



LJMU Research Online

Parker, LJF, Elliott-Sale, K, Hannon, MP, Morton, JP and Close, GL

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.

<http://researchonline.ljmu.ac.uk/id/eprint/18153/>

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Parker, LJF, Elliott-Sale, K, Hannon, MP, Morton, JP and Close, GL 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. Science & medicine in football.

LJMU has developed [LJMU Research Online](#) for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

<http://researchonline.ljmu.ac.uk/>



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

Lloyd J. F. Parker, Kirsty J. Elliott-Sale, Marcus Hannon, James P. Morton & Graeme L. Close

To cite this article: Lloyd J. F. Parker, Kirsty J. Elliott-Sale, Marcus Hannon, James P. Morton & Graeme L. Close (2022): 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, *Science and Medicine in Football*, DOI: [10.1080/24733938.2022.2123555](https://doi.org/10.1080/24733938.2022.2123555)

To link to this article: <https://doi.org/10.1080/24733938.2022.2123555>



© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Accepted author version posted online: 15 Sep 2022.



Submit your article to this journal [↗](#)



View related articles [↗](#)



View Crossmark data [↗](#)

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Science and Medicine in Football*

DOI: 10.1080/24733938.2022.2123555

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

LLOYD J. F. PARKER^{1,2}, KIRSTY J. ELLIOTT-SALE², MARCUS HANNON¹, JAMES P. MORTON¹ & GRAEME L. CLOSE¹

¹Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK

²Everton Football Club, USM Finch Farm, Halewood Liverpool, UK.

³Musculoskeletal Physiology Research Group, Sport Health and Performance Enhancement Research Centre, Nottingham Trent University, Nottingham, UK.

Correspondence*:

Professor Graeme L. Close,

g.l.close@ljmu.ac.uk

Language style: British English

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: (1) longitudinal profiling of the player, (2) nutrition to support her return from injury, and (3) 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 ¹, menstrual function ², energy availability ³, amenorrhea ⁴, doubly labeled water ⁵

INTRODUCTION

Low energy availability (LEA) is the cornerstone of both the Female Athlete Triad (1) and Relative Energy Deficiency in Sport (RED-S) (2) and results in a myriad of negative health and performance outcomes (3, 4). 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].

Nutrition intervention

The focus of the post injury nutrition intervention was to maintain the player's 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 (<2.5g·kg⁻¹) and higher protein (2-2.5g·kg⁻¹) (5), ingesting ~25g protein every ~3 hours throughout the day (6). Supplementation included omega-3 fatty acids (n-3FA) (1500 mg eicosapentaenoic acid and 750 mg docosahexaenoic acid) (7), creatine (5 g·d⁻¹) (8) and collagen (20 g·d⁻¹) (9). The player was provided the nutrition strategy and supplements to take at home and whilst it wasn't 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 (10). 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) (11). Menses was 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 (12). 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 x 10-minute duplicates). Data for the second 10 minute period were used to determine RMR and analysed as previously described (13). Based on the work done by O'Neil et al. (14) the following equations were used to predict RMR: Cunningham (CRMR) (15), Ten Haaf (16) and Watson (17). Ten Haaf (16) 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) (18, 19) over 14-days. DLW was administered using methods described previously (20).

Energy availability (EA)

EA was calculated using the equations of Loucks, Kiens (21).

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; (22)). The player completed at least one 24-hour dietary recall every other day (using the triple pass method) to ensure the player didn't omit food/drinks and to cross check the dietary intake information (23) 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 (24). The training load was captured using methods previously described (20)

Blood biomarkers

All venous blood samples were obtained by an accredited phlebotomist between 8.00 – 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

[Insert Figure 1 here]

Body composition:

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

[Insert Figure 2 here]

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.

[Insert Table 1 here]

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·d⁻¹. Table 2 shows the average and range of relative EA per day using the Loucks et al. (2011) and Torstveit et al. (2018) equations.

[Insert Table 2 here]

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.

[Insert Table 3 here]

Exercise training:

[Insert Table 4 here]

Biomarkers:

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

[Insert Table 5 here]

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; (21)). 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 (21), despite reporting regularly menstruating (Figure 1) and her blood profile supporting this (Table 4). One possible reason for this is that this level of EA can be tolerated for short periods of times without any negative outcomes (21). The other option is that DEI was underreported, despite employing measures to reduce underreporting including dietary recalls and multiple daily reminders (23). Measuring DEI in free-living athletes is difficult and places a great burden on athletes and practitioners (25).

