# Assessing the prevalence and factors affecting low energy availability in Women's Super League soccer players

Lloyd J. F. Parker

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# Abstract

The growth of female soccer in England has been substantial since the Football Association (FA) lifted its ban on female players using accredited facilities in 1971. The establishment of the Women's Super League (WSL) in 2010 marked a significant milestone in this growth, evolving into a fully professional league with 12 teams, known as WSL 1. Despite this progress, research in the field of female soccer, particularly concerning nutritional and menstrual health, remains relatively underdeveloped. For female soccer players it is imperative that their energy intake is aligned with their total energy requirements. If this does not occur players could suffer from low energy availability (LEA), which is the failure to provide the body with adequate energy to sustain homeostasis. Low energy availability has been shown to have detrimental health and performance outcomes including, but not limited to, altered menstrual function and poor bone health. With this in mind the aim of this thesis was to assess hormonal contraceptive use, menstrual function, energy intake, energy expenditure, blood biomarkers and body composition with a view to ascertain the prevalence and factors contributing to LEA among elite female soccer players in WSL 1.

Study 1 (Chapter 4) investigated the prevalence of hormonal contraceptive and non-hormonal contraceptive use and associated symptomatology among multiple squads in WSL 1 (N = 75). Results revealed that 28% of players reported current hormonal contraceptive use, with non-users experiencing more negative symptoms (74%) such as cramps (70%) during menstruation. Fourteen players (26%) reported menstrual dysfunction, though only one had been clinically diagnosed. These findings highlight that naturally menstruating female soccer players suffer from more negative symptoms associated with the menstrual cycle and that menstrual dysfunction could be a league wide issue.

Having quantified that 26% of players suffered from menstrual dysfunction in Study 1 (Chapter 4), Study 2 (Chapter 6) assessed resting metabolic rate (RMR), exercise energy expenditure (EEE), energy intake (EI), menstrual function (Low Energy Availability in Females Questionnaire (LEAF-Q)), blood biomarkers, and body composition during pre-season in a WSL 1 team. Despite the LEAF-Q classifying 32% of the squad being at risk of LEA, actual measurements of LEA did not support the presence of this or menstrual dysfunction. Additionally, players did not adjust their energy or carbohydrate intake based on the intensity of training or the match schedule, with all of the squad under consuming carbohydrates on

match day (3.7 ± 1.1g·kg·day$^{-1}$). Most of the squad were within normal clinical ranges for micronutrient markers, suggesting that players dietary intakes were providing sufficient nutrients.

Though none of the squad appeared to suffer from LEA or menstrual dysfunction in Study 2 (Chapter 5), this was only one time point in the season, therefore, the aim of Study 3 (Chapter 6) was a longitudinal assessment of RMR, EEE, EI, menstrual function, blood biomarkers, and body composition among elite female soccer players participating in a WSL 1 team throughout an entire season. Over the 10-month season where players were measured over four equally spaced time points, LEAF-Q continued to overestimate players at risk of LEA. Two players reported menstrual dysfunction over the season, with one player presenting with symptoms (menstrual dysfunction) and markers of suffering from LEA (energy availability of 34 kcal·kg FFM$^{-1}$·day$^{-1}$, decrease in FM, BF%, BM and RMR ratio). Players significantly periodised their energy and carbohydrate intake around training and match days, however, still under consumed carbohydrates on match days (4.5 g·kg·day$^{-1}$). Body composition notably changed over the season with a significant decrease in fat mass and increase in fat free mass, while bone health remained within normal clinical ranges throughout the season. Vitamin D concentrations significantly declined during the season, with 70% of the squad having insufficient concentrations (<75 nmol·L$^{-1}$) mid-season. Although remaining within clinical norms, ferritin concentrations dropped in 50% of players during the season, classifying them as stage 1 iron deficient according to athletic classifications (Peeling et al., 2007).

Although there was an absence of major issues related to LEA and menstrual dysfunction within the team in Studies 2 and 3 (Chapters 5 and 6), the aim of Study 4 (Chapter 7) was to provide a detailed two-year examination of one elite female soccer player suffering from secondary amenorrhea and anovulatory menstrual cycles. The player reported eight months without menstruation after suffering a serious injury and having surgical intervention. Initial indications suggested the possibility of LEA, with the player scoring 13 on the LEAF-Q. However, thorough investigations involving RMR, energy expenditure, energy intake, and biomarker analyses concluded that LEA was unlikely the cause of the player's condition. Unfortunately, due to the onset of the COVID-19 pandemic and the player relocating abroad, an official diagnosis could not be obtained. Subsequent follow-ups revealed that the player's menstrual function returned to normal, and she later became pregnant. As a result, it was concluded that despite initial suspicions of LEA, further investigation suggested other psychological and stress-related factors contributed to the menstrual dysfunction, highlighting the importance of practitioners considering multifactorial causes.

In summary, the findings of this thesis challenge previous estimations of LEA in elite female soccer players and underscores the need for a comprehensive, multidisciplinary approach to address menstrual disturbances. Moreover, it highlights the importance of education required to better fuel female soccer players for match day. Meanwhile, individualised supplement protocols are required to help achieve optimal nutritional status throughout the season, with continuous monitoring allowing for early identification and intervention to mitigate potential issues before they impact players' health and performance.

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# Declaration

I declare that the work in this thesis, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy is entirely my own. Additionally, all attempts have been made to ensure that the work is original, does not, to the best of my knowledge, breach any copyright laws and has not been taken from the work of others, apart from the works that have been fully acknowledged within the text.

# Publications

**Publications of the work listed within this thesis are as follows:**

1. Parker L.J, Elliott-Sale K.J, Hannon M.P, Morton J.P, Close G.L. (2021). An audit of hormonal contraceptive use in Women's Super League soccer players; implications on symptomology. *Science and Medicine in Football*. 6 (2), 153–158.
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# Abbreviations

| | |
|---|---|
| $^{18}O$ | Heavy oxygen |
| $^{2}H$ | Heavy hydrogen / deuterium |
| ACSM | American College of Sports Medicine |
| AEE | Activity energy expenditure |
| ANOVA | Analysis of variance |
| AUC | Area under the curve |
| β-CTX | β- Carboxyl-terminal cross-linked telopeptide of type I collagen |
| BDA | British Dietetic Association |
| BF | Body fat |
| BM | Body mass |
| BMC | Bone mineral content |
| BMD | Bone mineral density |
| BMR | Basal metabolic rate |
| $CO_2$ | Carbon dioxide |
| CV | Coefficient of variation |
| DI | Dietary intake |
| DIE | Desired initial enrichment |
| DLW | Doubly labelled water |
| DMPA | Depot medroxyprogesterone acetate |
| DXA | Dual-energy X-ray absorptiometry |
| EA | Energy availability |
| ED | Explosive distance |
| EDTA | Ethylenediaminetetraacetic acid |
| EEE | Exercise energy expenditure |
| EI | Energy intake |
| FA | Football Association |
| FFM | Fat-free mass |
| FHA | Functional hypothalamic amenorrhea |
| FIFA | Fédération Internationale de Football Association |
| FSH | Follicle-stimulating hormone |
| FBC | Full blood count |
| GnRH | Gonadotropin-releasing hormone |
| GPS | Global positioning system |
| Hb | Haemoglobin |
| HC | Hormonal contraceptive |
| HRpQCT | High-resolution peripheral quantitative computer tomography |
| HPA | Hypothalamic-pituitary-adrenal |
| HPO | Hypothalamic-pituitary-ovarian |
| HSR | High speed running |
| IDA | Iron-deficient anaemia |
| IDNA | Iron-deficient non-anaemia |
| IE | Initial enrichment |
| ISAK | International Society for the Advancement of Kinanthropometry |
| IUD | Intrauterine devices |
| IUS | Intrauterine systems |
| Kcal | Kilocalories |
| Kg | Kilogrammes |
| KJ | Kilojoules |
| Km | kilometres |
| $Km.h^{-1}$ | Kilometres per hour |
| LEAF-Q | Low Energy Availability in Females Questionnaire |

| | |
|---|---|
| LH | Luteinizing hormone |
| LEA | Low energy availability |
| LPD | Luteal phase deficiency |
| M | Metres |
| Min | Minutes |
| MJ | Megajoules |
| MPS | Muscle protein synthesis |
| NCAA | National Collegiate Athletic Association |
| NEAT | Non-exercise activity thermogenesis |
| NSAIDs | Nonsteroidal anti-inflammatory drugs |
| NTX | N-terminal telopeptide |
| $O_2$ | Oxygen |
| OC | Oral contraceptives |
| P1NP | Procollagen type 1 N-terminal propeptide |
| pQCT | Peripheral quantitative computer tomography |
| QCT | Quantitative computer tomography |
| RED-S | Relative Energy Deficiency in Sport |
| RFPM | Remote food photographic method |
| RMR | Resting metabolic rate |
| SD | Standard deviation |
| SENr | Sport and Exercise Nutrition register |
| $T_3$ | Triiodothyronine |
| $T_4$ | Thyroxine |
| TD | Total distance |
| TEE | Total energy expenditure |
| TEF | Thermic effect of food |
| TIBC | Total iron-binding capacity |
| TML | Training and match load |
| TRB | Tom Reilly Building |
| UEFA | Union of European Football Associations |
| UK | United Kingdom |
| UKAS | United Kingdom Accredited Services |
| US | United States |
| USA | United States of America |
| $\dot{V}CO_2$ | Carbon dioxide production |
| $\dot{V}O_2$ | Oxygen consumption |
| WSL | Women’s Super League |

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# CHAPTER 1

## GENERAL INTRODUCTION

*The aim of this General Introduction is to provide a brief overview of the subject area in order to provide the rationale for the aims and objectives of this thesis.*

## 1.1 Background

Female football (herein referred to in this thesis as soccer) has experienced remarkable growth worldwide in recent years (Manson, Brughelli, and Harris, 2014) with former Fédération Internationale de Football Association (FIFA) president, Joseph S. Blatter, announcing that the future of football is feminine in 1995 (Martínez-Lagunas, Niessen, and Hartmann, 2014). In England, significant growth has occurred since the Football Association (FA) lifted its ban on female soccer players using accredited facilities in 1971. Over the past two decades, this growth has been particularly pronounced. In 2010, the Women's Super League (WSL) was established as England's first semi-professional league, later evolving into a fully professional league with 12 teams (WSL 1). Despite the increased professionalism and growing interest in female soccer, research in this field remains relatively nascent, especially when compared to studies on male soccer players (Nassis et al., 2022). The number of research studies has risen from 20 in 2000 to 400 in 2020, although only a small fraction (10%) focused specifically on elite female soccer players (Kirkendall and Krustrup, 2022).

One of the reasons for the paucity of research in this area is due to the complexities conducting research around the menstrual cycle and hormonal contraceptive (HC) use (Elliott-Sale et al., 2021). Hormonal contraceptives consist of exogenous steroid hormones that suppress ovulation, leading to consistently low concentrations of endogenous oestrogen and progesterone (Elliott-Sale and Hicks, 2018). Recent research by Martin, Sale, Cooper, and Elliott-Sale (2018) revealed a higher percentage of athletes use HC (49.5%) compared to the general population (30%) (Cea-Soriano, García Rodríguez, Machlitt, and Wallander, 2014). It is important to determine the prevalence, reasons for usage, and any side effects of HCs to understand their potential implications. However, the prevalence of HC use among elite soccer players in England remains unknown.

During the menstrual cycle, fluctuations occur in reproductive hormone concentrations. Disruptions in these patterns can potentially impact athletes' health and performance (Constantini, Dubnov, and Lebrun, 2005). In the absence of menstrual irregularities, non-hormonal contraceptive users typically experience cycles lasting 28 days, although variations between 21 to 35 days are common (Lamina et al., 2013). Menstrual cycle abnormalities can affect 6% to 79% of female athletes (Warren and Perlroth, 2001), yet there is currently no available data on elite female soccer players in England.

The menstrual cycle is highly sensitive to both physiological and psychological stress, with energy deficiency being a key stressor (Redman and Loucks, 2005). Energy deficiency can lead to a downregulation of reproductive hormone concentrations, a condition known as hypothalamic amenorrhea (Gibbs, Williams, and De Souza, 2013). This occurs when limited energy is available to maintain physiological functions, either due to restricted dietary intake, excessive energy expenditure during exercise, or a combination of both (De Souza et al., 2014; Loucks, Kiens, and Wright, 2011). The combination of LEA and menstrual dysfunction has been linked to negative impacts on bone health, a relationship termed the Female Athlete Triad (Nattiv et al., 2007). More recently, the International Olympic Committee proposed a broader model called Relative Energy Deficiency in Sport (RED-S), suggesting that the negative effects of LEA extend beyond menstrual function and bone health to impact other aspects such as physiological function, immune system, growth and development, muscle strength, and cognitive function (Mountjoy et al., 2018). However, many factors proposed in the RED-S model lack robust evidence and are often based on anecdotal clinical observations (De Souza et al., 2014). While research on the Female Athlete Triad and energy availability has been well-studied in female athletes (Gibbs et al., 2013), investigations into this condition among female soccer players are primarily limited to collegiate athletes in the United States (US) (Prather et al., 2016; Reed, De Souza, and Williams, 2013) and international players (Sundgot-Borgen and Torstveit, 2007). For instance, the female Norwegian national team soccer players reported 9.3% experiencing menstrual dysfunction and 13% with a history of stress factors (Sundgot-Borgen and Torstveit, 2007). In comparison, Prather et al. (2016) reported menstrual disturbances in 19% of their squad, while Reed et al. (2013) found LEA in 26% of the squad during pre-season, 33% mid-season, and 12% post-season. These findings highlight the existence of LEA and the Female Athlete Triad in elite female soccer players. However, further research is needed among female soccer players in England to gain a comprehensive understanding of the issue in this population.

The dietary intake of an elite female soccer player is crucial for supplying the necessary nutrients to meet the energy demands of their training and match programme, optimising adaptations after training sessions, and enhancing recovery between sessions and matches (Ranchordas, Dawson, and Russell, 2017). Soccer is associated with significant utilisation of glycogen in both skeletal muscle and liver (Bangsbo, Mohr, and Krustrup, 2006). Despite recommendations for daily carbohydrate intakes ranging from 5-7 $g \cdot kg^{-1} \cdot d^{-1}$ on moderate training days to over 7 $g \cdot kg^{-1} \cdot d^{-1}$ around match days (Collins et al., 2021; Maughan and

Shirreffs, 2007), studies suggest that elite female soccer players often under consume carbohydrate (4.1–4.7 g·kg·day$^{-1}$) (Martin, Lambeth, and Scott, 2006; Mullinix, Jonnalagadda, Rosenbloom, Thompson, and Kicklighter, 2003). The carbohydrate recommendations of 7 g·kg$^{-1}$·d$^{-1}$ are advised to help fuel performance and recovery on match day (Collins et al., 2021; Williams and Rollo, 2015). Therefore, to ensure that female soccer players fuel adequately to perform in matches, energy and carbohydrate intake needs to be assessed in WSL 1 players.

Micronutrients play a pivotal role in physiological processes such as immune function, energy metabolism and growth and repair (de Sousa et al., 2022; Lukaski, 2004; Owens, Allison, and Close, 2018). Prolonged intense exercise, such as soccer, can increase micronutrient turnover, resulting in increased dietary requirements. Vitamins such as folate and vitamin B12 are essential for regulating energy metabolism and forming haemoglobin. Previous studies have shown that US female soccer players often under-consume these vitamins compared to recommended dietary intakes (Mullinix et al., 2003). Minerals such as magnesium and zinc are also critical, regulating energy metabolism, as well as aiding immune function, growth and development. Elite female soccer players have been reported to under-consume these minerals (Mullinix et al., 2003), with deficiencies negatively impacting physical performance (Lukaski, 2004; Lukaski and Nielsen, 2002). Iron and vitamin D are vital for immune function, energy metabolism, and muscle regeneration. Iron deficiency is prevalent in female athletes (15-35%) (Sim et al., 2019), with studies in female soccer players reporting reduced iron concentrations in 29-56% of players (Braun, von Andrian-Werburg, Schänzer, and Thevis, 2018; Landahl, Adolfsson, Börjesson, Mannheimer, and Rödjer, 2005; Tan, Dawson, and Peeling, 2012). Additionally, vitamin D concentrations have been reported to be lower in athletes who train during the winter (von Hurst and Beck, 2014) with elite male soccer players in the English Premier League suffering significant decreases in vitamin D concentrations from August to December, with 65% of players being insufficient (<50 nmol·L$^{-1}$) (Morton et al., 2012). Therefore, investigating the prevalence of micronutrient deficiencies among female soccer players in the WSL 1 is crucial for understanding their nutritional needs and aiding performance.

## 1.2 Aims and objectives of the thesis

The primary aim of this thesis was to assess hormonal contraceptive use, menstrual function, energy expenditure, energy intake, energy availability, blood biomarkers and body composition among elite female soccer in WSL 1.

This was achieved by completion of the following objectives:

1. Assessing hormonal contraceptive and non-hormonal contraceptive use and associated symptomatology among Women's Super League 1 players (Chapter 4).

2. Assessment of RMR, exercise energy expenditure, energy intake, menstrual function, blood biomarkers and body composition, among elite female soccer players participating in a WSL 1 team during pre-season. (Chapter 5).

3. Longitudinal assessment of RMR, exercise energy expenditure, energy intake, menstrual function, blood biomarkers and body composition, among elite female soccer players throughout an entire season in a WSL 1 team (Chapter 6).

4. Provide a detailed two-year examination of a professional internationally capped female soccer player suffering from secondary amenorrhea and anovulatory menstrual cycles (Chapter 7).

# CHAPTER 2

## LITERATURE REVIEW

*The aim of this chapter is to introduce key theoretical concepts and provide a summary and critical appraisal of the relevant current literature.*

### 2.1 History of female soccer

Female soccer has a long history dating back to the 1890's when several female soccer teams were formed. One of the earliest recorded female clubs was the British Ladies Football Club in 1895 (Figure 1), which garnered significant attention with games in North London drawing over 10,000 spectators. One of the most successful female teams, Dick, Kerr's Ladies of Preston, was established in 1917. This team comprised of female factory workers who were responsible for the production of ammunition during World War I (Skillen, Byrne, Carrier, and James, 2022). Whilst female soccer was initially discouraged, suggested due to a perceived threat to the 'masculinity' of the game, it was later encouraged in the belief that competitive sports would boost morale and productivity (Mårtensson, 2010). After defeating their male counterparts within the factory, Dick, Kerr Ladies began playing matches against other factories across the country. They played in front of a crowd of 10,000 fans at Deepdale (Preston North End Football Clubs ground) on Christmas Day in 1917. Their success continued into the 1920s, when they played the first recognised international game against France. They toured both the United Kingdom (UK) and France, and their matches attracted substantial attention. Often, these matches drew larger crowds than men's games played on the same day, including a record-breaking attendance of 53,000 at Goodison Park on Boxing Day in 1920 when they faced St Helens Ladies (Skillen et al., 2022). This attendance set a world record for a female club game, a record that lasted for 98 years and even surpassed Everton men's highest attendance that year, which was 39,400.

