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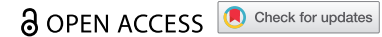


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CASE STUDY



Where do you go when your periods go?: A case-study examining secondary amenorrhea in a professional internationally-capped female soccer player through the lens of the sport nutritionist

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ABSTRACT

This case study follows a professional internationally capped female soccer player's two-year journey from eumenorrhea, through injury, to amenorrhea, and the challenges faced by the player and nutritionist. The two years are split into three sections: (Areta et al. 2013) longitudinal profiling of the player, (Baker et al. 2020) nutrition to support her return from injury, and (Beato et al. 2018) investigation into the observed secondary amenorrhea. The cause of amenorrhea was investigated through the assessment of energy availability via doubly labelled water, remote food photography, blood biomarkers and resting metabolic rate. Despite having secondary amenorrhea and anovulatory cycles, the player did not have low energy availability. This study shows the importance for practitioner's, particularly nutritionists, to not assume that all menstrual irregularities are caused by low energy availability and could be caused by a combination of factors (e.g., clinical, physiological, and psychological), which requires a multi-disciplinary investigation and intervention team. This study also showed that education needs to be provided about menstrual health to elite female soccer players as the player (i) believed that not having a period was beneficial for performance and unsure of possible health implications; (ii) was convinced that a one-day bleed indicated a regular menstrual cycle; and (iii) was reluctant to waste the practitioners time discussing menstrual issues and was nervous of finding out if she had an actual health issue. It is therefore crucial that players feel comfortable in discussing their menstrual status with practitioners to support their performance and long-term health.

KEYWORDS

Women's soccer; menstrual function; energy availability; amenorrhea; doubly labeled water

Introduction

Low energy availability (LEA) is the cornerstone of both the Female Athlete Triad (Joy et al. 2014) and Relative Energy Deficiency in Sport (RED-S) (Mountjoy et al. 2014) and results in a myriad of negative health and performance outcomes (Dipla et al. 2021, Logue et al. 2020). LEA is often identified and treated by nutritionists as they are largely responsible for an athlete's dietary energy intake (DEI). Menstruation, or rather the absence of menstruation (*i.e.*, secondary amenorrhea), is commonly used as an early warning sign for LEA, meaning that nutritionists are now routinely encountering and dealing with menstrual irregularities, often without specialist training. As such, where should athletes go when their periods go?

Methods

Athlete

The player was a Caucasian, professional, internationally capped female soccer player. She previously played soccer in international leagues, before moving to England to play in the Women Super League (WSL).

Research design

Figure 1 shows the timeline and outcomes of the study. In brief, the player was involved in a longitudinal profiling study (S1) before becoming injured [mid-substance full-thickness tear of the anterior cruciate ligament, plus a partial tear of the popliteal attachment of the posterior inferior meniscal popliteal fascicle and of the popliteofibular ligament of left knee]. Following the injury and resultant surgery [semitendinosus autograft], a nutrition intervention was undertaken to assist in recovery from surgery (S2), which led to the identification of secondary amenorrhea. An investigation into the underlying cause of the secondary amenorrhea was subsequently instigated (S3). The study ended in August 2019 when the player left the area/club, thus preventing further detailed investigation, although the player stayed in remote contact with the research team, thus facilitating additional non-invasive measures. The player read the case study and provided a written record of approval for publication. Ethical approval was granted by the Wales Research Ethics Committee [approval number: 17/WA/0228].

