549 (four), mid-career (three) and senior researchers (four). The effects of race/ethnicity or
550 socioeconomic status was not considered. We discuss the effect of sex on our findings.

551 **Data Availability Statement**

552 All data relevant to the study are included in the manuscript or uploaded as supplementary
553 material.

554 **Supplementary Material**

555 This content has been supplied by the author(s).

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769 **Table 1.** An overview of the assessment, training, and match schedule for the fourteen-day observational period.

		MICROCYCLE 1							MICROCYCLE 2							
		WEDNESDAY DAY 0	THURSDAY DAY 1	FRIDAY DAY 2	SATURDAY DAY 3	SUNDAY DAY 4	MONDAY DAY 5	TUESDAY DAY 6	WEDNESDAY DAY 7	THURSDAY DAY 8	FRIDAY DAY 9	SATURDAY DAY 10	SUNDAY DAY 11	MONDAY DAY 12	TUESDAY DAY 13	WEDNESDAY DAY 14
Team Schedule	Testing Procedures	07:00-10:00 BM, US, DLW Administration, RMR 14:00-15:30 US	07:00-09:00 BM, US GPS & Video sRPE	07:00-09:00 BM, US GPS & Video sRPE	07:00-09:00 BM, US GPS & Video sRPE	07:00-09:00 US sRPE	07:00-09:00 US sRPE	07:00-09:00 US GPS & Video sRPE	07:00-09:00 BM, US GPS & Video sRPE	07:00-09:00 BM, US sRPE	07:00-09:00 BM, US GPS & Video sRPE	07:00-09:00 US sRPE	07:00-09:00 US sRPE	07:00-09:00 US sRPE	07:00-09:00 US sRPE	07:00-09:00 BM, US GPS & Video sRPE
	Training & Match	Rest	11:00 Pitch S (Speed) 15:00 Gym	11:00 CR (Clarity)	12:00 Match	Rest	Gym - Remote	15:00 PS (Clarity)	11:00 PS (Physical) 14:30 PS (Units)	12:45 Travel (Air)	11:00 Walk 14:00 CR (Clarity)	16:45 Match	Rest	Gym - Remote	Rest *PS- Removed	11:00 PS (Physical) 14:30 PS (Units)
Individual Player Schedules	P1 (FR)	Rest	Rest			11:00 Walk (20)	07:30 Gym (40)			17:20 Mobility (15)			Rest	07:30 Gym (40)	Rest	
	P2 (FR)	16:00 Swim (30)	14:45 Mobility (45)			12:00 Walk (120)	12:00 Walk (60) 15:00 Gym (45)			15:30 Walk (45)			17:30 Walk (60)	12:00 Walk (100) 17:00 Gym (60)	Rest	
	*P3 (FR)	16:00 Swim (30)			INJ	Rest	16:30 Gym (60)	Rest	Rest	16:00 Upper Gym (60) 19:00 Arms Assault Bike (25)	Rest	Rest	Rest	15:30 Upper Gym (60) 17:45 Seated Ski Erg (25)	07:45 Seated Ski Erg (45) 19:00 Upper Body Gym (45)	Rest
	P4 (SR)	16:30 Walk (30)				11:30 Walk (15)	16:15 Gym (60)			16:00 Walk (20)			12:00 Walk (30)	12:00 Gym (60)	12:00 Walk (20)	
	P5 (SR)	Rest				12:00 Walk (30)	07:00 Gym (60)			17:00 Walk (40)			Rest	07:00 Gym (50) 12:00 Walk (30)	Rest	
	P6 (BR)	Rest				11:30 Walk (30)	10:00 Walk (60) 17:00 Gym (60)			17:00 Walk (30)			Rest	12:00 Gym (60)	Rest	
	P7 (BR)	16:30 Walk (25)				12:00 Walk (30)	10:15 Gym (45)			16:00 Walk (40)			11:45 Walk (100)	13:30 Walk (30) 14:15 Gym (50)		
	**P8 (BR)	16:00 Swim (20)				Positive PCR	Rest	Rest	Rest	15:00 Run (20)	14:00 Circuit Training (30)	18:00 Run (30)	17:00 Walk (20)	16:00 Gym (60) Negative PCR	09:00 Watt Bike & Core (30) 10:00 Walk (30)	
	P9 (SH)	16:00 Walk (30)				12:00 Walk (60)	12:00 Walk (30) 17:15 Gym (60)			16:30 Walk (30)			11:00 Walk (45)	14:00 Gym (80) 17:00 Walk (30)	Rest	
	P10 (IB)	14:30 Walk (30)				14:00 Walk (60)	10:00 Gym (70)			16:30 Walk (30)			12:00 Walk (30)	09:00 Gym (60) 11:00 Walk (30)	Rest	
	P11 (IB)	14:30 Walk (30)				12:00 Walk (60)	10:00 Gym (75) 12:30 Walk (45)			16:30 Walk (30)			Rest	12:00 Gym (60)	Rest	
	**P12 (IB)	15:00 Walk (30)				Positive PCR	Rest	Rest	Rest	13:00 Run (20)	10:00 Run (20) Negative PCR Travel		10:30 Run (30)	07:15 Run (40)	07:30 Run (30) 11:30 Gym (60)	Rest
	P13 (OB)	Rest	14:45 Bike & Mobility (45)			Rest	10:00 Gym (90)			16:00 Walk (30)			Rest	09:00 Gym (60) 12:00 Walk (100)	Rest	
	**P14 (OB)	Rest				Positive PCR	Rest	Rest	Rest	12:40 Walk (45)	14:00 Circuit Training (20)	11:35 Walk (40)	17:25 Walk (68)	12:20 Walk (70) Negative PCR	17:00 Gym (60)	
	P15 (OB)	15:45 Swim (15)				Rest	10:00 Gym (70)			Rest			Rest	09:00 Gym (60)	Rest	