**Figure 1.** The women of the 'South' football team, who played the 'North' team in the opening match of the British Ladies' Football Club at Nightingale Lane ground at Crouch End in March 1895 (FA, 2024).

Due to the growing popularity of female soccer, the Football Association (FA) took the controversial step of banning female soccer in December 1921. This ban was not an outright ban on female participation in soccer but instead applied to female teams playing at FA-accredited grounds and using FA-accredited training facilities (Skillen et al., 2022). While the official reason given was concerns about women's physical ability to play soccer, it is widely accepted that the ban was primarily motivated by the perception that female soccer posed a threat to the men's game (Harris, 2007). At that time, the FA released a statement that read:

> *"Complaints have been made as to football being played by women, the council feel compelled to express their strong opinion that the game is quite unsuitable for women and ought to be discouraged." (FA, 2024)*

The ban had significant consequences for female soccer. It resulted in the loss of official recognition for teams like Dick, Kerr's Ladies and others. This ban persisted for five decades, remaining in place until 1971. The ban inflicted substantial damage on the female game since only FA-accredited grounds had the capacity to meet the demands of female soccer (Williams, 2003). As a result, female teams were forced to play at venues with much smaller capacities, leading to lower revenue and reduced exposure for the games. It was not until 1993 that the FA took back control of the administration and funding of female soccer. By this time, the women's game had fallen many decades behind its male counterpart in terms of development and support.

Since 1993, female soccer in the UK has experienced significant growth. In 1997, the FA introduced plans to develop the sport from grassroots to the elite level. The following year, 20 centres of excellence for girls were established, and by 2002, female soccer had become the top participated sport for girls and women in England. In 2010, the FA Women's Super League (WSL) was formed, featuring eight teams in a semi-professional competition. The sport's popularity continued to soar in 2012 when Team Great Britain reached the quarterfinals of the London Olympics. England Women also played their first game at the new Wembley Stadium in 2014, attracting a crowd of over 45,000 spectators. In 2015, England Women achieved a third-place finish at the FIFA Women's World Cup in Canada. Additionally, the Women's FA Cup was held at the new Wembley Stadium for the first time, with over 30,000 in attendance. Notable female soccer players like Manchester City’s Steph Houghton and Liverpool’s Fara Williams were honoured with Member of the Order of the British Empires (MBEs) in recognition of their contributions to the sport. In the 2018/19 season, the FA WSL was rebranded, expanding to 12 teams, and becoming a fully professional league in England for the first time. Clubs were required to meet minimum standards to participate in the top league. A new second-tier league of 12 teams (WSL 2) was introduced, operating on a part-time or semi-professional basis, contributing to the continued growth and participation in female soccer across the country. The year 2022 marked a significant milestone as England Women won their first major trophy, the Union of European Football Associations (UEFA) Women's Euros. Record-breaking moments continued, with the 2023 FIFA Women's World Cup selling a historic 1.5 million tickets.

Despite the increasing professionalism and interest in female soccer, research in this field is still in its early stages. The ban on female soccer for several decades has left many unanswered questions regarding the physiology, training and match demands, and nutritional requirements specific to female soccer. Challenges also arise from the complexities of studying female athletes due to menstrual function and various hormonal contraceptives, making controlled trials more intricate. The combination of these factors and the limited research on female soccer means that the field is still in its infancy.

## 2.2 Training and match demands of female soccer

### *2.2.1 Monitoring training and match load in female soccer players*

There are several commonly used methods to quantify training and match load in female soccer players (Datson et al., 2014). The accuracy of measurements is influenced by several factors, including the technology used, the population studied, the manager's training philosophy, and tactical variations within games. In the early 2000's, methods in female soccer were traditionally selected due to financial constraints (e.g. video-based time motion analyses), however recently, more contemporary techniques have become more common e.g. optical tracking systems and global positioning systems (GPS) (Datson et al., 2017; Hewitt, Norton, and Lyons, 2014; Mara, Thompson, Pumpa, and Morgan, 2017; Trewin, Meylan, Varley, and Cronin, 2018). This coincided with player tracking devices being permitted by FIFA in competitive matches in 2015. The current research into the demands of female soccer has focused on describing volume and intensity of training and match play (Datson et al., 2014). These markers are important as they help determine the metabolic demands of soccer. High speed running and sprinting are key components of soccer (22-28% of total match load) (Vescovi and Favero, 2014) and require the involvement of different metabolic resources (e.g. anaerobic energy system) compared to lower intensities. Therefore, understanding the training and match demands of female soccer players is crucial, as these factors contribute significantly to energy expenditure (Westerterp, 2013). Without a comprehensive understanding of these requirements, it becomes challenging to optimise fuelling strategies for both training and match play.

#### *2.2.1.1 Global positioning systems*

Global positioning systems were originally developed in the United States in the late 1960's for military purposes. The technology is satellite-based, providing precise information on geolocation and time (Larsson, 2003). Global positioning system devices continually communicate with up to 27 satellites orbiting at varying trajectories to cover the surface of earth. The delay between the GPS device and the satellites enable the calculation of the signal travel time and distance between GPS devices and satellites (Larsson, 2003). Global positioning system units within a sporting context are programmed to require at least four different satellites as an acceptable level to collect movement data, however, the higher the number of connected satellites the more accurate the data (Larsson, 2003). The data collection rate of a GPS device is determined by its sampling frequency. In the past, GPS devices used in sports typically had a sampling frequency of 1 Hz, meaning they recorded one data point per second. However, advancements in technology have led to the availability and widespread use of devices with higher sampling frequencies, such as 10 Hz and 15 Hz (Cummins, Orr, O'Connor, and West, 2013; Malone, Lovell, Varley, and Coutts, 2017).

Given the use of GPS devices for monitoring in soccer, it is crucial that devices are valid and reliable. Numerous studies have evaluated different GPS devices in the context of team sport movement demands. While it is recognised that GPS devices can accurately measure total distance (TD), those sampling at 1 and 5 Hz may struggle to accurately quantify distance covered at higher speed thresholds (>19.8 km.h$^{-1}$), especially during multi-directional team sport movements (Coutts and Duffield, 2010; Scott, Scott, and Kelly, 2016). In contrast, devices sampling at 10 Hz are considered the most accurate and reliable, particularly for measuring at higher speed thresholds (>19.8 km.h$^{-1}$) and rapid directional change (Johnston, Watsford, Kelly, Pine, and Spurrs, 2014; Rampinini et al., 2015), of which there are >1300 changes of direction during a female soccer match (Mohr, Krustrup, Andersson, Kirkendal, and Bangsbo, 2008). While inter-unit reliability from the same manufacturer is considered good (CV: 0.2 -1.5% Thornton, Nelson, Delaney, Serpiello, and Duthie (2019)), it is advisable for players to consistently wear the same unit, especially for longitudinal monitoring (Malone et al., 2017). However, the reliability between different GPS devices (brands and models) and associated software versions is less certain and should be approached with caution (Thornton et al., 2019).

### *2.2.2 Profile of a match*

Early video-tape data showed that 7 female national team players covered a TD of 8.5 ± 2.2 km (mean ± standard deviation (SD)), with an average sprint distance of 14.9 ± 5.6 m, however, the velocity threshold was not defined (Davis and Brewer, 1993). More recent studies have reported higher TD covered (~10km) when measured using GPS technology and semi-automated camera systems (Andersson, Randers, Heiner-Møller, Krustrup, and Mohr, 2010; Bradley, Dellal, Mohr, Castellano, and Wilkie, 2014; Hewitt et al., 2014; Martínez-Lagunas et al., 2014). While the trend appears to be for higher TD covered compared to earlier data, these differences are likely to reflect variations in data collection methods (Randers et al., 2010).

Total distance provides a global estimation of overall movement demands of match play, however, it is high speed running (HSR) that is considered to be more informative and reflective of the match demands (Bradley et al., 2009; Di Salvo, Gregson, Atkinson, Tordoff, and Drust, 2009). The determination of distance covered in high-intensity actions typically involves applying specific speed thresholds to players' movements and calculating the amount of activity that exceeds these thresholds (Mara et al., 2017). However, variations in methodology for determining speed thresholds such as individual vs. absolute (Datson et al., 2017; Scott and Lovell, 2018)) and physiological vs. statistical approaches (Bradley and Vescovi, 2015; Park, Scott, and Lovell, 2019) contribute to ambiguity in defining high-intensity actions, making it challenging to reach a consensus on the data (Bradley and Vescovi, 2015). Additionally, different methodologies and technologies used for data collection result in variations in measurement accuracy (Mara et al., 2017). The available data on high-intensity activity and sprinting are variable (see Table 1), with factors such as the specific population studied, data collection and analysis methods, and game context likely contributing to this variation. Female specific HSR and sprint zones have been proposed (Bradley and Vescovi, 2015) and utilised in recent studies investigating the demands of female soccer matches (Hewitt et al., 2014) as well as other team sports (Clarke, Anson, and Pyne, 2015). However, these thresholds were derived from small samples of non-elite players (Dwyer and Gabbett, 2012; Krustrup, Mohr, Ellingsgaard, and Bangsbo, 2005). Consequently, these thresholds may not be more valid than arbitrary ones from professional male soccer players. Despite reported variability, high-intensity running and sprinting are crucial components of the physiological demands of soccer, constituting a significant portion (22–28%) of total match distance covered (Vescovi and Favero, 2014). These activities require additional metabolic and physiological resources, which will have implications for the fuelling strategies required for female soccer players.

**Table 1.** Match physical performance data and methodological details for elite female soccer players.

| Reference | N | Playing level | Measurement technology | HSR threshold | Total distance (km) | High speed running distance (m) |
|---|---|---|---|---|---|---|
| Andersson et al. (2010) | 17 | International<br>Domestic (highest division) | Video camera | 18 km.h$^{-1}$ | 9.9 ± 1.8<br>9.7 ± 1.4 | 1530 ± 100<br>1330 ± 900 |
| Datson et al. (2017) | 107 | International | Optical tracking | >19.8 km.h$^{-1}$ | 10.3 ± 0.9 | 608 ± 181 |
| Hewitt et al. (2014) | 58 | International | GPS | 12 - 19 km.h$^{-1}$ | 9.6 ± 0.2 | 2407 ± 125 |
| Krustrup et al. (2005) | 14 | Domestic (highest division) | Video tape | 18 km.h$^{-1}$ | 10.3 | 1310 |
| Mara et al. (2017) | 12 | Domestic (highest division) | Optical tracking | 12.2 – 19.1 km.h$^{-1}$ | 10.0 ± 0.8 | 2452 ± 636 |
| Mohr et al. (2008) | 19 | International | Video tape | 18 km.h$^{-1}$ | 10.3 ± 0.2 | 1680 ± 90 |
| | 15 | Domestic (highest division) | | | 10.4 ± 0.2 | 1300 ± 100 |
| Trewin, Meylan, Varley, and Cronin (2018) | 45 | International | GPS | >16.5 km.h$^{-1}$ | 10.4 ± 1.0 | 930 ± 348 |

HSR = high speed running. GPS = global positioning system.

### *2.2.3 Profile of training*

It is well known that individuals respond and are exposed to different training stimuli even within the same team. This is not only related to the individual but also their training position, tactical role and coaching methodology (Bangsbo et al., 2006). Improved technology such as GPS has allowed improved monitoring of players' training loads. However, research focused on training load and high-intensity training in elite female soccer players is lacking, with only a limited volume of female-specific studies available (Costa et al., 2022; Datson et al., 2014). Furthermore, existing research encounters similar challenges as match data, including issues with data collection methods (GPS vs indirect methods) and varying speed thresholds, which hinder meaningful comparisons. Therefore, it is important to gain a deeper understanding of the training loads experienced by elite female soccer players to establish effective strategies aimed at enhancing performance, facilitating recovery, and reducing injury risk. Consequently, accurately measuring the training loads of female soccer players will help inform and implement appropriate fueling strategies.

## 2.3 Endocrinology of female athletes

### *2.3.1 Menstrual cycle*

The menstrual cycle (Figure 2) is a complex process governed by reproductive hormones, which are regulated by the hypothalamic-pituitary-ovarian (HPO) axis. In this axis, gonadotropin-releasing hormone (GnRH) is produced by the hypothalamus, which then stimulates the anterior pituitary to release gonadotropins, including follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones, in turn, regulate the production of oestrogens, progesterone, and inhibin from the ovaries (Barbieri, 2014).

The first day of the menstrual cycle is marked by the onset of menstruation (menstrual bleeding), which occurs due to the shedding of the endometrial lining of the uterus. At the cycle's outset, GnRH pulse frequency prompts the production of FSH. Follicle stimulating hormone, in turn, stimulates the growth of primordial follicles in the ovary, transforming them into primary follicles (Reed and Carr, 2000). Over approximately 14 days, known as the follicular phase, these primary follicles progress into secondary and then tertiary follicles. During this phase, granulosa cells and thecal cells within the follicles proliferate and develop. These cells produce the steroid hormone androstenedione, which undergoes conversion into oestrogen, leading to a gradual increase in oestrogen concentrations throughout the follicular phase (Figure 2) (Ferin, Jewelewicz, and Warren, 1993). As oestrogen concentrations rise during the follicular phase, they inhibit the production of FSH and LH, halting additional follicular development, whilst stimulating further oestrogen synthesis in granulosa cells (Reed and Carr, 2000). Towards the end of the follicular phase, there is a sharp increase in oestrogen concentrations, and granulosa cells start secreting inhibin and progesterone (Barbieri, 2014). The high oestrogen concentrations and increasing progesterone enhance the pituitary gland's responsiveness to GnRH, leading to a sudden surge in LH production, often referred to as the 'LH surge' (Figure 2) (Reed and Carr, 2000). The LH surge plays a crucial role in ovulation by triggering the release of the secondary oocyte (egg) from the tertiary follicle. The released egg then travels to the fallopian tubes, where it awaits fertilisation and potential implantation (Ferin et al., 1993). After ovulation, the now-empty tertiary follicle undergoes collapse and is infiltrated by granulosa and thecal cells, a process termed 'luteinisation'. This transformation leads to the formation of an endocrine structure called the corpus luteum. The corpus luteum utilises lipids stored within it to synthesise steroid hormones, resulting in a significant increase in progesterone concentrations and elevated concentrations of oestrogen (Ferin et al., 1993;

Stricker et al., 2006). The primary role of increased progesterone concentrations is to prepare the uterus for potential pregnancy by promoting the maturation of the uterine lining. Typically, the lifespan of the corpus luteum is approximately 12 days. If pregnancy does not occur during this time, the corpus luteum undergoes apoptosis and transforms into an inactive structure known as the corpus albicans. Consequently, production of both progesterone and oestrogen decreases (Reed and Carr, 2000). As progesterone concentrations decline, the blood vessels in the endometrium contract, leading to the death of surface cells within the next 2 days. These cells are then shed, resulting in menstrual bleeding (Barbieri, 2014). This is known as the luteal phase, which spans the time between ovulation and the onset of menstruation, typically lasting 14 days (Reed and Carr, 2000). Towards the end of the luteal phase, decreasing concentrations of oestrogen and progesterone cease their negative feedback to the hypothalamus and anterior pituitary. This cessation prompts increased production of FSH, initiating the hormonal regulation of the subsequent menstrual cycle (Ferin et al., 1993).

The average length of the menstrual cycle is typically 28-29 days, although there is significant intra- and inter-individual variation (Fehring, Schneider, and Raviele, 2006; Stricker et al., 2006). Earlier records of menstrual cycle length suggest a typical variation of 26 to 30 days (Willson, 1966), while more recent studies indicate a broader range of 21 to 35 days as typical for a ‘regular’ menstrual cycle length (Lamina et al., 2013). In a study involving women aged 18 - 40 (n = 276), who self-reported as having 'regular' menstrual cycles (between 21 and 35 days), the mean menstrual cycle length was found to be 29.1 ± 3.5 days. When measured over 30 weeks, it was noted that 46% of these women had a menstrual cycle length range exceeding 7 days, and 20% had a cycle range exceeding 14 days during a 30-week prospective measurement period (Creinin, Keverline, and Meyn, 2004). Therefore, it is important when researching female athletes to gain an understanding of their menstrual cycle length in order to individualise the findings. Menstrual cycles that fall either below 21 days or exceed 35 days are typically considered irregular and may lead to variations in reproductive hormone concentrations (Fehring et al., 2006).

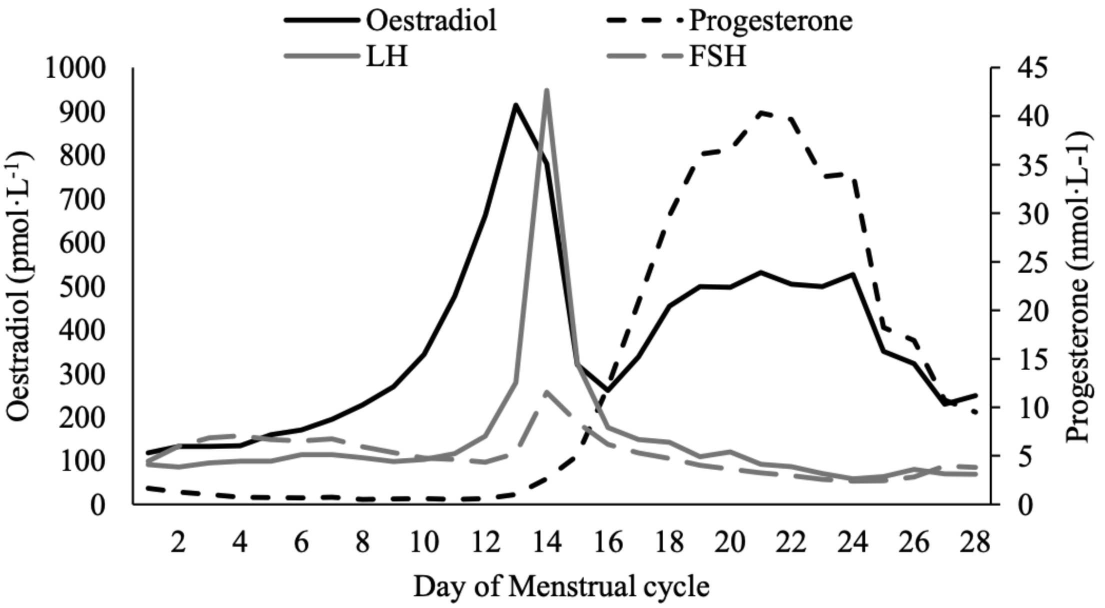


**Figure 2.** Progesterone, oestradiol, follicle stimulating hormone and luteinising hormone concentrations during the follicular, ovulation and luteal phase of the menstrual cycle. Adapted from Martin (2018).