	S1 Original Investigation			S2 Nutrition Intervention				S3 Additional Investigation						
	Aug-17	Oct-17	Jan-18	May-18	Jun-18	Aug-18	Oct-18	Jan-19	Feb-19	Mar-19	May-19	Aug-19	Nov-19	Mar-20
Age (y)	23	24	24	24	24	24	24	25	25	25	25	25	25	26
Body mass (kg) [Figure 2 Panel A]	55.4	54.6	55.4	53.8	54.2	54.6	54.8	56.2	56.2	55	54.6	56.4		
Fat mass (g) [Figure 2 Panel B]	11.0	10.8	10.7	11.4	12.1	12.9	12.2	11.7	11.8	10.6	10.1	12.3		
Fat free mass (g) [Figure 2 Panel C]	39.5	39.1	39.8	37.5	37.1	37.1	37.7	39.8	39.7	39.7	40	39.2		
Body fat (%) [Figure 2 Panel D]	21.0	20.9	20.6	22.6	23.8	24.9	23.7	21.9	22.1	20.4	19.6	23.1		
Hip Z score	1.7	1.6		1.6		1.7	1.7	1.6						
Lumbar Z score	1.2	1.2		1.2			1.5	1.4						
Bleeding	Normally menstruating			Not menstruating				Not menstruating or sporadic 1-day of menses					3-day menses	
Ovulation								No ovulation						
Low Energy Availability in Females Questionnaire score	8	10	8	10					13					
Resting metabolic rate (kcal·d ⁻¹) [Table 1]	1450	1474	1364	1375	1474				1450					
Energy expenditure [doubly labelled water] (kcal·d ⁻¹)								2062						
Energy availability (kcal·d ⁻¹) [Loucks; Table 2]								45						
Dietary energy intake (kcal·d ⁻¹) [Table 3]	1526	1517	1574	1697					2215	2035				
Exercise duration (mins) [Table 4]	416	290	451						290					
Exercise distance (m) [Table 4]	33361	26834	29041						24186					
17 beta oestradiol (pmol·L ⁻¹) [Table 5]	464	62	244	366	268				361					
Progesterone (nmol·L ⁻¹) [Table 5]	15.5	<1	7.3	14	<1				19					
Cortisol (nmol·L ⁻¹) [Table 5]	436	476	261	284	211				382					

Figure 1. Summary of the main findings.

Nutrition intervention

The focus of the post-injury nutrition intervention was to maintain the players lean mass and improve healing time, with a long-term goal of returning the player to peak performance for the FIFA Women's World Cup in May/June 2019. Nutrition education was provided with a food plan focused on overall energy balance (2200 kcal), lower carbohydrate (<2.5 g·kg⁻¹) and higher protein (2–2.5 g·kg⁻¹) (Close et al. 2019), ingesting 25 g protein every 3 hours throughout the day (Areta et al. 2013). Supplementation included omega-3 fatty acids (*n*-3FA) (1500 mg eicosapentaenoic acid and 750 mg docosahexaenoic acid) (Marques et al. 2015), creatine (5 g·d⁻¹) (Johnston et al. 2009) and collagen (20 g·d⁻¹) (Shaw et al. 2016). The player was provided the nutrition strategy and supplements to take at home and whilst it was not possible to measure adherence the player verbally reported that she followed the protocols provided.

Research protocol

Anthropometrical assessments

Body mass was assessed in minimal clothing and without shoes (SECA, model-875, Hamburg, Germany). Body composition was assessed using whole-beam dual-energy X-ray absorptiometry (DXA) (Hologic QDR Series, Discovery A, Bedford, MA, USA). All scans were performed and analysed by the same trained operator in accordance with the best practice guidelines (Nana et al. 2016). All scans were performed at the same time of day and in a fasted and euhydrated state.

Menstrual function

Menstrual function and risk of LEA was assessed using the Low Energy Availability in Females questionnaire (LEAF-Q) (Melin et al. 2014). Menses were tracked (electronic calendar) and verbally confirmed prior to DXA scanning. Ovulation was

assessed using a urinary detection kit (Clearblue, Digital Ovulation Test, SPD, Development Company, Bedford, UK).

Resting metabolic rate (RMR)

RMR was measured at the same time of day in a fasted state having avoided strenuous exercise for at least 24 hours. The measurement was carried out via open-circuit indirect calorimetry (GEM Nutrition Ltd, UK) using a standard protocol (Bone and Burke 2018). Before starting data collection, the player relaxed for 10 minutes under a transparent ventilated hood, in a supine position, in a dark, quiet, thermoneutral room. Data were collected over a 20-minute period (2 × 10-minute duplicates). Data for the second 10-min period were used to determine RMR and analysed as previously described (Hannon et al. 2020). Based on the work done by O'Neil et al (O'Neill et al. 2022), the following equations were used to predict RMR: Cunningham (CRM) (Cunningham 1980), Ten Haaf (ten Haaf et al. 2014) and Watson (Watson et al. 2019). Ten Haaf (ten Haaf et al. 2014) was used as the primary comparative equation.

Measurement of total energy expenditure using the doubly labelled water method

Measurement of total energy expenditure was quantified using doubly labelled water (DLW) (Lifson and McClintock 1966, Speakman and Hambly 2016) over 14 days. DLW was administered using methods described previously (Hannon et al. 2020).

Energy availability (EA)

EA was calculated using the equations of Loucks, Kiens (Loucks et al. 2011).