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 (26) 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 (27).

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 (Table 1, 5 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) (28), 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). 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 player's biomarkers were within normal clinical ranges. These clinical ranges are inflated by between-subject variability. Hecksteden and Meyer (29) 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.

Acknowledgements

The study was designed by LP, KES, JM and GC and data were collected and analysed by LP, KES, MH, JM and GC. The authors would like to thank Jack Clover and Matthew Taberner for their help with data collection.

Conflict of interest

LP, KES, MH, JM and GC have no conflict of interest to report.

Data availability statement

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

REFERENCES

1. Joy E, De Souza MJ, Nattiv A, Misra M, Williams NI, Mallinson RJ, et al. 2014 female athlete triad coalition consensus statement on treatment and return to play of the female athlete triad. *Curr Sports Med Rep.* 2014;13(4):219-32.
2. Mountjoy M, Sundgot-Borgen J, Burke L, Carter S, Constantini N, Lebrun C, et al. The IOC consensus statement: beyond the Female Athlete Triad--Relative Energy Deficiency in Sport (RED-S). *Br J Sports Med.* 2014;48(7):491-7.
3. Diplá K, Kraemer RR, Constantini NW, Hackney AC. Relative energy deficiency in sports (RED-S): elucidation of endocrine changes affecting the health of males and females. *Hormones (Athens).* 2021;20(1):35-47.
4. Logue DM, Madigan SM, Melin A, Delahunt E, Heinen M, Donnell SM, et al. Low Energy Availability in Athletes 2020: An Updated Narrative Review of Prevalence, Risk, Within-Day Energy Balance, Knowledge, and Impact on Sports Performance. *Nutrients.* 2020;12(3).
5. Close GL, Sale C, Baar K, Bermon S. Nutrition for the Prevention and Treatment of Injuries in Track and Field Athletes. *International Journal of Sport Nutrition and Exercise Metabolism.* 2019;29(2):189-97.
6. Areta JL, Burke LM, Ross ML, Camera DM, West DW, Broad EM, et al. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *J Physiol.* 2013;591(9):2319-31.
7. Marques CG, Santos VC, Levada-Pires AC, Jacintho TM, Gorjão R, Pithon-Curi TC, et al. Effects of DHA-rich fish oil supplementation on the lipid profile, markers of muscle damage, and neutrophil function in wheelchair basketball athletes before and after acute exercise. *Applied Physiology, Nutrition, and Metabolism.* 2015;40(6):596-604.
8. Johnston APW, Burke DG, MacNeil LG, Candow DG. Effect of Creatine Supplementation During Cast-Induced Immobilization on the Preservation of Muscle Mass, Strength, and Endurance. *The Journal of Strength & Conditioning Research.* 2009;23(1):116-20.
9. Shaw G, Lee-Barthel A, Ross ML, Wang B, Baar K. Vitamin C-enriched gelatin supplementation before intermittent activity augments collagen synthesis. *The American Journal of Clinical Nutrition.* 2016;105(1):136-43.
10. Nana A, Slater GJ, Hopkins WG, Halson SL, Martin DT, West NP, et al. Importance of Standardized DXA Protocol for Assessing Physique Changes in Athletes. *Int J Sport Nutr Exerc Metab.* 2016;26(3):259-67.
11. Melin A, Tornberg ÅB, Skouby S, Faber J, Ritz C, Sjödín A, et al. The LEAF questionnaire: a screening tool for the identification of female athletes at risk for the female athlete triad. *British Journal of Sports Medicine.* 2014;48(7):540-5.
12. Bone JL, Burke LM. No Difference in Young Adult Athletes' Resting Energy Expenditure When Measured Under Inpatient or Outpatient Conditions. *Int J Sport Nutr Exerc Metab.* 2018;28(5):464-7.
13. Hannon MP, Carney DJ, Floyd S, Parker LJF, McKeown J, Drust B, et al. Cross-sectional comparison of body composition and resting metabolic rate in Premier League academy soccer players: Implications for growth and maturation. *J Sports Sci.* 2020:1-9.
14. O'Neill J, Walsh CS, McNulty SJ, Gantly HC, Corish ME, Crognale D, et al. Resting Metabolic Rate in Female Rugby Players: Differences in Measured Versus Predicted Values. *J Strength Cond Res.* 2022;36(3):845-50.
15. Cunningham JJ. A reanalysis of the factors influencing basal metabolic rate in normal adults. *The American Journal of Clinical Nutrition.* 1980;33(11):2372-4.
16. ten Haaf T, Weijs PJ. Resting energy expenditure prediction in recreational athletes of 18-35 years: confirmation of Cunningham equation and an improved weight-based alternative. *PLoS One.* 2014;9(9):e108460.
17. Watson AD, Zabriskie HA, Witherbee KE, Sulavik A, Gieske BT, Kerksick CM. Determining a Resting Metabolic Rate Prediction Equation for Collegiate Female Athletes. *J Strength Cond Res.* 2019;33(9):2426-32.