### *2.3.2 Menstrual dysfunction*

The menstrual cycle is a finely balanced function, and its cyclic nature is indicative of normal reproductive activity (Roupas and Georgopoulos, 2011). The female reproductive system is highly sensitive to both physiological and psychological stress, often resulting in abnormalities of the menstrual cycle in a significant percentage of females engaged in sports, with those affected ranging from 6% to 79% (Warren and Perlroth, 2001). The prevalence of menstrual dysfunction depends on the specific athletic discipline and competition level (Warren and Perlroth, 2001). For example, athletes in atheistic disciplines such as ballet dancing have higher prevalence rate of menstrual dysfunction (34 – 79%) (Abraham, Beumont, Fraser, and Llewellyn-Jones, 1982; Brooks-Gunn, Warren, and Hamilton, 1987), while athletes in non-atheistic disciplines such as swimming exhibiting lower rates (6 – 26%) (Sanborn, Albrecht, and Wagner, 1987; Shangold and Levine, 1982). While research has extensively examined the menstrual function of athletes in numerous sports (Cobb et al., 2003; Fahrenholtz et al., 2018; Hoch et al., 2009; Hoch et al., 2011; Melin et al., 2015; Torstveit and Sundgot-Borgen, 2005a), limited data are available for female soccer players (Prather et al., 2016; Reed et al., 2013; Sundgot-Borgen and Torstveit, 2007), with no data available in the UK since soccer turned professional in 2018. Females athletes are at risk of developing various forms of menstrual dysfunction, which may include primary amenorrhea, secondary amenorrhea, oligomenorrhea, luteal phase deficiency, and anovulation (Roupas and Georgopoulos, 2011). Each of these conditions can impact upon a female’s menstrual function in various ways, these will be characterised in the subsequent sub-sections.

### *2.3.2.1 Primary amenorrhea*

Primary amenorrhea is defined as the absence of menarche by the age of 15 years, despite the presence of normal secondary sexual characteristics (e.g., pubic and axillary hair), or within 5 years of breast development (Mountjoy et al., 2014). Its prevalence is approximately 7% in collegiate-aged athletes and has been reported to be higher, reaching 54% in certain aesthetic sports such as gymnastics (Meng et al., 2020; Mountjoy et al., 2014). Besides the high prevalence in aesthetic sports, primary amenorrhea has been reported as 20% in female collegiate soccer players (Tenforde et al., 2017).

### *2.3.2.2 Secondary amenorrhea*

Secondary amenorrhea refers to the absence of three or more consecutive menstrual cycles following menarche (Mountjoy et al., 2014) or, for females with previously irregular menses, the absence of a menstrual cycle for six months or more (Klein and Poth, 2013). Amenorrhea is characterized by infrequent or absent LH pulses, leading to suppression of follicular development, ovulation, and luteal activity. This results in persistently low concentrations of oestrogen and progesterone, as well as the absence of endometrial proliferation (Roupas and Georgopoulos, 2011). The prevalence of secondary amenorrhea in collegiate women is estimated to be around 2-5%, but in certain athlete populations, it can be as high as 69% (Mountjoy et al., 2014). Sport disciplines such as cycling (56%) (Haakonssen, Martin, Jenkins, and Burke, 2015) and triathlon (40%) (Hoch, Stavrakos, and Schimke, 2007) demonstrate the highest prevalence rates. This high prevalence is attributed to athletes being in a calorie deficit, often due to dietary restrictions imposed by the pressures for low body mass (Gimunová, Paulínyová, Bernaciková, and Paludo, 2022). In female soccer players, the prevalence of secondary amenorrhea varies across studies, ranging from 0 – 19%. Mudd, Fornetti, and Pivarnik (2007) surveyed ten US collegiate players aged 19.8 ± 0.9, with 10% reporting secondary amenorrhea. In contrast, Tenforde et al. (2017) found that none of the five collegiate players aged 20.0 ± 1.3 self-reported secondary amenorrhea. Prather et al. (2016) surveyed 145 US collegiate athletes aged 16.4 ± 4.0 and reported that 19% of the players suffered from secondary amenorrhea. Additionally, 69 elite Norwegian soccer players aged 19.6 ± 4.1 self-reported a prevalence of 9% (Sundgot-Borgen and Torstveit, 2007). The differences in reported prevalence are likely due to varying definitions of secondary amenorrhea used in these studies, such as fewer than three or six menstrual cycles per year, or three missed cycles within 12 months. This highlights the importance of consistent definitions in research, as emphasised by Elliott-Sale et al. (2021). Therefore, as suggested by Elliott-Sale et al. (2021), secondary amenorrhea in this thesis will be defined as the absence of three or more consecutive periods in non-pregnant women with past menses.

#### *2.3.2.3 Oligomenorrhea*

Oligomenorrhea is characterised by infrequent menstrual periods, typically defined as having menstrual cycles longer than 35 days or fewer than six to eight periods per year. (Deligeoroglou and Tsimaris, 2010). The primary cause is a dysfunction of the HPO axis which can result from various factors, including hormonal imbalances, excessive exercise, stress and low body fat. The implications of oligomenorrhea can potentially lead to reduced bone density and increased risk of osteoporosis due to prolonged low oestrogen levels (He et al., 2020). Its prevalence in the general population is estimated to be ~13% (Riaz and Parekh, 2023). However, in athletes, the prevalence is not extensively documented but is estimated to range from 5-55%, depending on the sport and level of competition (Lamina et al., 2013). Boxing is reported to have the highest incidence at 55% (Trutschnigg, Chong, Habermayerova, Karelis, and Komorowski, 2008), with collegiate-level female soccer players having reported incidences of 10% (Mudd et al., 2007) and 20% (Tenforde et al., 2017).

#### *2.3.2.4 Luteal phase deficiency*

Luteal phase deficiency (LPD) is characterised by abnormal concentrations of progesterone production, resulting in a shortened luteal phase (less than 10 days in duration) and irregular menstrual bleeding (Schliep et al., 2014). While ovulation often occurs, the corpus luteum produces reduced progesterone, leading to stunted endometrial development and prevention of fertilisation (Roupas and Georgopoulos, 2011). There is currently a paucity of information regarding LDP in female athletes. This could be partly explained by the methodological rigor required to identify this subtle disorder. Unlike amenorrhea, which can be observed easily, LPD detection necessitates daily measurements of ovarian hormones (Klein and Poth, 2013), which are costly and time-consuming.

#### *2.3.2.5 Anovulation*

Anovulation is a dysfunction characterised by suppressed follicular maturation, resulting in the absence of ovulation (Manore, 2002). While anovulation often presents without symptoms, it is associated with low concentrations of oestrogen and progesterone. However, some proliferation of the endometrium may occur, leading to irregular bleeding patterns (Roupas and Georgopoulos, 2011). Anovulation affects up to 7% of females with normal cycle lengths but is more commonly observed in individuals with shorter or longer cycles (Mihm, Gangooly, and Muttukrishna, 2011). Similar to LPD, there is limited information on the prevalence of anovulation in female athletes. This is likely due to the time-consuming and costly methods required for identification, such as monitoring the LH surge using urinary testing kits (Behre et al., 2000).

### *2.3.3 Hormonal contraceptive*

Hormonal contraceptives (HC) are synthetic steroid hormones used to inhibit ovulation and prevent pregnancy (Rivera, Yacobson, and Grimes, 1999). They come in two main types: combined contraceptives, which contain synthetic oestrogen and progestin, and progestin-only contraceptives. The progestin component of these contraceptives inhibits the surge of LH, preventing ovulation, while the oestrogen component provides negative feedback to the anterior pituitary gland, reducing FSH production and inhibiting follicle development (Goldzieher and Rudel, 1974). Hormonal contraceptives are available in various delivery methods, including oral contraceptives (OC), injections, transdermal patches, vaginal rings, implants, and intrauterine systems (IUS). According to Cea-Soriano et al. (2014), approximately 30% of women of reproductive age in the UK use hormonal contraceptives, with 54% of these women using combined OC. Previous reports in elite athletes have indicated that 40-46% of them use oral contraceptives (OCs) (Brynhildsen, Hammar, and Hammar, 1997; Torstveit and Sundgot-Borgen, 2005b). This usage rate is higher than that observed in the general population. In addition to being used as a form of contraception, HCs can offer benefits such as reducing symptoms of dysmenorrhea and altering the bleeding profile, which may be advantageous for athletes during competition (Schindler, 2013). According to Schaumberg et al. (2018), 73% of competitive athletes using OCs deliberately manipulated the timing of their menstruation in the previous year, with 54% of athletes adjusting their OC use specifically due to competition demands. However, the specific proportion of athletes using combined OCs versus progestin-only preparations was not specified, nor was the prevalence of other non-orally administered contraceptive preparations among athletes. Since then, Martin et al. (2018) have reported that a great number of athletes use HCs (49.5%). Soccer players (n = 83) were measured within this study but a breakdown by sport was not provided. Furthermore, according to Martin et al. (2018), 43.5% of competitive athletes using OCs intended to manipulate menstruation, often citing the benefits of predicting and/or controlling the timing, frequency, and amount of menstrual bleeding as the primary reasons. Currently, the number of female soccer players using HCs, their reasons for use, and any associated side effects remain unknown.

#### *2.3.3.1 Combined pill*

Combined OCs typically contain a synthetic oestrogen, primarily ethinyl oestradiol, along with a progestin. There are various types of progestins, each differing in potency and androgenicity (Benagiano, Primiero, and Farris, 2009). The prevention of ovulation is considered the primary mechanism of action, as the combined OC inhibits follicular development, ovulation and consequently corpus luteum formation (Rivera et al., 1999). In addition, the OC has effects on the cervical mucus and endometrium. The cervical mucus remains thick and viscous with tests showing that sperm penetration is inhibited due to the progestin's effect on the mucus (Shoupe, 1994). Combined OC regimens typically involve 21 days of pill consumption, followed by a 7-day pill-free interval, which is then repeated in a continuous manner. However, some formulations may use shorter pill-free interval or a longer period of pill consumption (Coffee et al., 2014). During the 7-day pill-free interval, concentrations of oestradiol, FSH, and LH increase due to the withdrawal of negative feedback to the anterior pituitary (van Heusden and Fauser, 1999; Willis, Kuehl, Spiekerman, and Sulak, 2006). Figure 3 illustrates the circulating concentrations of ethinyl oestradiol and endogenous oestradiol throughout a combined OC cycle. Martin et al. (2018) report that of the athletes (49.5%) using HCs, 68% used combined OCs.

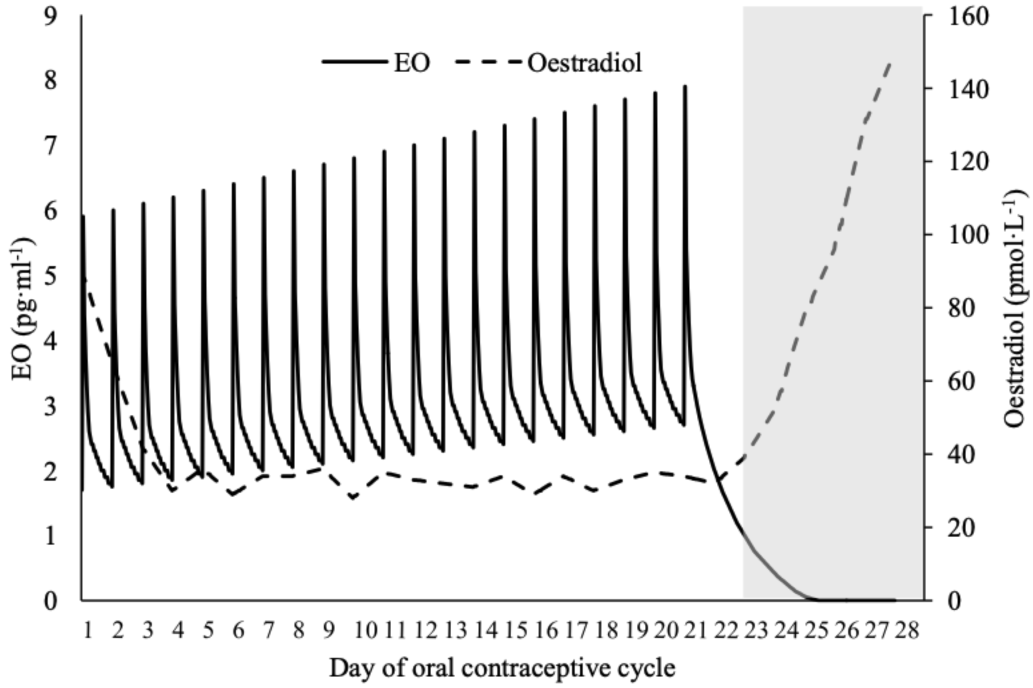


**Figure 3.** Ethinyl oestradiol (EO) and oestradiol concentrations during a combined oral contraceptive cycle. Grey area indicates the 7-day pill free interval. Pmol = picomole, pg = petagram. Adopted from Martin (2018).

*2.3.3.2 Progesterone pill*

Progestin-only OCs operate through various mechanisms, with one of the primary modes being the disruption of the HPO axis, leading to the suppression of ovulation. However, due to the lower concentration of progestin in progestin-only OCs compared to combined OCs, ovulation is not consistently prevented, with approximately 40% of women using progestin-only OCs still experiencing ovulation (Landgren and Diczfalusy, 1980). Among those who do not ovulate, there may still be follicular activity without adequate corpus luteum development or insufficient luteal function, while in some users, ovarian function is completely suppressed (Kim-Björklund, Landgren, and Johannisson, 1991). Another mechanism of action, like combined OCs, involves the effect on cervical mucus. Progestins significantly increase the viscosity of cervical mucus and decrease its receptivity to sperm, inhibiting sperm penetration (Benagiano et al., 2009). This change in cervical mucus resembles the characteristics observed during menopause or towards the end of pregnancy (Chretien and Dubois, 1991). Martin et al. (2018) report that of the athletes (49.5%) using HCs, 10% used progestin-only OCs.

#### *2.3.3.3 Implant*

A recent study by Martin et al. (2018) suggested that the implant is the second most popular form of HC in athletes after OCs (13% in HC users). The implant, another form of progestin-only contraception, can remain in place for up to 3 years and is typically inserted into the upper arm. Its mechanism of action is similar to progestin-only OCs, involving ovulation suppression (Rivera et al., 1999), whilst also thickening cervical mucus (Brache, Faúndes, Johansson, and Alvarez, 1985), and altering the endometrium to prevent pregnancy (Shaaban et al., 1993).

#### *2.3.3.4 Contraceptive injection*

Progestin-only injections are a less common but still utilised option, particularly among athletes. Depot medroxyprogesterone acetate (DMPA) (e.g., Depo-Provera®) is the most prevalent injection used for this purpose, requiring administration every 12 weeks. DMPA is a long-acting progestin with a mechanism of action that interrupts ovulation. DMPA functions at the pituitary and hypothalamic concentrations by inhibiting the mid-cycle surge of LH which is necessary for ovulation (Mishell, 1967). Similar to other hormonal contraceptives, DMPA also affects cervical mucus, causing it to thicken and reducing the likelihood of sperm penetration (Rivera et al., 1999). Martin et al. (2018) report that of the athletes (49.5%) using HCs, 4% used progestin-only injection.

#### *2.3.3.5 Patch*

The transdermal patch is applied to the woman's arm for a continuous 7-day period, repeated for three weeks, followed by a 7-day patch-free interval. These patches typically contain a combination of oestrogen and progestin, with the most popular formulation containing ethinylestradiol and norelgestromin (Galzote, Rafie, Teal, and Mody, 2017). The mode of action of the patch is threefold: 1) thickening of cervical mucus to inhibit sperm penetration, 2) alter the endometrium to reduce likelihood of implantation, and 3) inhibit ovulation by supressing GnRH, FSH and LH (Galzote et al., 2017). In the most recent survey of 430 elite female athletes in the UK, none reported using the patch (Martin et al., 2018).

#### *2.3.3.6 Ring*

The vaginal ring is a flexible device that is inserted into the vagina. It contains the hormones oestrogen and progestin and operates similarly to combined OCs by releasing active hormones for 21 days, followed by 7 days without (Lopez, Grimes, Gallo, Stockton, and Schulz, 2013).

The ring cycle consists of three weeks with the ring in place, followed by a ring-free week. There are also other vaginal rings emerging on the market that contain only progesterone. These rings can remain in place for up to 3 months and are beneficial for women who prefer to avoid oestrogen-containing contraceptives (Jensen, 2013). Martin et al. (2018) report that of the athletes (49.5%) using HCs, only one athlete was using the vaginal ring (<1%).

#### *2.3.3.7 Intrauterine device and system*

Intrauterine devices and IUSs are both long acting and quickly reversible forms of contraception. Intrauterine systems consist of plastic T-shaped frames with a sleeve of progesterone mounted on them (Nelson, 2017), while IUDs are copper wires, coils, or sleeves mounted on T-shaped plastic frames and not considered HC's (Nelson, 2017). The effects of IUDs and IUSs on menstrual function differ significantly. IUDs are associated with increased volume of menstrual flow and dysmenorrhea, whereas IUSs are known to reduce menstrual flow and alleviate menstrual pain (Suhonen, Haukkamaa, Jakobsson, and Rauramo, 2004). The exact mechanisms of action of IUDs and IUSs are still not fully understood. For women with IUDs, it has been hypothesised that the copper component induces an inflammatory response in the endometrium, creating an environment hostile to sperm and interfering with sperm motility, thus preventing fertilisation of the ovum (Alvarez, Brache, Tejada, and Faúndes, 1986). For women with IUSs, the high concentrations of levonorgestrel are believed to cause changes in cervical mucus, making it hostile to sperm movement (Jonsson, Landgren, and Eneroth, 1991). Additionally, women with IUSs tend to have abnormally thin endometrium, which may make the uterus inhospitable for implantation (Guttinger and Critchley, 2007). Martin et al. (2018) report that of the athletes (49.5%) using HCs, 3% were using IUS, with none using the copper non-HC IUD.

## **2.4 Nutrition and female soccer**

### *2.4.1 Energy expenditure*

All metabolic processes in the body ultimately lead to the production of heat. This heat production defines the rate of energy metabolism. Energy expenditure (EE) involves the generation of energy through the combustion of energy-containing substrates such as carbohydrates, fats, proteins, and alcohol (FAO/WHO/UNU, 2001). This metabolic process requires oxygen and results in the production of carbon dioxide and heat. This concept was first discovered by Lavoisier in the late 18th century:

$$(\text{substrate} + O_2 \rightarrow CO_2 + H_2O + \text{heat})$$

Energy expenditure, along with energy intake (EI), is typically quantified in kilojoules (KJ) or megajoules (MJ). However, the kilocalorie (kcal), which is equivalent to 1000 calories, is the most widely recognised unit of energy (Council, 1988), with one kcal equal to 4.184 KJ. One calorie represents the amount of energy required to raise the temperature of one gram of water by one degree Celsius (Council, 1988). Energy expenditure and requirements of female athletes consists of three main components, basal metabolic rate (BMR), thermic effect of food (TEF) (also known as dietary induced thermogenesis) and activity energy expenditure (AEE) (Manore and Thompson, 2006).