Assessment of dietary energy intake (DEI)

The player electronically tracked dietary intake for 3 days (S1 and S2) or 14 days (S3) using the validated remote food photographic method (RFPM) (Costello et al. 2017). The player completed at

least one 24-hour dietary recall every other day (using the triple pass method) to ensure the player did not omit food/drinks and to cross-check the dietary intake information (Capling et al. 2017). Dietary intake was analysed by a British Dietetic Association and Sports and Exercise Nutrition Register (SENr) accredited dietitian using dietary analysis software (Nutritics, v5, Ireland). To ensure reliability, a second SENr accredited nutritionist independently analysed the players dietary intake (Nutritics, v5, Ireland).

Exercise training

The player's training load was monitored using global positioning system (GPS) technology (Apex, STATSports, Newry, Northern Ireland). The portable GPS unit sampled at 10 Hz (Beato et al. 2018). The training load was captured using methods previously described (Hannon et al. 2020).

Blood biomarkers

All venous blood samples were obtained by an accredited phlebotomist between 8.00 and 9.00 hours in a rested and fasted state. Blood samples were collected into vacutainers containing ethylenediaminetetraacetic acid (EDTA), lithium heparin, thixotropic gel, fluoride/oxalate, and silica and stored on ice. All samples (except full blood count) were centrifuged immediately and separated. Samples were either stored at -20°C until analysed or tested on the same day. A full blood count, electrolytes, liver function, iron profile, and endocrine panel (including luteinising hormone, follicle-stimulating hormone, prolactin, progesterone, oestradiol, testosterone, sex hormone-binding globulin,

cortisol, insulin growth factor-1 thyroxine, triiodothyronine, and thyroid stimulating hormone) were measured.

Results

Body composition

Fat-free mass decreased by 2.6 kg within three weeks of injury, returning to pre-injury levels within nine months (Figure 2).

Resting metabolic rate

The players RMR varied by 7% over the two-year period. All three prediction equations were within 10% of the measured RMR and >0.90 ratio.

Energy expenditure and availability

The mean daily energy expenditure for week 1, week 2, and over the 14-day period was 2054, 2070, and 2062 kcal $\cdot\text{d}^{-1}$. Table 1 shows the average and range of relative EA per day using the Loucks et al. (2011) equation.

Daily estimated intake

Estimated energy intake increased by 30% post intervention (February and May 2019), with the increase being made up from protein and fat intake.

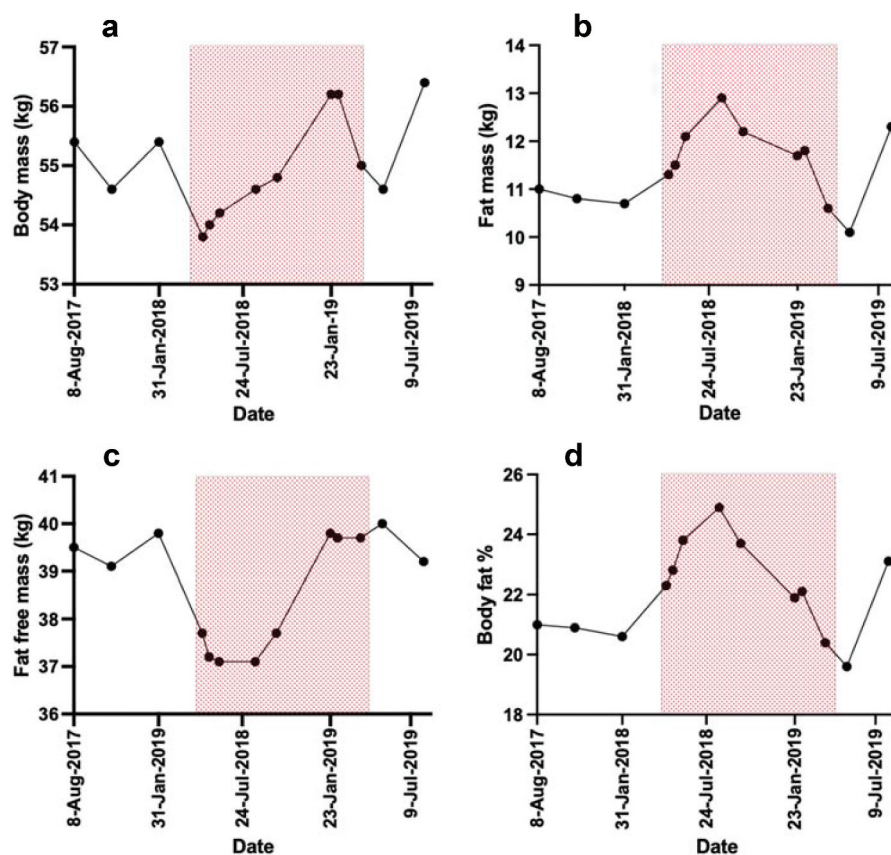


Figure 2. Changes in body mass(a), fat mass (B), fat free mass (C) and body fat percent (%) (D). The red zone indicates the time between the injury occurring to returning to full team training.