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771

772 Table highlights research testing and prescribed training and match schedules. Modified or home-based loads completed away from the training environment are detailed for
773 each participant. Number in parentheses represents duration in minutes. Four participants (P3, P8, P12 and P14) had altered schedules due to injury or illness. '*' denotes
774 injured participant. '**' denotes participants with Covid-19. Participant positions are shown as; FR, Front Row; SR, Second Row; BR, Back Row; SH, Scrum Half; IC, Inside
775 Centre and OC, Outside Centre. INJ (the participant sustained an injury). PS (pitch session). CR (captains run). RMR (resting metabolic rate). DLW (doubly labelled water). US
776 (urine sample). GPS (global positioning system). sRPE (sessional ratings of perceived exertion). PCR (polymerase chain reaction test).

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781 **Table 2.** Baseline characteristics of international female rugby union players

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	Forwards (<i>n</i> = 8)	Backs (<i>n</i> = 7)	Mean (<i>n</i> = 15)
Age (Years)	27.5 ± 2.5	26.4 ± 2.8	27.0 ± 2.6
Stature (cm)	170.2 ± 6.2	168.9 ± 5.5	169.6 ± 5.7
Body mass (kg)	81.9 ± 7.2	69.7 ± 9.9	76.2 ± 10.4
Total body water (L) ² H	45.2 ± 2	40.5 ± 4.0	43.0 ± 4.0
Fat-free mass (kg) ² H	62.0 ± 3.7	55.5 ± 5.5	58.9 ± 5.5
Fat mass (kg) ² H	19.9 ± 4.6	14.2 ± 6.4	17.2 ± 6.1
Percent body fat (%) ² H	24.1 ± 3.9	19.7 ± 6.3	22.1 ± 5.5

783 Body composition assessment technique is labelled in *italics* (deuterium (²H)).

784 **Figure Legends**

785

786 **Figure 1:** (A) Training duration (B) match duration, (C) total distance, (D) average speed, (E)
787 high speed running (> 5 m/s), (F) player load, (G) contact count and (H) sRPE for forwards
788 and backs, microcycle 1 (white bars) and microcycle 2 (grey bars). Load data is the summed
789 values for the 14-day period. Bars represent mean \pm SD (n=11) in accordance with the players
790 who attended all training and games. * $p < 0.05$. ** $p < 0.01$.

791

792 **Figure 2:** (A) Total energy expenditure (MJ.day⁻¹) and (B) *adjusted* total energy expenditure
793 (*adj.* MJ.day⁻¹), for forwards and backs, microcycle 1 (white bars) and microcycle 2 (grey bars).
794 Adjusted TEE represents TEE controlled for differences in body composition (FFM and FM).
795 Bars represent mean \pm SD (n=11) in accordance with the players who attended all training
796 and games. * $P < 0.05$.

797

798 **Figure 3:** A heat map depicting individual participant data for anthropometric, energy
799 expenditure and training load variables over the 14-day observational period. Columns
800 represent each participant and rows represent variables. All load variables refer to 14-day
801 summed values. Participant positions are shown as; FR, Front Row; SR, Second Row; BR,
802 Back Row; SH, Scrum Half; IC, Inside Centre and OC, Outside Centre.

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