#### *2.4.1.1 Basal Metabolism*

Basal metabolism refers to the heat produced over a period of time in a rested, fasted, and thermoneutral state. It represents the energy required to sustain normal physiological functions (cellular, central nervous system, and organ homeostasis) at rest (Manore and Thompson, 2015). Basal metabolic rate is measured in the same bed where the individual slept, although in practice, resting metabolic rate (RMR) measurements are more common. Resting metabolic rate is assessed under similar conditions as BMR (i.e., rested, fasted, and thermoneutral), but the individual does not need to remain in the bed they normally sleep in. However, a period of rest prior to measurement is still required. Therefore, RMR encompasses basal metabolism plus the energy cost of arousal (Manore and Thompson, 2015). Factors such as food/drink intake (including energy and macronutrient content), caffeine, alcohol, and exercise can influence RMR. Consequently, RMR assessments should be conducted under standardised conditions (Bone and Burke, 2018; Compher, Frankenfield, Keim, and Roth-Yousey, 2006).

RMR typically accounts for the largest portion of EE in the general population, ranging from 60% to 75% (Carpenter, Poehlman, O'Connell, and Goran, 1995; Speakman and Selman, 2003). Studies on RMR have a century-long history, showing early correlations between RMR and factors like stature and body mass (BM) (Harris and Benedict, 1918). Recent research highlights fat-free mass (FFM) as the primary influencer of RMR in untrained individuals (Cunningham, 1991; Nelson, Weinsier, Long, and Schutz, 1992). Sex has also been proposed as a contributing factor to RMR, with males showing approximately 23% higher RMR values compared to females (Arciero, Goran, and Poehlman, 1993; Poehlman, Toth, Ades, and Calles-Escandon, 1997; Redman et al., 2014). However, in athletes, when RMR is normalised to BM and FFM, sex differences in RMR become insignificant (Jagim et al., 2019; Reale, Roberts, Lee, Bonsignore, and Anderson, 2020; Thompson and Manore, 1996). This suggests that sex itself may not directly influence RMR, but rather females often have lower RMR due to their typically lower BM and FFM. Given RMR contributes significantly to EE, it can impact BM and performance in female athletes. Therefore, it is vital to determine female soccer player's RMR to help optimise body composition and fuelling strategies around training and matches. However, there is currently a lack of data quantifying RMR in elite female soccer players. A study involving a Finnish female soccer team comprising twelve players with a mean mass of 60.8 ± 5.9 reported a mean RMR of 1383kcal·day$^{-1}$ (Fogelholm et al., 1995). However, it is important to note that the participants in this study were not full-time professional players. Consequently, further research is warranted to investigate RMR in this specific population of athletes.

#### *2.4.1.2 Thermic effect of food*

The TEF refers to the increase in metabolism (above RMR) following the consumption of food or drink (i.e., energy). It represents the energy required to digest, absorb, transport, metabolise, and store nutrients after consumption (Manore and Thompson, 2015). Thermic effect of food typically peaks around 60-180 minutes after food or drink intake and fully subsides within approximately eight hours (Compher et al., 2006). The magnitude of TEF varies depending on the energy content and macronutrient profile of the ingested food or drink: it accounts for approximately 5-10% of the energy content in carbohydrates, 0-3% in fat, 20-30% in protein, and 10-30% in alcohol (Westerterp, 2004). For individuals consuming a mixed diet, which is common among female soccer players (Martin et al., 2006), TEF typically represents around 10% of the total energy expenditure (Westerterp, 2004).

#### *2.4.1.3 Activity energy expenditure*

Activity energy expenditure refers to the amount of energy expended above RMR and the TEF due to physical activity (Poehlman and Horton, 1989). It is the most variable component of total energy expenditure (TEE) and is influenced by factors such as body size and locomotion (Westerterp, 2013). AEE can be further divided into two categories: non-exercise activity thermogenesis (NEAT), which includes the energy cost of activities such as maintaining posture, daily living activities, fidgeting, and spontaneous muscle contraction (e.g., shivering); and planned exercise-related energy expenditure (EEE) (Levine, 2002). In athletic populations, AEE often constitutes the largest component of TEE in adults (Morehen et al., 2016). While there is currently no research on AEE in female soccer players, Brewer (1994) conducted initial studies of EEE, finding that players expended approximately 1100 kcal during a professional match. More recently, Mara, Thompson, and Pumpa (2015) assessed EEE during pre-season and reported expenditure of ~644kcal during match play and ~607kcal during training. It's important to note that the match play in their study involved a simulated friendly game consisting of three periods of 20 minutes each, which may not accurately represent the EE in a typical 90-minute match. Furthermore, in both sets of findings, it was unclear whether the energy expended during sedentary activity (RMR) was subtracted from the EEE calculation (Loucks, 2014). Without accounting for this, there is a risk of overestimating EEE, particularly during longer bouts of exercise such as a 90-minute game.

#### *2.4.1.4 Total energy expenditure*

Total energy expenditure is the combined sum of the three previously mentioned components (RMR, TEF and AEE) and determines an individual's energy requirements to maintain physical function and BM (Manore and Thompson, 2015). The initial investigation into the energy expenditures of elite English soccer players (male adults) dates back to the late 1970s (Reilly and Thomas, 1979). Since then, numerous studies have gone on to quantify TEE in elite male soccer players (Anderson et al., 2019; Anderson et al., 2017; Briggs et al., 2015). However, in female soccer players, only Mara et al. (2015) have reported TEEs of 2925 $\text{kcal}\cdot\text{day}^{-1}$, 2794 $\text{kcal}\cdot\text{day}^{-1}$ and 2274 $\text{kcal}\cdot\text{day}^{-1}$ for match days, training days and rest days respectively. This study was conducted in eight players from the same national league team in the US, therefore, more research is needed across various teams to attain a representative depiction of the energy needs of female soccer players. In addition, as previously reported, data collected on a match day was during a simulated game consisting of three periods of 20 minutes each, which may not accurately represent the TEE in a typical 90-minute match day. Therefore, more research is needed in the area of TEE in elite female soccer players.

### *2.4.2 Methods to assess energy expenditure*

To accurately prescribe EI for any athlete it is important to accurately quantify or estimate EE. Various methods can be employed for this purpose, each differing in terms of their validity, reliability, and practicality within the applied setting (Donahoo, Levine, and Melanson, 2004; Levine, 2005). These methods are typically classified into two categories:

- Calorimetric methods, including direct and indirect calorimetry.
- Non-calorimetric methods, which involve estimation techniques.

#### *2.4.2.1 Direct calorimetry*

As mentioned previously all metabolic processes (energy expenditure) in the body ultimately lead to the production of heat, therefore direct calorimetry measures heat loss from the body through convection, evaporation, and radiation, representing the gold standard for quantifying EE in humans (Ainslie, Reilly, and Westerterp, 2003; Levine, 2005). However, direct calorimetry requires highly sophisticated metabolic chambers, which are expensive to set up and maintain, and necessitate skilled technicians for operation. Additionally, individuals being assessed must remain confined to the chamber for the duration of the measurement, limiting their activities. These practical and logistical challenges make direct calorimetry seldomly used in research or applied practice, especially in assessments of EE in soccer players (Ainslie et al., 2003).

#### *2.4.2.2 Indirect calorimetry*

Indirect calorimetry involves assessing oxygen ($O_2$) consumption ($\dot{V}O_2$) and carbon dioxide ($CO_2$) production ($\dot{V}CO_2$) via pulmonary ventilation, as opposed to directly measuring heat. $\dot{V}O_2$ reflects the amount of oxygen required for substrate oxidation, while $\dot{V}CO_2$ represents the amount of $CO_2$ produced during metabolism. By calculating the respiratory exchange ratio as the ratio of $\dot{V}CO_2$ to $\dot{V}O_2$, one can discern which substrates are being oxidised (Patel, Kerndt, and Bhardwaj, 2024). Different substrates yield varying $\dot{V}O_2$ and $\dot{V}CO_2$ values, thus leading to distinct respiratory exchange ratio values (see Table 2). Subsequently, energy expenditure can be calculated using the modified Weir equation (Weir, 1949).

$$\textit{Energy expenditure } (kcal \cdot day^{-1}) = [(\dot{V}O_2 \times 3.941) + (\dot{V}CO_2 \times 1.11)] \times 1440$$

**Table 2.** Comparison of oxygen uptake, carbon dioxide released, respiratory exchange ratio and heat generation during oxidation of 3 main biological substrates. Adopted from Haugen, Chan, and Li (2007).

| Substrate | Oxygen consumed ($L \cdot g^{-1}$) | Carbon dioxide produced ($kcal \cdot g^{-1}$) | Respiratory exchange ratio | Heat produced per gram oxidised ($kcal \cdot L^{-1}$) |
|---|---|---|---|---|
| Glucose | 0.746 | 0.746 | 1.00 | 3.75 |
| Lipid | 2.029 | 1.430 | 0.69 | 9.30 |
| Protein | 0.966 | 0.782 | 0.81 | 4.30 |

Kcal = kilocalorie

Indirect calorimetry, particularly ventilated metabolic carts, is the predominant method for quantifying EE across both research and practical applications (Levine, 2005; Pinheiro Volp, Esteves de Oliveira, Duarte Moreira Alves, Esteves, and Bressan, 2011). This technique has been employed previously to establish RMR in female soccer players (Fogelholm et al., 1995). With an accuracy ranging from 0.5% to 2.0%, and a typical coefficient of variation (CV) of ~2-3%, this method offers reliable measurements (Donahoo et al., 2004; Levine, 2005). RMR can typically be measured within a brief duration of approximately 10 minutes, under conditions of steady state in ventilated metabolic carts (CV <10% for $\dot{V}O_2$ and $\dot{V}CO_2$) (Compher et al., 2006). However, it is crucial to note that factors such as recent food intake, alcohol or caffeine consumption, and preceding exercise can influence both $O_2$ and $\dot{V}CO_2$, thus impacting EE assessments (Compher et al., 2006). Therefore, adhering to standardised conditions is imperative to ensure the accuracy and reliability of RMR assessments (Compher et al., 2006).

#### *2.4.2.3 Prediction equations*

Where specialised equipment like direct or indirect calorimetry is unavailable, along with the requisite technical expertise or constraints due to cost or time, various prediction equations have been devised to estimate RMR. These equations offer a practical and time-efficient alternative for practitioners working with athletes. However, these equations are frequently developed using data from non-athletic populations (Cunningham, 1980; Harris and Benedict, 1918; Henry, 2005). Numerous studies have indicated that prediction equations derived from non-athletic populations can underestimate (Morehen et al., 2016; O'Neill et al., 2022) and overestimate RMR in athletes (O'Neill et al., 2022) due to not including FFM in the equation (Schofield, Thorpe, and Sims, 2019). Previous prediction equations have included athletic populations (De Lorenzo et al., 1999; Wong et al., 2012) but failed to include FFM (Wong et al., 2012) or female athletes (De Lorenzo et al., 1999). The marked differences in body stature and composition between male and female athletes (Fields, Merrigan, White, and Jones, 2018) render these equations less suitable for application in female soccer players. Recent advancements have been made in developing prediction equations for estimating RMR in female athletes by including FFM (Jagim et al., 2019; Watson et al., 2019) and elite female Dutch athletes (Ten Haaf and Weijs, 2014). A recent review focusing on elite female rugby players found that the FFM equation proposed by Ten Haaf and Weijs (2014) provided the best accuracy in this population (O'Neill et al., 2022). While it is recognised that elite female rugby players typically have higher BM and FFM compared to female soccer players, there is currently no available data on estimated RMR using prediction equations specifically tailored for female soccer players. Therefore, given the success of the Ten Haaf equations in other female athlete populations, this equation seems to be the most suitable option for estimating RMR in elite female soccer players.

#### *2.4.2.4 Doubly labelled water*

The doubly labelled water (DLW) technique is widely regarded as the gold standard for assessing TEE in free-living conditions (Ainslie et al., 2003). It was first employed nearly 40 years ago to evaluate TEE in humans (Schoeller, 1988) and has since been utilised in various athletic populations, including elite male soccer teams (Anderson et al., 2017; Brinkmans et al., 2019). Doubly labelled water enables the measurement of TEE over a period ranging from 4 to 20 days (Ainslie et al., 2003) and exhibits a precision of 2-8% when compared to direct calorimetry (Schoeller, 1988).

The DLW method is based on the turnover of oxygen and hydrogen isotopes in the body. This technique involves enriching the body's water pool with two stable isotopes, heavy oxygen ($^{18}O$) and heavy hydrogen ($^{2}H$ or deuterium), administered through an oral bolus dose of 'doubly labelled water' ($^{2}H_2{}^{18}O$) (Ainslie et al., 2003; Speakman, 1998). The dosage of $^{2}H_2{}^{18}O$ is calculated based on the subject's body mass using the formula:

$$\text{dose (ml)} = 0.65\ (\text{body mass, grams}) \times \text{DIE} / \text{IE}$$

Where 0.65 represents the approximate proportion of the body comprised of water, DIE denotes desired initial enrichment (DIE = 618.923 × body mass, kg$^{-0.305}$), and IE represents initial enrichment (10% or 100,000 parts per million) (Speakman, 1998). Once ingested, these isotopes ($_{18}$O and $_{2}$H) mix with the total body water pool and reach equilibrium after several hours (Speakman, 1998).

Oxygen turnover in the body is influenced by respiration (oxygen consumption and carbon dioxide production) as well as water flow through the body (excreted via urine, sweat, saliva, or breath). On the other hand, hydrogen turnover is solely determined by water flow through the body (Speakman, 1998). Consequently, the elimination rate of $_{18}$O is faster than that of $_{2}$H, as illustrated in Figure 4. The disparity in elimination rates between these isotopes reflects the rate of carbon dioxide production (Ainslie et al., 2003). This allows for the estimation of TEE under the assumption of a respiratory exchange ratio of 0.85 for a mixed diet, a value representative of female soccer players (Martin et al., 2006).

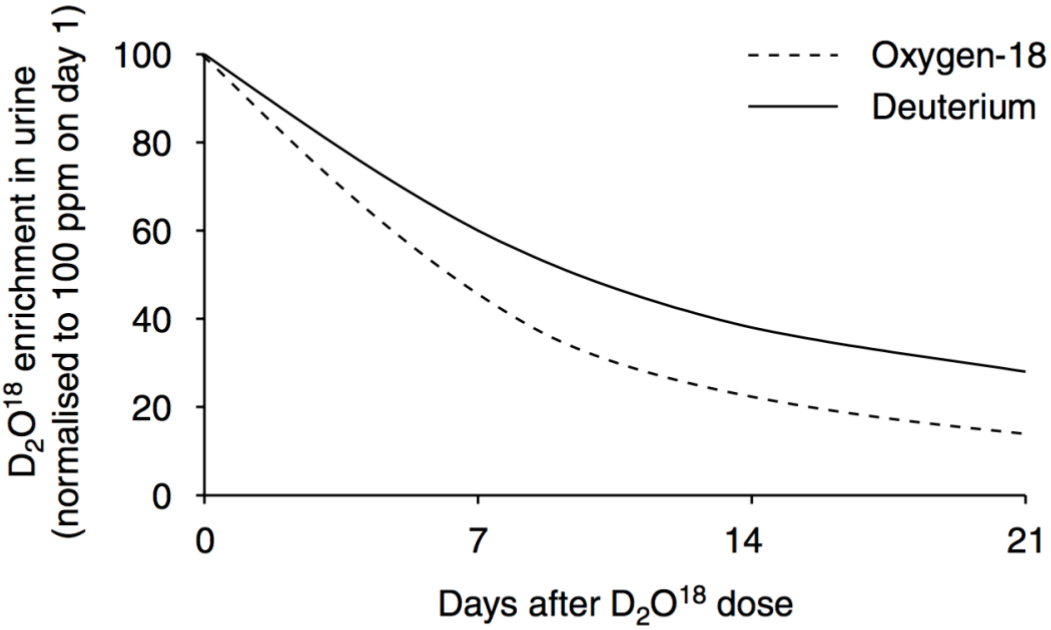


**Figure 4.** Decline of 2H (deuterium) and 18O (oxygen-18) in body fluids (urine, plasma, or saliva) during a hypothetical doubly labelled water experiment. Adopted from Ainslie et al. (2003).

The DLW technique allows for the calculation of mean daily TEE over a specified period. However, it does not provide insight into the energy expended during individual training sessions (AEE) or the TEE for a particular day (Ainslie et al., 2003). Due to the substantial costs associated with acquiring isotopes and the complex analysis required to determine isotope concentrations using isotope ratio mass spectrometry, this method is expensive (approximately £1,000 per subject) (Westerterp, 2017). Despite these drawbacks, it remains the gold standard for quantifying TEE and is particularly valuable in athletes due to it being non-invasive and not disrupting daily activities such as soccer training or matches (Westerterp, 2017).

There are several other non-calorimetric methods available to estimate EE, each with its own set of advantages and disadvantages (refer to Table 3). These methods are validated against the DLW technique (Westerterp, 2013).

**Table 3.** Strengths and limitations of various non-calorimetric methods to estimate energy expenditure.

| Method | Overview of method | Strengths | Limitations |
|---|---|---|---|
| Accelerometery (tri-axial) | Detects movement, particularly accelerations, across three different planes of motion: anterior-posterior, mediolateral, and longitudinal. It monitors the frequency, velocity, and duration of movement. Participants typically wear a small device, often on the hip, for a specified period (Hills, Mokhtar, and Byrne, 2014; Ridgers and Fairclough, 2011) | Easy to use.<br>Non-invasive.<br>Cost effective.<br>Portable.<br>Low subject burden. | Differences in physical activity intensity thresholds across studies pose challenges for comparisons. Currently, there is no consensus on these thresholds (Hills et al., 2014). Subject compliance may also present an issue (Ridgers and Fairclough, 2011). Underestimates EE in intermittent team sports (Taylor, Nagle, Goss, Rubinstein, and Simonson, 2018) |
| Physical activity diaries | Participants record their activities undertaken within specific time intervals, often 15 minutes. Various activities are assigned an estimated EE (MET) and are then totalled to calculate TEE for a 24-hour period (Hills et al., 2014). | Cost effective.<br>Simple. | High subject burden and relies upon accurate and honest recoding (Hills et al., 2014). |
| Heart rate | There is a strong correlation between heart rate and oxygen consumption (and consequently, energy expenditure) during submaximal exercise (Ainslie et al., 2003) | Non-invasive.<br>Cost effective.<br>Relatively low subject burden. | Validity of applying steady-state exercise principles, such as those observed in linear treadmill running, to the dynamic and unpredictable heart rate responses during soccer training and matches has been questioned (Drust, Atkinson, and Reilly, 2007). In high-intensity intermittent exercises like soccer, heart rate changes relatively slowly compared to abrupt alterations in work rate (Achten and Jeukendrup, 2003). |

| | | | |
|---|---|---|---|
| GPS devices | Estimates of EE are calculated using various external load metrics collected from GPS devices, which include velocity, accelerations, and decelerations. These metrics are often referred to as 'metabolic power' (Buchheit, Manouvrier, Cassirame, and Morin, 2015) | Portable.<br>Non-invasive.<br>Low subject burden.<br>Easily accessible if GPS already worn. | When compared to indirect calorimetry, metabolic power underestimates the oxygen cost of soccer training in soccer players (Buchheit et al., 2015) |

EE = energy expenditure, TEE = total energy expenditure, GPS = global positioning system, MET = metabolic equivalent of task.