Table 1. Relative mean and per day energy availability.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean
Loucks	49	53	53	28	40	40	39	63	65	68	41	58	40	43	45

Exercise training

Table 2 provides an overview of combined absolute training and match load during data collection periods.

Biomarkers

Iron markers were out of range (low) at the second testing time-point, otherwise all markers were within the normal clinical ranges (Table 5).

Discussion

Findings

This case study provides a detailed two-year examination of a professional internationally capped female soccer player. The major finding from this study was that although the player had secondary amenorrhea and anovulatory menstrual cycles, she did not have LEA (defined as ≤ 30 kcal/kg LBM/day (Loucks et al. 2011)). This study shows that practitioners, particularly nutritionists, should not assume that menstrual irregularities are always caused by LEA, and are likely caused by a combination of factors (e.g., clinical, physiological, and psychological), which requires a multi-disciplinary investigation and intervention team.

During the initial study (S1), the player was classified as 'at risk' of LEA [LEAF-Q score ≥ 8 ; calorie intake 1517–1697 kcal-d⁻¹; EA 34 kcal·kg·d⁻¹], placing her in the subclinical EA range (Loucks et al. 2011), despite reporting regularly menstruating (Figure 1) and her blood profile supporting this (Table 5). One possible reason for this is that this level of EA can be tolerated for short periods of times without any negative outcomes (Loucks et al. 2011). The other option is that DEI was underreported, despite employing measures to reduce underreporting including dietary recalls and multiple daily reminders (Capling et al. 2017). Measuring DEI in free-living athletes is difficult and places a great burden on athletes and practitioners (Burke et al. 2018).

During the nutrition intervention (S2) the player did not menstruate (i.e., since being injured and undergoing surgery). Eight months of amenorrhea had little impact on the player's bone health (Figure 1; Z scores), which might be due to the loading nature of soccer (Baker et al. 2020) and the fact that the player was not suffering from long-

term LEA. In addition, at this time (S2 into S3), DEI increased (1526 kcal·d⁻¹ pre-injury to 2215 kcal·d⁻¹ post injury) and provided greater than 45 kcal·kg·d⁻¹ (i.e., above the threshold for LEA). In contrast, her LEAF-Q score was 13, categorising her 'at risk' of LEA. This score has its limitations as the LEAF-Q was designed as a screening tool in female endurance runners. Recent evidence suggests that it should not be used to classify 'at risk' athletes or as a surrogate diagnostic tool in soccer due to its low specificity and validity in team sports (Rogers et al. 2021).

Measurements	Aug 2017	Nov 2017	Feb 2018	Feb 19 Wk 1	Feb 19 Wk 2
Duration (mins)	416	290	451	283	298
Distance (m)	33,361	26,834	29,041	23,056	25,315
HSR (m)	792	1891	1088	431	756
ED (m)	3649	2068	3264	2899	2741

Abbreviations: HSR – high speed running; ED – explosive distance, Wk – week; Aug – August; Nov – November; Feb – February.

term LEA. In addition, at this time (S2 into S3), DEI increased (1526 kcal·d⁻¹ pre-injury to 2215 kcal·d⁻¹ post injury) and provided greater than 45 kcal·kg·d⁻¹ (i.e., above the threshold for LEA). In contrast, her LEAF-Q score was 13, categorising her 'at risk' of LEA. This score has its limitations as the LEAF-Q was designed as a screening tool in female endurance runners. Recent evidence suggests that it should not be used to classify 'at risk' athletes or as a surrogate diagnostic tool in soccer due to its low specificity and validity in team sports (Rogers et al. 2021).