18. Lifson N. Theory of use of the turnover rates of body water for measuring energy and material balance. *J Theor Biol.* 1966;12(1):46-74.
19. Speakman JR, Hambly C. Using doubly-labelled water to measure free-living energy expenditure: Some old things to remember and some new things to consider. *Comp Biochem Physiol A Mol Integr Physiol.* 2016;202:3-9.
20. Hannon MP, Parker LJF, Carney DJ, McKeown J, Speakman JR, Hambly C, et al. Energy Requirements of Male Academy Soccer Players from the English Premier League. *Med Sci Sports Exerc.* 2020.
21. Loucks AB, Kiens B, Wright HH. Energy availability in athletes. *J Sports Sci.* 2011;29 Suppl 1:S7-15.
22. Costello N, Deighton K, Dyson J, McKenna J, Jones B. Snap-N-Send: A valid and reliable method for assessing the energy intake of elite adolescent athletes. *European Journal of Sport Science.* 2017;17(8):1044-55.
23. Capling L, Beck K, Gifford J, Slater G, Flood V, O'Connor H. Validity of Dietary Assessment in Athletes: A Systematic Review. *Nutrients.* 2017;9(12):1313.
24. Beato M, Coratella G, Stiff A, Iacono AD. The Validity and Between-Unit Variability of GNSS Units (STATSports Apex 10 and 18 Hz) for Measuring Distance and Peak Speed in Team Sports. *Front Physiol.* 2018;9:1288.
25. Burke LM, Lundy B, Fahrenholtz IL, Melin AK. Pitfalls of Conducting and Interpreting Estimates of Energy Availability in Free-Living Athletes. *Int J Sport Nutr Exerc Metab.* 2018;28(4):350-63.
26. Baker BS, Chen Z, Larson RD, Bembien MG, Bembien DA. Sex differences in bone density, geometry, and bone strength of competitive soccer players. *J Musculoskelet Neuronal Interact.* 2020;20(1):62-76.
27. Rogers MA, Drew MK, Appaneal R, Lovell G, Lundy B, Hughes D, et al. The Utility of the Low Energy Availability in Females Questionnaire to Detect Markers Consistent With Low Energy Availability-Related Conditions in a Mixed-Sport Cohort. *Int J Sport Nutr Exerc Metab.* 2021;31(5):427-37.
28. Elliott-Sale KJ, Minahan CL, de Jonge XAKJ, Ackerman KE, Sipilä S, Constantini NW, et al. Methodological Considerations for Studies in Sport and Exercise Science with Women as Participants: A Working Guide for Standards of Practice for Research on Women. *Sports Medicine.* 2021;51(5):843-61.
29. Hecksteden A, Meyer T. Blood-borne fatigue markers during major international football tournaments – a retrospective analysis of data from the FIFA World Championships and UEFA European Championships 2006 – 2016. *Science and Medicine in Football.* 2020;4(2):135-41.

Table 1. 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.

	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

Table 2. Relative mean and per day energy availability.

Numbers *in italics* indicate a rest day

ACCEPTED MANUSCRIPT

Table 3. Mean energy and macronutrient intake expressed in absolute and relative terms during a 3-

	Aug 17*	Nov 17*	Feb 18*	May 18*	Feb 19**	May 19*	day*
Energy (kcal)	1526	1517	1574	1697	2215	2035	and
Energy (kcal/kg FFM)	38.6	38.8	39.5	45.6	55.8	50.9	14-
Carbohydrate (g)	197	205	201	146	251	200	day*
Carbohydrate (g/kg)	3.6	3.8	3.6	2.7	4.5	3.7	*
Protein (g)	91	73	96	82	116	143	data
Protein (g/kg)	1.6	1.3	1.7	1.5	2.1	2.6	colle
Fat (g)	46	45	43	87	82	70	
Fat (g/kg)	0.8	0.8	0.8	1.6	1.5	1.3	

ction timescale.