### *2.4.3 Dietary intake*

The daily diet of a female elite soccer player serves several crucial purposes: supplying the necessary nutrients to meet the energy demands of their training programme, optimising adaptations achieved during and after training sessions and matches, and enhancing recovery between sessions and matches (Ranchordas et al., 2017). It is also important for players to eat in a way that maintains good health and avoids practices that could compromise their well-being (Reed, De Souza, Kindler, and Williams, 2014). There is strong evidence supporting the importance of diet for optimal performance and its role in modulating adaptations to training (de Sousa, Madsen, Fukui, Santos, and da Silva, 2012; de Sousa, Pereira, Fukui, Caparbo, and da Silva, 2014). Studies examining EI during the season of female players at sub-elite to elite level show a range between 1865 ± 530 and 2794 ± 233 kcal per day (Clark, Reed, Crouse, and Armstrong, 2003; Gravina et al., 2012; Reed et al., 2014; Reed et al., 2013). Given that most soccer training sessions and matches last 60–120 minutes and involve high glycolytic activity in skeletal muscles (Krustrup et al., 2006), especially early in a match (Bendiksen et al., 2012), soccer is associated with significant utilisation of glycogen in both skeletal muscle and liver (Bangsbo et al., 2006).

#### *2.4.3.1 Carbohydrate*

Muscle glycogen stores depend on the amount of carbohydrate consumed in the diet (Burke, Kiens, and Ivy, 2004; Fritzen, Lundsgaard, and Kiens, 2019). Previous studies involving female soccer players have reported daily carbohydrate intakes ranging from 4.1–4.7 $g \cdot kg^{-1} \cdot day^{-1}$ (Martin et al., 2006; Mullinix et al., 2003). It is worth noting that these studies (Martin et al., 2006; Mullinix et al., 2003) were conducted with international teams, and currently, there is no available data for WSL 1 players. Recommended carbohydrate intakes for female soccer players have been based on data from male soccer players (Krustrup et al., 2006). However, it is important to consider insights from studies specifically focused on female glycogen storage physiology (Thomas, Erdman, and Burke, 2016). Research indicates that glycogen stores may be lower during the mid-luteal phase of the menstrual cycle compared to the mid-follicular phase (McLay, Thomson, Williams, and Rehrer, 2007). Nevertheless, studies have shown that female athletes can effectively increase muscle glycogen stores and reduce the difference in glycogen storage between these phases with adequate carbohydrate intake ($>8\ g \cdot kg^{-1} \cdot d^{-1}$) (McLay et al., 2007; Tarnopolsky et al., 2001). Due to this and the known demands of the game, it is therefore recommended for female soccer players to consume 5-7 g·kg$^{-1}$·d$^{-1}$ of carbohydrates on moderate training days, increasing to over 7g·kg$^{-1}$·d$^{-1}$ around match days (Maughan and Shirreffs, 2007). These carbohydrate intakes are advised to optimise performance during both training sessions and matches (Williams and Rollo, 2015). However, previous studies focusing on elite female international teams suggest that female soccer players may be consuming inadequate amounts of carbohydrates (4.1 - 4.7g·kg$^{-1}$·d$^{-1}$) (Martin et al., 2006; Mullinix et al., 2003). These studies both utilised self-reported food diaries, which, as discussed later has many limitations. Further research, employing more robust dietary intake assessment methods is needed to ascertain if insufficient carbohydrate intakes are also a concern among WSL players.

### *2.4.3.2 Protein*

Female soccer involves high-intensity intermittent activity, with players performing as many as 200–300 intense actions during a match or intense training session, including fast running, sprinting, jumping, accelerations, decelerations, changes of direction, and various other movements (Bradley et al., 2014; Mohr et al., 2008). The majority of these actions have a significant eccentric component, leading to exercise-induced muscle damage. Therefore, for professional players facing a demanding match schedule along with daily training, adopting a high-protein diet on match and training days may be beneficial. Dietary protein plays a vital role in stimulating muscle protein synthesis (MPS), as it provides the necessary building blocks for muscle remodelling and repair post-exercise. This process is crucial for optimal post-exercise muscle recovery and growth (Wooding et al., 2017). Previous studies have reported varied protein intake among international female soccer players. For instance, protein intakes of 1.2g·kg$^{-1}$·day$^{-1}$ (Martin et al., 2006) and 1.3g·kg$^{-1}$·day$^{-1}$ (Mullinix et al., 2003) have been documented. Conversely, higher protein intakes averaging 1.6 g·kg$^{-1}$·day$^{-1}$ over a season were observed in US collegiate players (Reed et al., 2013). Despite the importance of protein for supporting optimal recovery, there is a lack of studies assessing protein requirements specifically in female soccer players. Consequently, recommendations for female protein intake are primarily based on data from male athletes (Thomas et al., 2016). Recent evidence suggests that females engaging in intermittent exercise may benefit from a protein intake of approximately 1.7g·kg·day$^{-1}$ (Wooding et al., 2017). Additionally, a study involving female dancers found that a protein intake of 1.8 g·kg·day$^{-1}$, compared to 1.3g·kg·day$^{-1}$, accelerated the recovery of muscle function following intermittent sprint exercise (Brown, Stevenson, and Howatson, 2018). Based on current knowledge, there appears to be no need to adjust protein recommendations according to the menstrual cycle or hormonal contraceptive use (Wooding et al., 2017).

### *2.4.3.3 Fat*

Dietary fat serves several important functions, including providing essential fatty acids (e.g. α-linoleic acid and linoleic acid) and fat-soluble vitamins. Moreover, it serves as an important energy source, especially for athletes with high energy expenditures, given its higher energy density compared to other macronutrients (Table 2). In elite female soccer players, previous reported intakes have been around $0.9\text{g}\cdot\text{kg}\cdot\text{day}^{-1}$ (Martin et al., 2006; Mullinix et al., 2003). In athletes, polyunsaturated fatty acids (PUFAs) have been shown to have beneficial effects on the immune system by alleviating post-exercise immunosuppression and exerting anti-inflammatory actions (Corder, Newsham, McDaniel, Ezekiel, and Weiss, 2016; DiLorenzo, Drager, and Rankin, 2014). These effects may improve muscle recovery and tolerance to strenuous exercise programmes, as demonstrated in studies involving omega-3 fatty acid supplementation following eccentric resistance training (Corder et al., 2016; DiLorenzo et al., 2014), leading to better training adaptations. Unlike protein and carbohydrate recommendations, there are no specific guidelines tailored for fat intake in male or female soccer players (Collins et al., 2021). Instead, players are generally advised to adjust their fat intake to ensure they meet their protein and carbohydrate requirements. Typically, this results in a diet where fat intake constitutes around 20-35% of total dietary intake. Further research is necessary to assess the fat intake of female soccer players in the WSL, as reduced fat intakes could potentially lead to an overall caloric deficit, increasing the risk of LEA and subsequent health and performance issues.

### *2.4.3.4 Micronutrients*

Micronutrients and macronutrients have distinct characteristics. Macronutrients such as proteins, carbohydrates, and fats are consumed in large quantities (over 100 grams per day), while vitamins and minerals are ingested in much smaller amounts (milligrams to micrograms per day). These differences in intake reflect their turnover rates in the body and their specific physiological functions. Despite being present in smaller amounts in the diet and the body, micronutrients play a pivotal role in processes such as immune function, energy metabolism and growth and repair (de

Sousa et al., 2022; Lukaski, 2004). Prolonged intense exercise, such as soccer training and matches, may potentially increase micronutrient turnover (de Sousa et al., 2022), resulting in increased dietary requirements. Therefore, it is crucial to prioritise nutrient-rich foods to minimise the risk of nutrient deficiencies (Maughan and Shirreffs, 2012). This risk can be increased with any dietary restrictions, such as vegetarianism or avoidance of dairy products (Maughan and Shirreffs, 2007). A feature of most vitamins is that the human body is not able to synthesise them, therefore must be obtained from the diet (Fogelholm and Mursu, 2021). Inadequate micronutrient availability may also occur when athletes are in a state of LEA. With potentially reduced EI, micronutrient intakes are also likely to be reduced. Furthermore, LEA may negatively impact the regulation and absorption of certain micronutrients (e.g. iron) (McKay, Pyne, Burke, and Peeling, 2020), however the interactions are not well understood and more likely due to factors such as macronutrient restrictions and training loads. Some micronutrients are more likely to be of a concern in particular sports or sexes. For instance, iron deficiency is reportedly three times more likely in female athletes (15-35%) compared to male athletes (3-11%) (Sim et al., 2019). Moreover, poor bone health is prevalent among female athletes (Chen, Tenforde, and Fredericson, 2013), with stress fractures occurring in 9.7% of females athletes compared to 6.5% of males, thus, making adequate calcium and vitamin D intake paramount. Additionally, vitamin D concentrations may be lower in athletes who train during the winter (von Hurst and Beck, 2014). Other key micronutrients that female athletes could be low in if dietary intake is sub-optimal are zinc, vitamin B12 and folate (Manore, 2017). These micronutrients are essential for regulating energy metabolism and forming haemoglobin. Previous studies have shown that US female soccer players often under-consume these vitamins compared to recommended dietary intakes (Mullinix et al., 2003).

### *2.4.4 Methods to assess dietary intake*

Assessing EI is considered one of the most challenging physiological measurements due to the difficulty in obtaining accurate and reliable data (Burke, Lundy, Fahrenholtz, and Melin, 2018; Capling et al., 2017). Since there is no universally accepted gold standard tool for assessing EI the choice of method depends on the characteristics of the population being studied (Magkos and Yannakoulia, 2003). In a recent systematic review in athletes by Capling et al. (2017), it was found that self-reported EI in athletic populations was under-reported by approximately 19% when compared to TEE assessed by DLW, and changes in body mass. This under-reporting may occur consciously or subconsciously. Additionally, assessments of dietary intake in athletic populations often reveal a tendency for athletes to under-report consumption of what they perceive as "unhealthy" foods while over-reporting consumption of what they consider "healthy" foods (Burke et al., 2018). The analysis of an athlete's diet can be conducted either retrospectively or prospectively. Retrospective methods, such as dietary recall, food frequency questionnaire (FFQ) and diet history, rely on the athlete's memory and honesty to recall recent or past food intakes. On the other hand, prospective methods, such as food diaries and the remote food photographic method (RFPM), track current and ongoing food consumption but can often lead to underreporting (Burke et al., 2018). As a result, researchers and practitioners should carefully select the dietary intake method(s) that are most appropriate for the specific population and situation at hand. It is essential to recognise and consider the limitations inherent in the chosen method(s). An overview of dietary assessment methods is provided in Table 4.

**Table 4.** Dietary assessment methods for collecting dietary intake. Adapted from Burke (2015).

| Method | Overview of method | Period of interest | Advantages | Disadvantages |
|---|---|---|---|---|
| 24-hour recall | Subject describes food consumed over the last 24-hour period or a typical day. | 24 hours | Speedy to implement.<br>Low burden for subject.<br>Interviews can be structured around daily activities.<br>Does not alter intake. | Relies on subject's honesty, memory, and food knowledge.<br>Requires trained interviewer.<br>Day for recall may be 'atypical'. |
| Food Frequency Questionnaire | Subjects asked how often they eat foods from a standardised list and to estimate portion sizes often using photos of food models as a prompt. | From 24-hour period to open-ended. | Can be self-administered to lower burden on investigator.<br>Can be used to cross-check data obtained from other methods.<br>Validated for ranking individuals.<br>Can be modified to target certain nutrients.<br>Can be automated to allow quick processing for investigator. | Relies on responders' honesty, memory literacy and food knowledge.<br>Validity dependent on the food list and the quantification method. |
| Diet history | Open ended interview concerning food use, food preparation, portion sizes, food like/dislikes and food checklist. | Open ended or over a specific period. | Account for daily variation in food intake by investigating a 'typical' day.<br>Can target contrasts between periods of interest as a sub-theme.<br>Collects information on timing of intake and factor that influence food patterns. | Relies on responders' memory and food knowledge.<br>Labour intensive and time consuming.<br>Requires training interviewer.<br>Mostly appropriate for qualitative assessments rather than quantitative. |
| Food diary | Weighed. | Undertaken for 1-7 days, with increasing ability to track usual intake as duration increases, but reduced compliance. | Provides a more accurate quantification of foods than household measures. | Relies on participants honesty and food knowledge.<br>Time consuming for subjects to keep and investigator to process.<br>Distorts food choices and quantity: subject alters their diet to improve their intake or to reduce the workload of recording. |
|  | Household measures (cups, teaspoons etc). | See weighed food diary. | Improved compliance with subjects compared with weighed record.<br>Less alteration of normal eating pattern compared to weighed record. | See comments for weighed record.<br>Requires checking by trained person.<br>Needs standardised set of household measures.<br>Subjective/inaccurate assessment of portion sizes. |
| Remote food photography method | Capturing photos of food alongside descriptions. | See weighed food diary. | Alleviates burden on subjects to estimate portion sizes.<br>Allows real tine communication/feedback with investigator. | See comments for weighed record.<br>Difficult to administer with multiple people at once. |

#### *2.4.4.1 Food diary*

The food diary is the most common method for assessing dietary intake in athletic populations, including female soccer players (Manore and Thompson, 2015; Martin et al., 2006; Mullinix et al., 2003). This approach necessitates the individual to record specific details such as food/drink consumed, quantity, brand, preparation and cooking method, and any leftovers with as much precision as possible over a period of 3-7 days (Dao et al., 2019; Manore and Thompson, 2015). For the most accurate food diary, food/drink quantities (and individual components of a meal) should be weighed and recorded at time of consumption to avoid reliance on memory. While weighed food diaries offer higher accuracy in assessing dietary intake, they can impose a substantial burden on the athlete (Dao et al., 2019). Consequently, athletes may fail to report consumed foods/drinks (i.e., under-reporting) or may alter their typical dietary intake and habits (Kristjansdottir et al., 2006). Alternatively, foods/drinks can be estimated using common household measures (e.g. tablespoon). Employing common household measures to assess dietary intake is less demanding but may lack accuracy due to subjective assessments of portion size (Manore and Thompson, 2015). To gain a comprehensive insight into players' nutritional practices, a 7-day period is ideally used to assess EI from training, competition, and recovery days. However, this extended period increases the reliability of collected data at the expense of increased demands on the players. Therefore, when investigating a players EI, it is important to balance the reliability of data and the demand placed on the players.

#### *2.4.4.2 Remote food photography method*

The RFPM is an innovative prospective technique that involves the individual being assessed capturing photos of their food and drink before and after consumption (Boushey, Spoden, Zhu, Delp, and Kerr, 2017). These photos, along with descriptions of the items, are then sent to investigators via a smartphone application. This method alleviates the burden on the individual to estimate food quantities accurately. Additionally, real-time communication of these photos and descriptions provides a timestamp, indicating when the food or drink was consumed (Costello, Deighton, Dyson, McKenna, and Jones, 2017). The RFPM offers high ecological validity and mitigates memory bias, thus reducing under-reporting compared to traditional food diaries (Boushey et al., 2017; Williamson et al., 2004; Williamson et al., 2003). Portion sizes estimated from digital photography exhibit a strong correlation with weighed portion sizes ($r > .90$, $p <$

.0001), and the mean difference between directly weighing foods and using digital photography is minimal (less than 6 g) (Williamson et al., 2003). Furthermore, the RFPM has demonstrated high correlation with weighed energy intake in both controlled laboratory settings and real-life conditions ($r > .62$, $p < .0001$) (Martin et al., 2009). More recently, Costello et al. (2017) introduced a variation called the 'snap-n-send' method, which enhances the original approach. Alongside capturing photos and descriptions, individuals are consistently reminded of the importance of accurately reporting all food and drink intake during the assessment period. Costello et al. (2017) demonstrated that the 'snap-n-send' method is valid for assessing dietary intake in an athletic population, revealing only a small mean bias for under-reporting (CI = -5.7% to - 2.2%) compared to researcher-observed weighed measures method.

#### *2.4.4.3 24-hour recall*

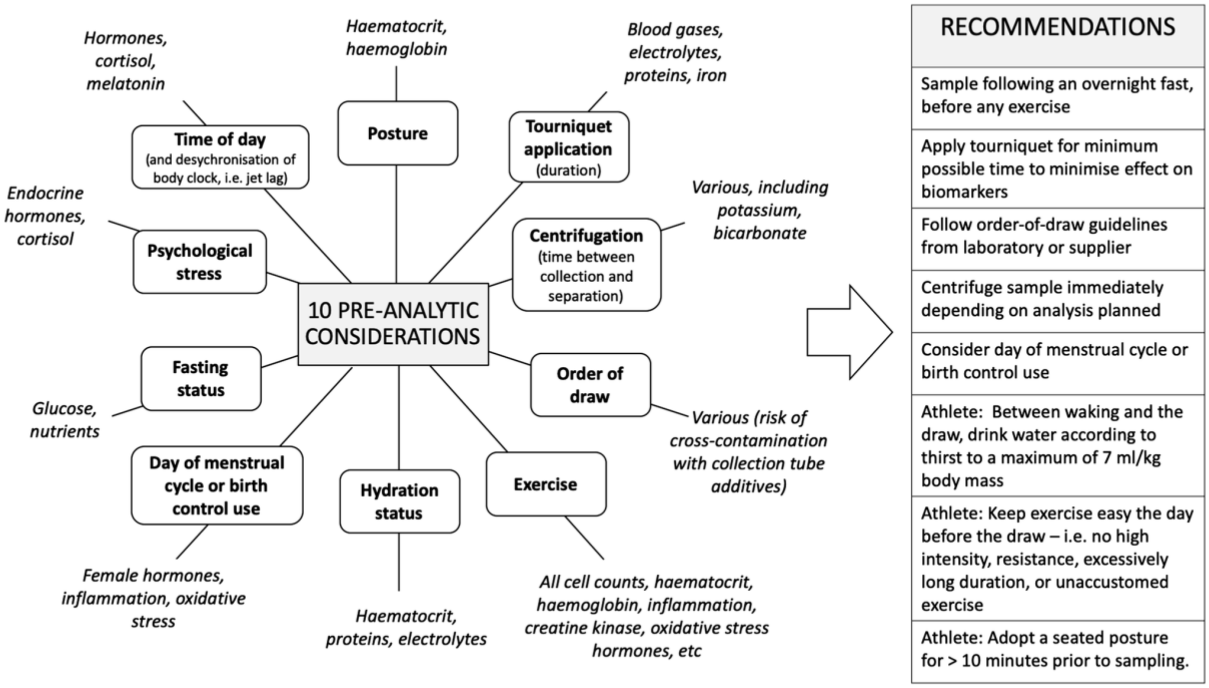
The 24-hour recall method is a retrospective approach used to estimate dietary intake by collecting information on an individual's food and drink consumption from the previous day (Baranowski and Willett, 2012). This method offers quick implementation, imposes minimal burden on the individual being assessed, and can be adapted to fit the athlete's schedule. Employing the triple or multiple pass technique allows interviewers to revisit the initial recall, extracting additional details to enhance the accuracy of dietary intake data, albeit requiring skilled interviewers (Nightingale et al., 2016). As this method relies on memory recall, it does not influence the athlete's dietary habits, but it does necessitate honest and precise recollection (Baranowski and Willett, 2012). However, since the 24-hour recall only captures a snapshot of the previous day's intake, it may not fully represent the athlete's typical diet. Therefore, conducting multiple 24-hour recalls may be necessary to obtain a comprehensive understanding of the athlete's dietary patterns, especially considering the potential variations in dietary intake, particularly carbohydrate consumption, in response to training and match demands. Combining the 24-hour recall with another prospective method for assessing dietary intake has been demonstrated to enhance the accuracy of data collection (Briggs et al., 2015).