During S3, the player's RMR ratio (measured vs predicted RMR >0.90) and biomarkers (LH, FSH, oestradiol, prolactin, FT3, FT4, and IGF-1) were within normal ranges (Tables 3 and 4 and Figure 1) indicating that the player was not in LEA. During this time, she transitioned from amenorrhea to anovulatory cycles with sporadic bleeding. During S3, the player participated in three matches for her country at the 2019 World Cup. After the World Cup, the player left her WSL club and the area, however, due to the continued menstrual irregularities the research team kept supporting the player. The research team had arranged for further medical investigations (e.g., polycystic ovary syndrome, hyperprolactinemia, primary ovarian insufficiency) (Elliott-Sale et al. 2021); however, the COVID-19 global pandemic prevented these tests from occurring. In November 2020, the player left England for Australia, preventing any further exploration and resolution for this case study.

Nutritionists' reflections

- (1) The player did not initially present with menstrual disturbances, meaning that the focus of this case-study changed over time; from a profiling study (S1), to a post-injury/surgery anthropometric-based nutrition intervention (S2), to a menstrual irregularities investigation (S3).

Table 3. Resting metabolic rate indirect measurements, predictions, and ratios.

RMR method	Aug 2017	Nov 2017	Feb 2018	May 2018	June 2018	Feb 2019
Indirect calorimetry	1450	1474	1364	1375	1474	1450
Watson BM	1409	1422	1429	1415	1419	1455
Cunningham	1369	1360	1376	1329	1316	1373
Ten Haaf	1384	1375	1391	1343	1329	1388
RMR ratio Watson BM	1.03	1.04	0.95	0.97	1.04	1.00
RMR ratio Cunningham	1.06	1.08	0.99	1.03	1.12	1.06
RMR ratio Ten Haaf	0.95	0.93	1.02	0.98	0.90	0.96

Abbreviations: RMR – resting metabolic rate; Aug – August; Nov – November; Feb – February, BM – body mass.

Table 4. Mean energy and macronutrient intake expressed in absolute and relative terms during a 3-day* and 14-day** data collection timescale.

	Aug 17*	Nov 17*	Feb 18*	May 18*	Feb 19**	May 19*
Energy (kcal)	1526	1517	1574	1697	2215	2035
Energy (kcal/kg FFM)	38.6	38.8	39.5	45.6	55.8	50.9
Carbohydrate (g)	197	205	201	146	251	200
Carbohydrate (g/kg)	3.6	3.8	3.6	2.7	4.5	3.7
Protein (g)	91	73	96	82	116	143
Protein (g/kg)	1.6	1.3	1.7	1.5	2.1	2.6
Fat (g)	46	45	43	87	82	70
Fat (g/kg)	0.8	0.8	0.8	1.6	1.5	1.3

Abbreviations: FFM – fat free mass; Aug – August; Nov – November; Feb – February. Numbers in italics indicate a rest day.

Table 5. Blood biomarker data over an 18-month study timescale.

Blood markers	Aug-17	Oct-17	Jan-18	May-18	Jun-18	Feb-19
Cortisol nmol·L ⁻¹	436	476	261	284	211	382
Luteinising hormone U·L ⁻¹	2.2	7	15.2	19.5	11.5	5.4
Follicle stimulating hormone U·L ⁻¹	1.7	5.4	6.8	5.2	8	4.1
17 beta oestradiol pmol·L ⁻¹	464	62	244	366	268	361
Total testosterone nmol·L ⁻¹	1.1	1.3	1	1.6	1	1.1
Sex hormone binding globulin nmol·L ⁻¹	152	31	137	145	93	136
Insulin growth factor 1 nmol·L ⁻¹	29	40	29	35	30	29
Insulin pmol·L ⁻¹	48	65	41	42	36	53
Thyroid stimulating hormone mU·L ⁻¹	3.6	1.3	2.3	2.2	2.1	3.4
Free thyroxine pmol·L ⁻¹	15.5	15.5	15.3	16.4	19.6	15.2
Free triiodothyronine pmol·L ⁻¹	5.5	4.3	5.4	4.9	5	5.1
Prolactin mU·L ⁻¹	370	329	283	317	316	343
Progesterone nmol·L ⁻¹	15.5	<1	7.3	13.7	<1	18.5
Ferritin ug·L ⁻¹	36	20	39	40	88	35
Vitamin B12 ng·L ⁻¹	436	391	537	456	661	496
Folate ug·L ⁻¹	5.1	4.5	7.9	8.3	8.7	11.9
Iron umol·L ⁻¹	13.3	6.9	25.6	24.5	21.6	16.4
Transferrin g·L ⁻¹	2.69	3.42	2.61	2.45	2.65	2.63
Total iron binding capacity umol·L ⁻¹	61	78	59	56	60	60
% Ferritin saturation	22	9	43	44	36	27
Zinc umol·L ⁻¹	14.6	18.9	17.9	23.9	17.8	13
Vit D nmol·L ⁻¹	91	75	70	84	95	59
Haemoglobin g·L ⁻¹	140	133	139	146	142	145
Haematocrit L·L ⁻¹	0.4	0.4	0.398	0.417	0.407	0.424
Magnesium nmol·L ⁻¹	0.75	0.93	0.75	0.74	0.86	0.82