Abbreviations: FFM - fat free mass; Aug – August; Nov – November; Feb – February.

ACCEPTED MANUSCRIPT

Table 4. An overview of combined absolute training and match load during a 7-day data collection period. The first three time-points in Table 4 were undertaken with the team pre-injury, with the last two time-points undertaken post-injury as individual sessions. These data [February 2019] were

Measurements	Aug 2017	Nov 2017	Feb 2018	Feb 19 Wk 1	Feb 19 Wk 2
Duration (mins)	416	290	451	283	298
Distance (m)	33361	26834	29041	23056	25315
HSR (m)	792	1891	1088	431	756
ED (m)	3649	2068	3264	2899	2741

aligned with the DLW assessment.

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

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	<u>20</u>	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	<u>78</u>	59	56	60	60
% Ferritin saturation	22	<u>9</u>	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

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

Abbreviations: Aug – August; Oct – October; Jan- January; Jun – June; Feb – February.

Numbers *in italics* and underlined indicate out of range.

	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					

Apr-19 injury

Figure 1. Summary of the main findings.

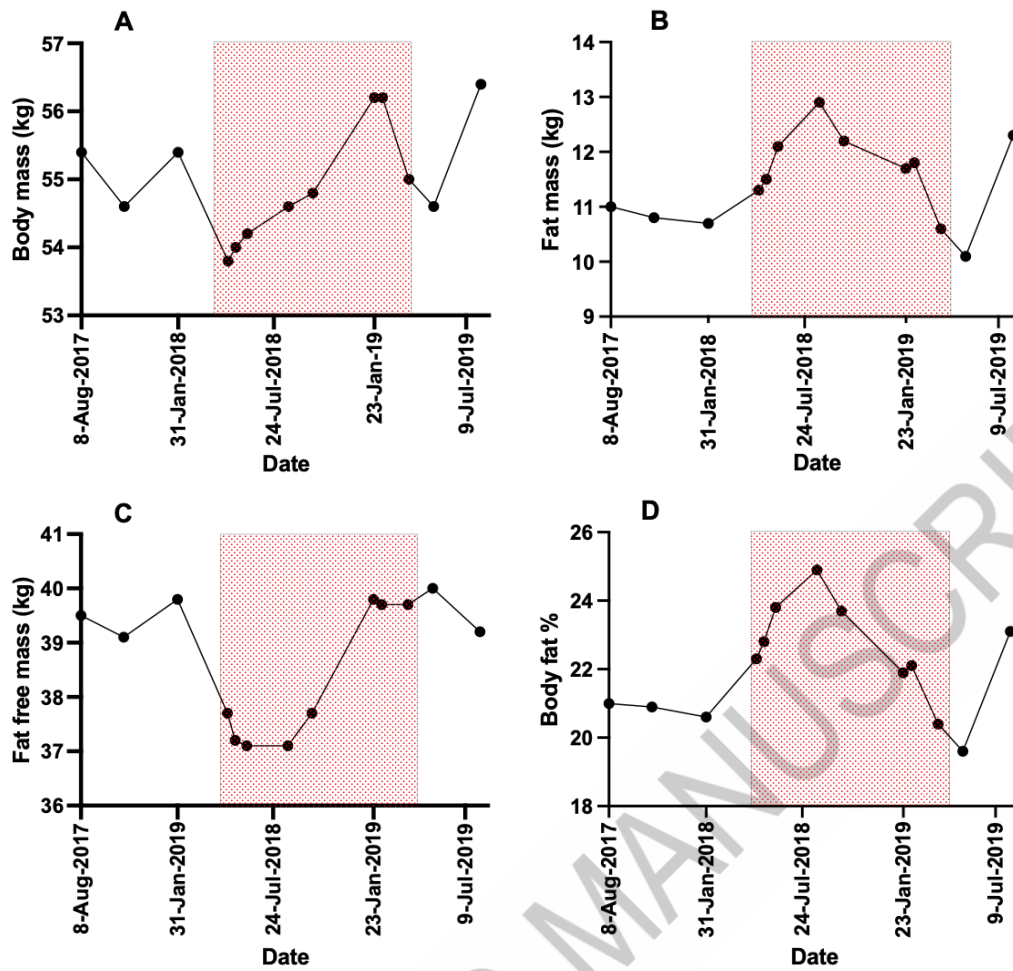


Figure 2. Changes in body mass(A), fat mass (B), fat free mass (C) and body fat percent (%) (D). The red zone indicates the time between the injury occurring to returning to full team training.