### *2.4.5 Blood biomarkers*

The term "biomarker" refers to a molecule or measurable parameter that reflects changes in an individual's physiological state due to various factors like health, disease, medication, environmental toxins, or other challenges (Zolg and Langen, 2004). The utilisation of biomarkers as surrogate indicators of bodily states is well-established, particularly in disease diagnosis such as cancer (Zhang, Li, Wei, Yap, and Chen, 2007). The evidence supporting the use of blood biomarker analysis in sports has been steadily growing for over three decades (Pedlar, Newell, and Lewis, 2019). Screening and monitoring blood biomarkers offer objective insights into overall health, recovery, metabolic, nutritional status and deficiencies (Lee et al., 2017). However, it is important to note that even though these biomarkers are useful, they also have limitations when it comes to tracking athletes. Therefore, prior to conducting measurements, it is crucial to thoroughly assess pre-analytical factors.

#### *2.4.5.1 Pre-analytic considerations*

The composition of blood is highly dynamic and undergoes continuous changes in vivo. After collection, various factors come into play, including the type of collection tube used, leading to ongoing metabolic processes within blood cells, potential separation of cells from plasma, and the risk of coagulation. Therefore, careful consideration of pre-analytical factors is essential to obtain reliable specimens and ensure robust data (Banfi and Dolci, 2003). These factors encompass various elements such as posture (supine, seated, standing), clinicians proficiency, duration of tourniquet application for venous samples, timing of centrifugation to separate cells from plasma, time of day, psychological stress, fasting status, menstrual cycle phase, and the timing, intensity, and mode of prior exercise (Dugué, Lombardi, and Banfi, 2018; Shaskey and Green, 2000; Statland, Bokelund, and Winkel, 1974). The significance of each factor depends on the specific biomarker measured. While it may be tempting to disregard these procedures for the sake of convenience in sports settings, doing so can lead to significant inaccuracies in the data, thereby impacting subsequent data analysis (Pedlar et al., 2019). An overview of pre-analytic considerations is found in Figure 4.



- Sample following an overnight fast, before any exercise
- Apply tourniquet for minimum possible time to minimise effect on biomarkers
- Follow order-of-draw guidelines from laboratory or supplier
- Centrifuge sample immediately depending on analysis planned
- Consider day of menstrual cycle or birth control use
- Athlete: Between waking and the draw, drink water according to thirst to a maximum of 7 ml/kg body mass
- Athlete: Keep exercise easy the day before the draw – i.e. no high intensity, resistance, excessively long duration, or unaccustomed exercise
- Athlete: Adopt a seated posture for > 10 minutes prior to sampling.

**Figure 5.** Pre-analytic considerations for the measurement of blood biomarkers from a venous blood sample. Adopted from Pedlar et al. (2019).

### *2.4.5.2 Hormonal markers*

Hormones play crucial roles in various physiological processes such as development, reproduction, maintaining homeostasis, and regulating metabolic demand. They are synthesised and secreted by endocrine glands like the hypothalamus, adrenal glands, pituitary glands, gonads (ovaries in females), thyroid gland, parathyroid gland, and pancreas (Hiller-Sturmhöfel and Bartke, 1998). Transported through the bloodstream, hormones act on target cells, with different classes including steroids, amino acid derivatives, and polypeptides/proteins. To ensure homeostasis, hormone production and secretion are tightly regulated, often involving multiple hormones. For athletes, training is essential for adaptation but excessive intensity or volume can lead to inappropriate hormonal responses (Hackney and Lane, 2015). Furthermore, several rigorously executed trials have established that LEA can disrupt hormonal balance in female athletes (Loucks, 2003; Loucks and Heath, 1994; Loucks and Thuma, 2003; Loucks, Verdun, and Heath, 1998). As mentioned previously (section 2.3.2), menstrual dysfunction is prevalent in athletes (6-79%). This dysfunction can negatively impact numerous hormones, including EI related hormones (adipokines, ghrelin, leptin), insulin, growth hormone (GH), insulin-like growth factor 1(IGF-1), thyroid hormones (triiodothyronine ($T_3$) and thyroxine ($T_4$)), cortisol, oestrogen and progesterone (Elliott-Sale, Tenforde, Parziale, Holtzman, and Ackerman, 2018). While hormonal blood marker measurements are common in male soccer players to measure fatigue and training load (Koundourakis et al., 2014; Meyer and Meister, 2011; Romagnoli et al., 2016; Silva et al., 2014) and common in elite female athletes (Ackerman, Putman, et al., 2012; Heikura, Burke, et al., 2018; Melin et al., 2015; Thong, McLean, and Graham, 2000), their utilisation in female soccer players has been less prevalent (Reed et al., 2013). Given the energetic demands of soccer (section 2.2) over an eleven-month season, additional research is warranted in elite female soccer players to better understand their hormonal profiles and aid in the diagnosis of menstrual dysfunction. Table 5 provides an overview of the body of work conducted in elite female athletes.

**Table 5.** Overview of studies investigating hormonal markers associated with energy availability in female athletes.

| **Study** | **Participants** | **EA definition** | **Hormonal marker** |
|---|---|---|---|
| Ackerman, Slusarz, et al. (2012) | 39 junior elite athletes | Athlete reported amenorrhea. | LH ↓<br>Leptin ↓ |
| Heikura, Uusitalo, et al. (2018) | 35 elite distance athletes | <30 kcal·kg FFM$^{-1}$·day$^{-1}$ | $T_3$ ↓<br>Insulin →<br>IGF-1 →<br>Oestrogen ↓ |
| Melin et al. (2015) | 40 elite endurance athletes | <30 kcal·kg FFM$^{-1}$·day$^{-1}$ | LH ↓<br>Leptin →<br>$T_3$ →<br>Cortisol →<br>Insulin →<br>IGF-1 → |
| Reed et al. (2013) | 19 elite female soccer players | <30 kcal·kg FFM$^{-1}$·day$^{-1}$ | $T_3$ → |
| Thong et al. (2000) | 18 elite national level athletes | Athlete reported amenorrhea. | Leptin ↓<br>$T_3$ ↓<br>$T_4$ ↓<br>Insulin ↓<br>Oestrogen ↓<br>Progesterone → |
| Vanheest, Rodgers, Mahoney, and De Souza (2014) | 10 junior national level athletes | Athlete reported amenorrhea and serum progesterone concentrations | $T_3$ ↓<br>IGF-1 ↓<br>Oestrogen ↓<br>Progesterone ↓ |

FFM = fat free mass, LH = luteinising hormone, $T_3$ = triiodothyronine, IGF-1 = insulin-like growth factor 1, $T_4$ = thyroxine, EA = energy availability.

### *2.4.5.3 Micronutrient markers*

As previously emphasised (section 2.4.3.4), micronutrients play pivotal roles as regulators of both health and performance (Lukaski, 2004). Micronutrient biomarkers are key tools for assessing internal nutrient status, often detecting either toxic concentrations or deficiencies before clinical signs and symptoms manifest (de Sousa et al., 2022). Moreover, they offer an efficient and reliable quantitative method for assessing nutritional status, unaffected by reporting bias (Pedlar et al., 2019). Table 6 provides an overview of commonly utilised biomarkers for key nutrients, detailing their reference ranges and associated manifestations of deficiency. Given that iron is particularly prone to suboptimal status in female athletic populations (Larson-Meyer, Woolf, and Burke, 2018) the subsequent section will provide additional comprehensive information concerning iron markers.

**Table 6.** Micronutrient biomarkers for nutritional assessment; references ranges, exercise related function, common manifestations, and limitations. Adapted from Larson-Meyer et al. (2018)

| Nutrient | Biochemical marker | Reference | Function in exercise | Physical manifestation | Limitations and precautions |
|---|---|---|---|---|---|
| Vitamin D | Serum 25(OH)D | Deficient <50 nmol/L<br>Insufficient <75 nmol/L Sufficient >75 nmol/L[a] | Interact with muscle and the immune system to modulate recovery from damaging exercise and infection risk[a] | Unexplained muscle weakness and pain, joint pain, bowing of legs[b] | Cut-offs for insufficiency/ sufficiency and optimal somewhat controversial; more research needed. |
| Vitamin B12 | Serum or plasma vitamin B12.<br>Additional markers: serum methylmalonic acid (MMA), and plasma homocysteine | 160 – 800 ng·L$^{-1}$ | Haemoglobin formation and neurological function[c] | Glossitis, macrocytic anaemia, fatigue, peripheral neuropathy (numbness, nerve cell degeneration)[d] | Ideally results from vitamin B12 and either MMA or homocysteine tests should be used to assess status[e] |
| Folate | Serum or plasma folate (indicative of recent intake) | 3.9 – 20 ug·L$^{-1}$ | Regulate energy metabolism and haemoglobin and nucleic acid formation[c] | Macrocytic anaemia, fatigue, anorexia, angular cheilosis, glossitis, insomnia, and pallor of skin[c] | Potential reduced plasma folate when taking oral contraceptives[f] |
| Magnesium | Serum magnesium | 0.7 – 1.0 nmol·L$^{-1}$ | Energy metabolism, nerve conduction, muscle contraction[c] | Muscle weakness, muscle spasm, confusion, loss of appetite, nausea[c] | Serum magnesium concentration has low sensitivity and specificity |
| Zinc | Serum zinc concentration | 10.7 – 24.5 umol·L$^{-1}$ | Nucleic acid and protein synthesis, glycolysis, wound healing[c] | Delayed physical growth, poor appetite, impaired immune function, poor wound healing[g] | Serum zinc maintained at the expense of tissue zinc and decreased with stress, infection, and inflammation and increased with fasting; serum zinc also altered by exercise[h] |

Sources: [a]Owens et al. (2018); [b]Larson-Meyer and Willis (2010); [c]Lukaski (2004); [d]Peake (2003); [e]White, Guenter, Jensen, Malone, and Schofield (2012); [f]Shere, Bapat, Nickel, Kapur, and Koren (2015); [g]Larson-Meyer et al. (2018); [h]Soria, González-Haro, Ansón, López-Colón, and Escanero (2015). MMA = methylmalonic acid.

#### *2.4.5.4 Haematological markers*

Iron is vital for various cellular functions and physiological systems, essential for both human health and athletic performance (Beard, 2001). Of particular interest to athletes are the iron dependent metabolic pathways, which involve haemoglobin (Hb) and myoglobin for oxygen transport to the exercising muscle, and oxidative production of adenosine triphosphate at the electron transport chain. These processes heavily rely on iron-containing enzymes and haem-containing cytochromes (Beard and Tobin, 2000; Sim et al., 2019). Despite its importance, iron deficiency is a prevalent issue among athletes, particularly impacting female athletes, with reported prevalence ranging from 15% to over 50% (Fallon, 2004, 2008; Koehler et al., 2012; Malczewska, Szczepańska, Stupnicki, and Sendecki, 2001; Parks, Hetzel, and Brooks, 2017). Athletes may have elevated iron requirements due to factors such as haemolysis from ground impact forces and muscle contraction, haematuria, gastrointestinal bleeding, sweating, and inflammatory responses (DellaValle, 2013; Telford et al., 2003). The risk of iron deficiency is further compounded in female athlete's due to menstrual bleeding and lower EI compared to men (de Sousa et al., 2022).

Compromised iron status can lead to symptoms such as lethargy, fatigue, and negative mood states. In more severe cases, such as iron deficiency anaemia (IDA), work capacity (aerobic performance) may be compromised, impacting an athlete's ability to train effectively and achieve competitive performances (Nielsen and Nachtigall, 1998; Pasricha et al., 2010; Patterson, Brown, and Roberts, 2001; Woodson, Wills, and Lenfant, 1978). Therefore, routine monitoring of an athlete's iron status is imperative to ensure optimal performance. A variety of haematological variables are available to assess iron status (Clénin et al., 2015). However, for routine clinical assessment of iron deficiency, analysis of ferritin, Hb, and transferrin saturation should be included as a minimum (Peeling et al., 2007). Peeling et al. (2007) proposed the following classification for various stages of iron deficiency in athletic populations based on these three haematological markers:

- Stage 1 – iron deficiency: ferritin < 35 µg/L, Hb > 115 g/L, transferrin saturation > 16%
- Stage 2 – iron-deficient non-anaemia (IDNA): ferritin < 20 µg/L, Hb > 115 g/L, transferrin saturation < 16%
- Stage 3 – iron-deficient anaemia (IDA): ferritin < 12 µg/L, Hb < 115 g/L, transferrin saturation < 16%

It is worth noting that depleted iron concentrations (stage 1) can have a minimal impact on performance, but early correction of iron depletion is crucial to prevent progression to more severe stages (Burden et al., 2015; Rubeor, Goojha, Manning, and White, 2018). However, ferritin, a commonly used marker of iron status, has limitations, such as being an acute phase protein with concentrations increased during periods of inflammation and intense exercise (Bermejo and García-López, 2009), thus resulting in a falsely high reading. Furthermore, Hb measurements can also be affected by shifts in plasma volume, leading to lower Hb readings and issues like sports anaemia, which does not necessarily impair performance (Clénin et al., 2015; Cowell, Rosenbloom, Skinner, and Summers, 2003; Eichner, 1992). These considerations underscore the complexity of iron status assessment in athletes and the need for thorough pre-analytic considerations, as depicted in Figure 4.

Landahl et al. (2005) conducted a study on elite Swedish female soccer players, revealing that before a Women's World Cup, 59% of the squad exhibited iron deficiency, with 29% suffering iron deficiency anaemia. Similarly, young elite female German players (Braun et al., 2018) and elite female Australian players (Tan et al., 2012) displayed low ferritin concentrations in 50-56% of their respective squads. In contrast, Walker et al. (2019) studied US collegiate female soccer players across a season and discovered that these players maintained haematological markers (iron, ferritin, transferrin saturation, total iron-binding capacity (TIBC) and haematocrit) above clinical norms. However, despite remaining within clinical norms, significant decreases in ferritin, TIBC, transferrin saturation, and haematocrit were observed over the course of their three-month season. The authors argued that while these measurements did not fall below clinical norms, the observed changes were likely sufficient to represent a suboptimal range for performance. Direct comparison of these findings is challenging due to methodological differences; notably, Landahl et al. (2005) included Hb as one of the diagnostic markers, whereas Walker et al. (2019) did not report this specific marker. Additionally, iron measurements in these studies were either taken as a one-off measurement or over a truncated 3-month season, making it difficult to interpret these results in WSL 1 players whose season is much longer at 10 months. Iron markers have not been reported in WSL 1 players to date. Therefore, to gain a better understanding of iron status in this population, a minimum assessment of ferritin, Hb, and transferrin saturation should be conducted over a whole season. This comprehensive evaluation will provide insight into the potential risk of iron deficiency among WSL 1 players and thus its potential impact on performance.

## 2.5 Body composition

Anthropometric measurements of athletes can contribute to optimising performance and maintaining health (Meyer et al., 2013). By assessing body composition on professional female soccer players, these data could offer valuable insights for coaches and practitioners, aiding in assessment of athletic status and monitoring effectiveness of injury rehabilitation (Milsom, Barreira, Burgess, Iqbal, and Morton, 2014), nutrition (Devlin, Kingsley, Leveritt, and Belski, 2017), and training interventions (Ackland et al., 2012). Within anthropometry, body mass is a key measure that can be further segmented into distinct compartments, each composed of varying tissues. These compartments include fat mass (FM), FFM, and bone mineral content (BMC). Several methods are available for measuring body composition, ranging from direct to indirect approaches. The direct method, cadaveric dissection, is impractical in live subjects. Therefore, indirect methods have been developed, including dual-energy X-ray absorptiometry (DXA), skinfold thickness measurements, hydro-densitometry, air displacement plethysmography (ADP), and ultrasound. Additionally, there are doubly indirect methods that utilise predictive regression equations. These methods include bioelectric impedance analysis (BIA), 3-dimensional photonic scanning, and estimations of body fat percentage using ultrasound or skinfold thickness measurements.

### *2.5.1 Bioelectrical impedance*

Bioelectric impedance analysis is commonly utilised in general populations for assessing body composition due to its quick procedure, minimal expertise required for administration, portability, and cost-effectiveness compared to other methods (Kasper et al., 2021). Bioelectric impedance analysis measures a current generated and measured using electrodes or metal contacts, which transmit a small voltage through the body to indirectly assess total body water volume (TBW) (Moon, 2013). Fat free mass contains more water and exhibits less resistance than FM, which contains less water and is therefore more resistant. Bioelectric impedance analysis quantifies this resistance and, in conjunction with height, sex, and body mass, employs regression equations to estimate FFM and body fat percentage (BF%) (Larson-Meyer et al., 2018). However, BIA has several limitations, including: (1) outputs being affected by temperature and hydration status (Koulmann et al., 2000); (2) makes assumptions of the body composition using formula and calculations, irrespective of the population (Ackland et al., 2012); (3) sensitivity to conductive surface of electrodes and electrode placement

(Baumgartner, Chumlea, and Roche, 1990). Given these limitations, it is important to consider alternative methods for assessing body composition in the applied setting.