Abbreviations: Aug – August; Oct – October; Jan- January; Jun – June; Feb – February. Numbers in italics and underlined indicate out of range.

- As such, nutritionists need to be adaptive to support players in a timely and dynamic fashion.
- (2) Sport nutritionists presented with a player with more than eight months without menses might default to investigating DEI-induced LEA without considering other options. The culture of soccer (and potentially other sports) was to turn to LEA as the initial cause rather than ruling out other medical issues. This can lead to costly (*i.e.*, DLW) and burdensome (*e.g.*, training and food diaries) investigations, without resolution (as in this case study). On reflection, a thorough medical assessment by other relevant domains (*e.g.*, physician, endocrinologist and gynaecologist) should have been carried out before exploring LEA in such detail. As such, nutritionists must work as part of a multi-disciplinary team, maintaining an open dialogue with players and other support staff (*e.g.*, clinicians, psychologists, and coaches) in order to gain a 360° perspective of the athlete's behaviours and health.
 - (3) Sports nutritionists and support staff need to be cautious when using or interpreting the LEAF-Q due to it being designed for female endurance runners and not being validated in female soccer players.
 - (4) Apart from iron markers, the players biomarkers were within normal clinical ranges. These clinical ranges are inflated by between-subject variability. Hecksteden and Meyer (Hecksteden and Meyer 2020) work around intraindividual analysis of fatigue markers provides an insight into the future of longitudinal biomarker interpretation. However, due to only having six time points over an eighteen-month period and with the athlete presenting with clinical symptoms, we did not feel confident interpreting the data to this level.
 - (5) Due to the nutritional intervention (S2) and DLW results (S3) the player changed her eating habits (*e.g.*, increasing total calories, protein, and fat intake and adherence

to protein shakes around training sessions) whilst reducing her fat mass and increasing her fat-free mass. This highlights the importance and impact of nutrition interventions/awareness/education in players.

- (6) The following verbatim quotes by the player highlight the need for menstrual cycle education: (i) talking about not having a period - *"its handy not having a period coming up to a World Cup", "what harm can it really do", "I can get my period back later"*; (ii) talking about her periods - *"I bled for 1 day this month again, isn't that a period"*; talking about further investigation into the cause of her menstrual disturbances - *"I'm waiting on my period at the moment. I just feel like surely my period is just a one-day bleed so don't want to waste your time", "Don't know if I'm just nervous to find out"*.
- (7) During this case study, the player sustained a career threatening injury, moved club (which involved moving away from her home and boyfriend), had a run of poor results (e.g., conceded 23 goals in three games) and travelled internationally (encountered different foods). As such, nutritionists also need to consider 'stress' and signpost athletes to appropriate resources rather than assume menstrual disturbance is always linked to LEA.

Conclusion

Players should be encouraged to discuss their menstrual status with appropriate support staff. Nutritionists should be mindful that secondary amenorrhea is not exclusively caused by LEA and should work as part of a multi-disciplinary team to support female players with menstrual disturbances.

Epilogue

Upon final drafting of the case study, we sent the final version to the player to gain permission to submit for publication. At this time, the player reported that her menstrual cycle had returned (January 2021) to that of pre-injury (April 2018) and she was ovulating (confirmed using an ovulation kit). She highlighted *'eating foods I grew up with'* and *'am much happier'* as reasons for this. The player had returned close to home, family and friends and was enjoying her football again. Therefore, the menstrual dysfunction was unlikely due to the pathology we were unable to measure and likely down to other factors such as nutrition and psychology. It also emphasises that the return of menstruation may take a prolonged period of time. As of May 2022, the player was not diagnosed with any clinical condition, is still playing topflight football with menses returning to that of pre-injury.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data that support the findings of this study are available from the corresponding author, GC upon reasonable request.

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