### *2.5.2 Air Displacement Plethysmography*

ADP determines whole body density by estimating body volume through air displacement (Larson-Meyer et al., 2018). Machines such as the BOD POD (COSMED, Rome, ITA), utilise Poisson's Law to calculate air displacement and, subsequently, volumetric calculation. Isothermal air is then measured using built-in systems or generated through prediction formulas and combined to calculate corrected body volume and body composition using various predictive equations (Higgins, Fields, Hunter, and Gower, 2001). However, ADP has several limitations: (1) inability to detect in-competition changes in body composition for elite athletes (Eston and Reilly, 2001); (2) sensitivity to factors such as clothing, body hair, air movement, moisture, pressure, and temperature changes, which can affect measurement accuracy (Fields, Higgins, and Hunter, 2004; Fields, Hunter, and Goran, 2000; Higgins et al., 2001; Peeters and Claessens, 2011); (3) limited ability to differentiate FM and FFM distribution, which may be of interest to practitioners when monitoring changes over multiple time points in a season (Kasper et al., 2021). Given these limitations, it is important to explore other more appropriate techniques for assessing body composition.

### *2.5.3 Skinfolds*

Skinfold thickness assessments involve the use of a caliper to measure a double fold of gripped skin across various anatomical sites to determine subcutaneous adiposity (Martin, Ross, Drinkwater, and Clarys, 1985). This method is cost-effective and requires minimal equipment, making it suitable for assessment in diverse field-based settings (Meyer et al., 2013). However, discrepancies in collected data can arise due to variations in the number of anatomical sites measured and the equations used to predict body density and FM. To address these discrepancies, the International Society for the Advancement of Kinanthropometry (ISAK) was established in 1986. International Society for the Advancement of Kinanthropometry provides training courses and accreditation worldwide, establishing professional standards for utilising skinfold thickness measurements in assessing body composition. The eight-site method, which includes measurements at the tricep, bicep, subscapular, iliac crest, supraspinale, abdominal, front thigh, and medial calf, is now commonly used in applied settings (Stewart, Marfell-Jones, Olds, and De Ridder, 2011). However, skinfold thickness assessments have limitations: (1) it only samples subcutaneous fat and cannot accurately determine visceral fat; (2) some athletes, particularly females, may find the procedure intrusive; (3) when calculating FFM and BF% using skinfold thickness, assessment is doubly indirect and relies on prediction equations (Ackland et al., 2012); (4) standardising measurement sites can be challenging, and even small differences in site locations, such as 1 cm, can yield significantly different results in the same athlete (Ackland et al., 2012).

### *2.5.4 Dual-energy X-ray absorptiometry*

DXA is considered to be the gold standard measurement for assessing body composition in free-living individuals (Santos et al., 2010). It has been established as a valid and reliable method for assessing body composition in team sport athletes (Beato et al., 2023; Fornetti, Pivarnik, Foley, and Fiechtner, 1999; Santos et al., 2010). Dual-energy X-ray absorptiometry offers detailed measures of FM, FFM, BF%, BMC, and bone mineral density (BMD) (Nana, Slater, Stewart, and Burke, 2015), enabling the detection of subtle differences in body composition between athletes and across time points (Stanforth, Crim, Stanforth, and Stults-Kolehmainen, 2014). However, DXA machines are predominantly found in clinical settings, making regular use by sports teams challenging due to their high cost. Consequently, many studies on female soccer players have utilised alternative methods such as skinfold measurements (Sedano, Vaeyens, Philippaerts, Redondo, and Cuadrado, 2009; Sjökvist et al., 2011), hydrostatic weighing (Clark et al., 2003), bioelectrical impedance (Parpa and Michaelides, 2020) and air-displacement plethysmography (Fields et al., 2018). While monetary investment in female soccer teams still lags behind that of their male counterparts, an increasing number of teams are now gaining access to DXA scanners for body composition assessment. At the time of this thesis design, DXA body composition had only been carried out in US collegiate teams (Reed et al., 2013; Stanforth et al., 2014) with the two studies looking at BM, FM, FFM and BF% over a season (Reed et al., 2013; Stanforth et al., 2014). Subsequently studies by Emmonds, Nicholson, Begg, Jones, and Bissas (2019) and Moss et al. (2020) expanded on this by assessing body composition pre-season and towards the end of the season, respectively. While these studies offer valuable snap-shot insights into the body composition of WSL 1 players, further research should aim to replicate comprehensive season-long assessments conducted in the US (Reed et al., 2013; Stanforth et al., 2014). This would provide a comprehensive understanding of the body composition changes that can occur during a WSL season. Table 7 provides a comparison of body composition results obtained from DXA assessments in female soccer players.

**Table 7.** Anthropometric and dual-energy X-ray absorptiometry data from elite female soccer players competing in the highest national leagues.

| **Reference** | **N** | **Country and time point** | **Standard** | **Age (years)** | **Stature (m)** | **Body mass (kg)** | **% body fat** | **Fat mass (kg)** | **Fat free mass (kg)** |
|---|---|---|---|---|---|---|---|---|---|
| Emmonds et al. (2019) | 10 | England<br>Start of season | Highest division (WSL 1) | 25.4 ± 7.0 | 1.67 ± 0.05 | 62.6 ± 5.1 | 21.3 ± 3.9 | 12.9 ± 2.3 | 46.3 ± 4.5 |
| Minett, Binkley, Weidauer, and Specker (2017) | 24 | USA<br>Pre-season | Highest division (NCAA D1) | 19.0 ± 0.2 | 1.65 ± 0.10 | 64.0 ± 1.5 | 22.0 ± 0.7 | 14.0 ± 0.8 | 48.0 ± 0.9 |
| Moss et al. (2020) | 13 | England<br>End of season | Highest division (WSL 1) | 23.7 ± 3.4 | 1.69 ± 0.08 | 63.7 ± 7.0 | 17.8 ± 4.4 | 11.5 ± 3.5 | 49.5 ± 5.3 |
| Reed et al. (2013) | 19 | USA<br>Pre-season<br>Mid-season<br>Post-season | Highest division (NCAA D1) | 19.2 ± 0.3<br>-<br>- | 165.6 ± 1.2<br>-<br>- | 60.6 ± 1.4<br>61.3 ± 1.4<br>61.0 ± 1.4 | 22.5 ± 1.1<br>22.3 ± 0.3<br>22.2 ± 0.3 | 13.2 ± 0.9<br>13.6 ± 0.9<br>13.3 ± 0.9 | 44.6 ± 0.7<br>44.9 ± 0.7<br>44.9 ± 0.7 |
| Stanforth et al. (2014) | 47 | USA<br>3-year average | Highest division (NCAA D1) | - | 1.66 ± 0.01 | 62.5 ±0.5 | 24.1 ± 0.4 | 15.2 ± 0.3 | 44.4 ± 0.3 |

USA = United States of America. NCAA = National Collegiate Athletic Association. WSL = Women’s Super League

## 2.6 Female Athlete Triad

The female athlete triad is a syndrome commonly observed in physically active females, characterised by three components: LEA, menstrual dysfunction, and low BMD (Nattiv et al., 2007). It was first identified by the American College of Sports Medicine (ACSM) in 1992, prompted by observed trends among adolescent and young adult female athletes (Yeager, Agostini, Nattiv, and Drinkwater, 1993). The ACSM released a position stand on the triad in 1997 (Otis, Drinkwater, Johnson, Loucks, and Wilmore, 1997), followed by an updated version in 2007 (Nattiv et al., 2007). A notable change between the two versions was the inclusion of LEA instead of focusing solely on disordered eating. This shift highlighted that intentional or unintentional energy deficiency can disrupt hormonal balance and bone metabolism (Barrack, Ackerman, and Gibbs, 2013). Furthermore, the 2007 position stand portrayed the triad as three interconnected spectrums, ranging from optimal health to disease (Figure 6). In a state of optimal energy availability, the model (Figure 6) predicts normal menstrual function (eumenorrhea) and optimal bone health. However, when energy availability is reduced, sub-optimal energy availability may lead to various endocrine and physiological responses, identifiable by changes in the hormonal milieu (Nattiv et al., 2007). These responses can result in sub-clinical menstrual disturbances, such as oligomenorrhea (section 2.3.2.3), anovulation (section 2.3.2.5), or LPD (section 2.3.2.4), as well as altered hormonal profiles, including the suppression of oestrogen. Additionally, reduced energy availability may lead to uncoupling of bone turnover (formation and resorption). Sustained uncoupling of bone turnover, in conjunction with a history of nutritional deficiencies, hypoestrogenism, stress fractures, or other secondary clinical fracture risk factors, can contribute to impaired bone health and low BMD (Nattiv et al., 2007). At the opposite end of the spectrum from a healthy state, prolonged LEA may lead to functional hypothalamic amenorrhea (FHA) and osteoporosis. This condition is directly linked to the impairment of reproductive function, affecting the pulsatile release of GnRH from the hypothalamus and subsequent pulsatility and amplitude of LH release (Loucks, 2003; Loucks and Heath, 1994; Loucks et al., 1998). Similarly, LEA may lead to changes in key bone-protective hormones in the endocrine milieu, ultimately contributing to the uncoupling of bone resorption and formation (Ihle and Loucks, 2004), which is believed to cause osteoporosis at the extreme end of the Female Athlete Triad continuum.

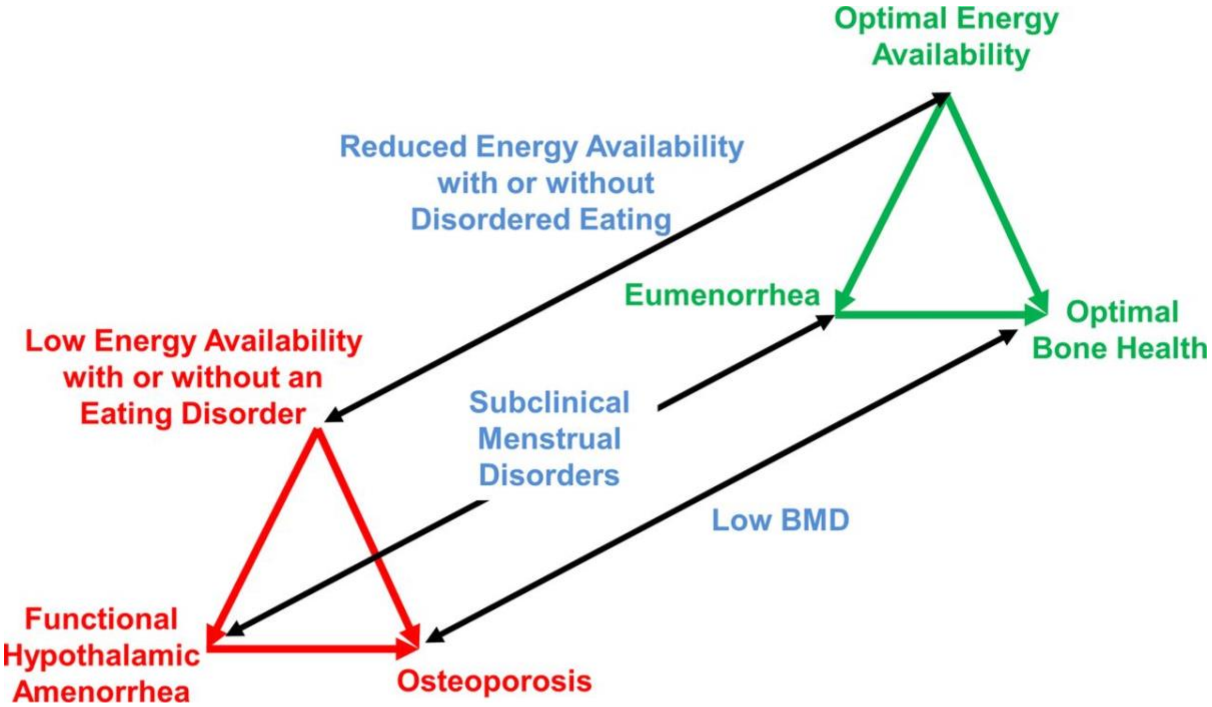


**Figure 6.** Components of the Female Athlete Triad presented on a continuum from optimal health to the most serious clinical sequelae. Adopted from De Souza et al. (2014). BMD = bone mineral density.

Female athletes often present with more than one component of the three triad components. However, it is sufficient to exhibit only one component to be diagnosed with the Female Athlete Triad (Nattiv et al., 2007). Prevalence rates of athletes with one Triad component range from 16% to 60%, while those with any two components range from 2.7% to 27%, and those with all three components range from 0% to 16% (Gibbs et al., 2013). Participation in lean or aesthetic-based sports further elevates prevalence rates, with adolescent athletes experiencing a higher incidence due to increased prevalence of menstrual disturbances (Gibbs et al., 2013). Despite the potential negative implications of the Female Athlete Triad, such as menstrual dysfunction, poor bone health leading to increased risk of fracture and osteoporosis, research on this condition among female soccer players is limited. Sundgot-Borgen and Torstveit (2007) examined 17 Norwegian national soccer players and found that 24% had an eating disorder, 9.3% had menstrual dysfunction, and 13% had a history of stress fractures.

### *2.6.1 Energy availability*

Energy availability represents the amount of dietary energy remaining for physiological function after deducting EEE (Nattiv et al., 2007). Energy availability is calculated by subtracting EEE from energy intake, then normalised relative to FFM (e.g. using FFM from

DXA scan). The critical threshold where these changes occur is an energy availability of <30 kcal·kg FFM$^{-1}$·day$^{-1}$, which often aligns with RMR (Ihle and Loucks, 2004; Loucks, 2003; Loucks and Thuma, 2003). Low energy availability typically arises from issues such as disordered eating, intentional weight loss without disordered eating, inadvertent undereating, or clinical eating disorders (De Souza, Koltun, Etter, and Southmayd, 2017). When energy availability falls below a certain threshold, it leads to unfavourable physiological changes affecting metabolic, reproductive function, and bone health (Loucks, 2003; Loucks et al., 2011; Nattiv et al., 2007).

### *2.6.2 Menstrual function and energy availability*

In instances of LEA, the body may undergo physiological adaptations aimed at preserving energy for critical physiological functions, with reproductive function being deprioritised (De Souza et al., 2017; Elliott-Sale et al., 2018; Wade and Schneider, 1992). This preservation mechanism involves the downregulation of metabolic pathways, potentially leading to disruptions in the HPO axis (Cano Sokoloff, Misra, and Ackerman, 2016; Elliott-Sale et al., 2018). Consequently, energy resources are redirected towards vital metabolic processes such as locomotion, energy conservation, thermoregulation, and cell maintenance (Gordon et al., 2017; Wade and Schneider, 1992). These physiological adjustments may manifest as FHA.

Loucks and Heath (1994) conducted a study in seven female participants where they were provided with two different dietary energy intake levels: 45 kcal·kg FFM$^{-1}$·day$^{-1}$ (balanced energy availability) or 10 kcal·kg FFM$^{-1}$·day$^{-1}$ (LEA) for five days during the follicular phase of the menstrual cycle, while performing no exercise. The results demonstrated that in the LEA condition, LH pulse frequency decreased by 23% and LH pulse amplitude increased by 40%. When exercise (30 kcal·kg FFM$^{-1}$·day$^{-1}$) was added to both energy availability conditions, similar reductions in LH pulse frequency and increases in LH pulse amplitude were observed in the LEA condition (Loucks et al., 1998). This suggests that energy availability, rather than the stress of exercise, regulates changes to the hypothalamic-pituitary-adrenal (HPA) axis, crucial for menstrual function regulation (Reame, Marshall, and Kelch, 1992).

Loucks and Thuma (2003) investigated the impact of energy availability on LH pulsatility during exercise equivalent to 15 kcal·kg FFM$^{-1}$·day$^{-1}$. They assigned energy availability of 10, 20, 30 or 45 kcal·kg FFM$^{-1}$·day$^{-1}$ for five days in the early follicular phase and found that LH pulsatility was not affected at energy availability of 45 or 30 kcal·kg FFM$^{-1}$·day$^{-1}$ but was significantly affected at energy availability of 20 and 30 kcal·kg FFM$^{-1}$·day$^{-1}$. This suggests a threshold between 20-30 kcal·kg FFM$^{-1}$·day$^{-1}$ at which energy availability impairs HPA axis function. Therefore, an energy availability of ≥30 kcal·kg FFM$^{-1}$·day$^{-1}$ was established as the threshold for LEA. However, recent evidence by Lieberman, De Souza, Wagstaff, and Williams (2018) suggests that there is no specific threshold of energy availability below which menstrual disturbances occur, but rather the frequency of menstrual disturbances increases linearly as energy availability decreases. The importance of energy availability on reproductive status has been demonstrated in several studies where increasing EI or reducing training volume resulted in the resumption of menses in previously amenorrheic athletes (Areta, 2020; Dueck, Matt, Manore, and Skinner, 1996; Kopp-Woodroffe, Manore, Dueck, Skinner, and Matt, 1999; Mallinson et al., 2013).

It is evident that both short-term and long-term reductions in energy availability can lead to impaired reproductive function. This is particularly evident in the disruption of LH pulsatility, which serves as a critical regulator of menstrual function. The alterations in menstrual function can have significant implications for the overall health and performance of female athletes (De Souza et al., 2014). Elite soccer players have reported menstrual dysfunction between 9.3 – 19.3% (Prather et al., 2016; Sundgot-Borgen and Torstveit, 2007), with LEA experienced in 26.3% during pre-season and 33.3% during mid-season (Reed et al., 2013).

### *2.6.3 Bone mineral density*

Bones are constantly being renewed through a process of resorption (breaking down of old bone tissue) and formation (building new bone tissue). Normally, the rates of bone resorption and formation are coupled, ensuring the maintenance of bone mass and structure. However, this balance can be disrupted for various reasons, leading to an imbalance favouring resorption over formation (Delaisse, 2014). When bone turnover becomes skewed in favour of resorption, it results in decreased bone mass, compromised bone structure, and a higher risk of fractures (Vasikaran, 2008).

The decrease in reproductive hormone concentrations along with other endocrine and metabolic disturbances caused by LEA can have adverse effects on bone health (De Souza et al., 2014; Nattiv et al., 2007). Bone mineral density is commonly assessed using DXA scanning. Bone mineral density Z-scores are utilised to evaluate bone density due to them comparing DXA results with those of age-matched peers. According to ACSM low BMD is defined as Z-scores falling between -1.0 and -2.0 along with clinical risk factors for fracture (e.g., LEA, eating disorders, amenorrhea, previous fractures). Osteoporosis is considered in athletes with a Z-score ≤ -2.0 along with clinical risk factors for fracture (Nattiv et al., 2007).

The significance of menstrual function for athletes' bone health was initially highlighted by Drinkwater et al. (1984), who found that 14 amenorrheic athletes had significantly lower lumbar vertebrae BMD compared to 14 eumenorrheic athletes. In a follow-up study over 15 months, amenorrheic athletes who resumed menses showed improved lumbar BMD (+6.3%), while those who remained amenorrheic continued to experience BMD loss (-3.4%) (Drinkwater, Nilson, Ott, and Chesnut, 1986). However, subsequent investigations, spanning two (Jonnavithula, Warren, Fox, and Lazaro, 1993) and eight (Keen and Drinkwater, 1997) years, revealed that BMD remained lower in previously amenorrheic athletes compared to eumenorrheic athletes and controls. Studies have shown that athletes with a history of optimal menstrual function have greater BMD (1.27 $g \cdot cm^2$) compared to those with a history of oligomenorrhea/amenorrhea (1.18 $g \cdot cm^2$), and the lowest BMD (1.05 $g \cdot cm^2$) was observed in athletes who never had regular menstrual function (Drinkwater, Bruemner, and Chesnut, 1990). Without intervention, BMD is estimated to decline by 2-3% per year in amenorrheic women (Audí et al., 2002; De Souza et al., 2014), and the duration of menstrual dysfunction is negatively associated with BMD (Drinkwater et al., 1990; Myburgh, Bachrach, Lewis, Kent, and Marcus, 1993). Stress fractures are more prevalent in women with menstrual dysfunction compared to those with regular menstrual cycles, irrespective of training volume (Bennell, Matheson, Meeuwisse, and Brukner, 1999; Duckham et al., 2012). However, exercise is a lifestyle intervention capable of enhancing bone mass and strength, as bone responds to mechanical strain via osteocytes (MacKnight, 2017). During adolescence, variable, dynamic, and progressive mechanical stress is crucial for maximal bone density acquisition. These advantages persist into adulthood. However, excessive exercise can result in LEA, weight loss, and amenorrhea, undermining the protective effects of exercise (De Souza et al., 2017).

The prevalence of low BMD in athletes ranges from 0% to 40% when defined as a Z-score between -1.0 and -2.0 (Gibbs et al., 2013). Unfavourable bone density outcomes may arise from disrupted bone turnover, characterised by decreased bone formation, increased bone resorption, or a combination of both, in response to LEA. While the initial bone metabolic responses to LEA remain unclear, studies have shed light on certain aspects. In sedentary women, a dose-response relationship between LEA levels (30, 20, and 10 kcal·kg LBM$^{-1}$·d$^{-1}$) and decreased bone formation markers has been observed. Additionally, severely restricted energy availability (10 kcal·kg LBM$^{-1}$·d$^{-1}$) over a span of 5 days has been associated with increased bone resorption markers compared to balanced energy availability (45 kcal·kg LBM$^{-1}$·d$^{-1}$). However, these data were from sedentary females who likely have different bone characteristics (Papageorgiou, Dolan, Elliott-Sale, and Sale, 2018). A more recent study in 11 eumenorrheic physically active females found similar results with LEA (15 kcal·kg LBM$^{-1}$·d$^{-1}$) resulting in a significantly higher area under the curve (AUC) of β-CTX (a reference marker of bone resorption) and a notably lower P1NP AUC (a reference marker of bone formation) compared to a control group of energy availability at 45 kcal·kg LBM$^{-1}$·day$^{-1}$ (Papageorgiou et al., 2017). These results confirm that altered bone metabolism is evident in physically active females with LEA.

While DXA is considered the clinical gold standard for measuring BMD, it has limitations due to being two-dimensional. DXA tends to underestimate BMD of smaller bones and overestimate BMD of larger ones because it cannot account for variations in bone size and depth (Barrack et al., 2013). Quantitative computer tomography (QCT), peripheral QCT (pQCT), and high-resolution pQCT (HRpQCT) offer direct measurement of volumetric BMD as well as assessment of cortical and trabecular bone microarchitecture (Radetti et al., 2006). HRpQCT has revealed compromised bone microarchitecture in amenorrheic athletes compared to eumenorrheic athletes and control groups, including decreased trabecular volume, density, and number, increased trabecular spacing, and cortical thinning (Ackerman, Putman, et al., 2012).

### *2.6.4 Low Energy Availability in Females questionnaire*

In an effort to effectively screen athletes at risk of developing health issues caused by LEA and The Female Athlete Triad, Melin et al. (2014) developed the Low Energy Availability in Females Questionnaire (LEAF-Q). The questionnaire consists of 25 items categorised into 'injuries' (2), 'gastrointestinal function' (4), and 'menstrual function and use of contraceptives' (19). Total and subscale scores have distinct cut-off values associated with an increased risk of the Triad/LEA. According to Melin et al. (2015) participants are classified as either at risk of LEA (total score ≥ 8) or not at risk (total score < 8) for the Triad and LEA. Initially validated for endurance athletes and dancers, the LEAF-Q demonstrated a specificity of 90% and sensitivity of 78% in successfully identifying LEA (Melin et al., 2014). Since then, the LEAF-Q has been widely used in various sports, including Australian rules football players (Condo, Lohman, Kelly, and Carr, 2019), elite rock climbers (Monedero, Duff, and Egan, 2023), paralympic athletes (Pritchett et al., 2021) and elite female soccer players (Moss et al., 2020). However, the interpretation of the LEAF-Qs in soccer players should be approached cautiously due to the different physical demands of soccer compared to the endurance sports for which the questionnaire was initially designed. Additionally, the LEAF-Q was developed in sports that primarily experience overuse injuries (Lopes, Hespanhol Júnior, Yeung, and Costa, 2012; van Gent et al., 2007), while soccer is a high-impact sport with potential for both acute and overuse injuries (Ekstrand, Hägglund, and Waldén, 2011). Nevertheless, LEA and The Female Athlete Triad can significantly impact a player's health and performance, and practitioners require a screening tool to quickly assess the risk of these conditions. Therefore, the LEAF-Q offers a quick and cost-effective method to assess the risk of LEA in female soccer players.

### 2.7 Relative Energy Deficiency in Sport

In 2014, the International Olympic Committee released a consensus statement on the Relative Energy Deficiency in Sport (RED-S) model, which aimed to expand upon the existing knowledge of the Triad (Mountjoy et al., 2014). Unlike the Triad, which consists of three components, the RED-S model proposes that relative energy deficiency is the primary factor that can impact various aspects of health and performance, beyond just menstrual function and bone health (Mountjoy et al., 2014). Additionally, the RED-S model suggests that male, non-Caucasian, and disabled athletes may also be affected, broadening the scope of individuals susceptible to its effects (Mountjoy et al., 2014). The model outlined several other potential factors, as illustrated in Figure 7, including immune function, cardiovascular health, metabolic rate, decreased muscle strength, and aspects related to impaired cognition. Since the conception of RED-S in 2014, there have since been two updates, one in 2018 (Mountjoy et al., 2018) which provided an update on the latest literature that had been conducted in the field, with the second in 2023 (Margo et al., 2023). The latest update has brought about significant change to the model as seen by Figure 8. The revised RED-S model suggests that LEA is on a continuum rather than a threshold. The continuum shifts from adaptable LEA through to problematic LEA. Adaptable LEA is described as having benign effects, typically a short-term experience with minimal or no impact on long term health, well-being or performance (Margo et al., 2023). Problematic LEA is associated with greater and potentially persistent disruption to health or performance that often present with signs or symptoms according to the body system and the individual (Margo et al., 2023). However, it is acknowledged that there is a need for further long-term prospective and controlled intervention studies to develop our understanding of this model, as much of the data underpinning it is cross-sectional or observational (Margo et al., 2023). Due to this model only being released in the past year, the studies conducted in this thesis will refer to the model previously described in 2014 and 2018 (Figure 7) (Mountjoy et al., 2014; Mountjoy et al., 2018).

Low energy availability has been shown to impact several of the health and performance factors of RED-S including impaired immune function (Hagmar, Hirschberg, Berglund, and Berglund, 2008), increased risk of cardiovascular disease (Oosthuyse and Bosch, 2010; Punnonen et al., 1997), endothelial dysfunction (Hoch et al., 2011; Rickenlund, Eriksson, Schenck-Gustafsson, and Hirschberg, 2005), reduced muscle protein synthesis (Areta et al., 2013), alteration to metabolism (Loucks and Thuma, 2003), increased injury risk (Hackney and Walz, 2013; Thein-Nissenbaum, Rauh, Carr, Loud, and McGuine, 2011) and diminished training response and impaired performance (Hackney, 2006; Vanheest et al., 2014). However, it is important to note that many of the factors proposed in the RED-S model lack robust evidence and are often based on anecdotal clinical observations (De Souza et al., 2014).

The field of RED-S research has primarily focused on leanness and endurance sports, where the prevalence of RED-S is believed to be higher due to the importance of body mass for optimal performance and its connection with energy availability (Logue et al., 2018; Mountjoy et al., 2014). Additionally, studies have investigated the prevalence of individual symptoms like LEA and BMD, rather than examining the occurrence of clustered RED-S indicators, which ultimately define the syndrome. Despite studies exploring the prevalence of LEA (Reed et al., 2014; Reed et al., 2013) and stress fractures (Prather et al., 2016) in female soccer players, there is limited research on the occurrence of RED-S indicators in this population. As outlined in the RED-S model (Figure 7), potential health consequences that will be looked at in this thesis include diminished BMD, menstrual dysfunction, hormonal imbalances, haematological function, and metabolic changes.

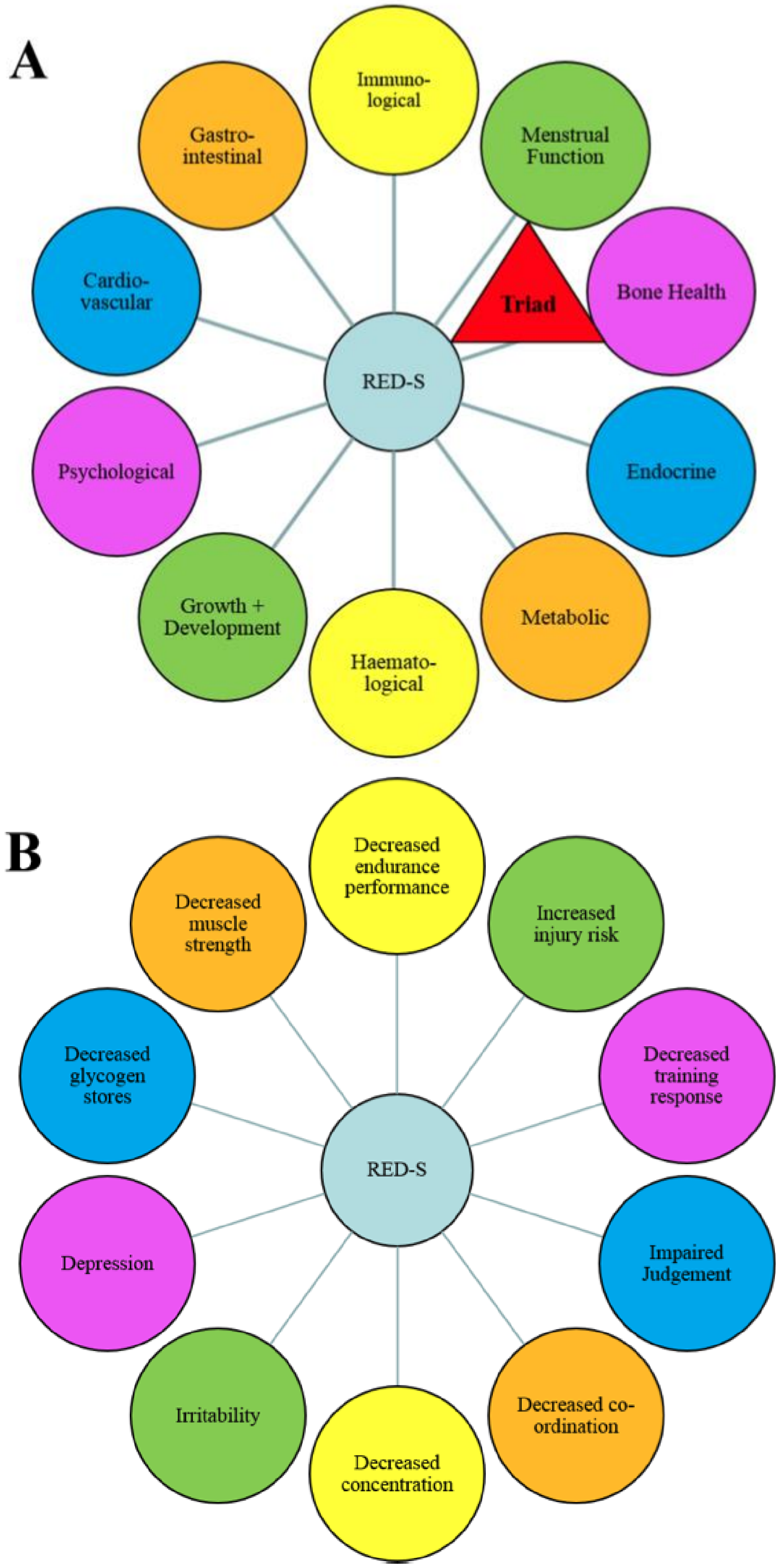


**Figure 7.** Health (A) and Performance (B) factors purportedly affected by relative energy deficiency in sport (RED-S), adapted from Mountjoy et al. (2018).

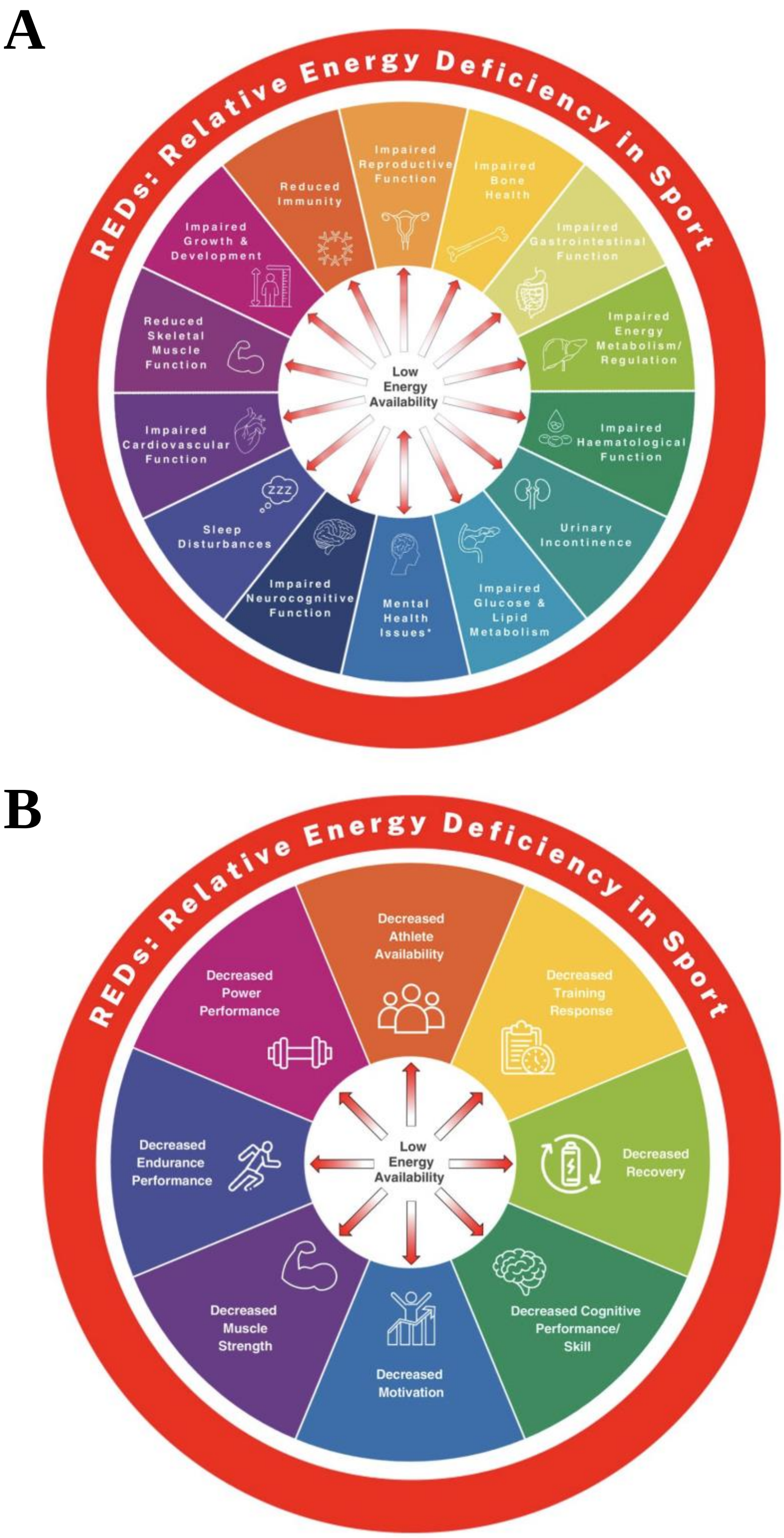


**Figure 8.** Relative energy deficiency in sport. The health (A) and performance (B) conceptual models associated with problematic LEA proposed in 2023. Energy availability is described on a continuum from mild (white arrows) through to problematic (red arrows) adapted from Margo et al. (2023).

## 2.8 Summary and directions for future research

This literature presented, highlights the significant transformation witnessed in female soccer over the past three decades. Unfortunately, research has not kept pace with the surge in participation and increased professionalism during this period. While menstrual function and HC use have been extensively studied in various sports (Cobb et al., 2003; Fahrenholtz et al., 2018; Hoch et al., 2009; Hoch et al., 2011; Martin et al., 2018; Melin et al., 2015; Torstveit and Sundgot-Borgen, 2005a), there exists a notable absence of data pertaining to WSL 1 players. Therefore, future research examining HC and non-HC use, as well as associated symptoms would help practitioners benchmark their players and implement strategies to support players with their menstrual health.

With the increase in professionalism, the demands of matches and training have increased (Datson et al., 2014), potentially leading to changes in body composition. While the gold standard DXA method has been utilised to assess body composition in US collegiate female soccer players over a season (Reed et al., 2013; Stanforth et al., 2014), its application has been limited to single time points in WSL players (Emmonds et al., 2019; Moss et al., 2020). To gain a more comprehensive understanding of body composition changes during a WSL 1 season, it is important to conduct DXA scans at multiple time points in this population, thereby facilitating effective monitoring and assessment of any training, nutritional, or injury interventions.

As female soccer continues to grow, more research is focusing on issues related to LEA, the Female Athlete Triad and RED-S among female soccer players. Studies indicate that menstrual dysfunction rates range from 9.3% to 19.3% among elite female soccer players (Prather et al., 2016; Sundgot-Borgen and Torstveit, 2007), with LEA experienced by 26.3% during pre-season and 33.3% during mid-season (Reed et al., 2013). However, there is a need for further research specifically focusing on WSL players throughout a season to determine the prevalence of LEA in this population. To achieve a comprehensive assessment of LEA in WSL 1 players, it is essential to employ high-quality methods to measure markers, including menstrual health (LEAF-Q), body composition (DXA), blood biomarkers (e.g. $T_3$), energy intake (RFPM), energy expenditure (GPS), and RMR (indirect calorimetry). These measurements assessed longitudinally over a season will provide a more comprehensive understanding of the prevalence and factors affecting LEA in WSL 1 players, addressing a significant gap in the current literature.

# CHAPTER 3

## GENERAL METHODS

*The aim of this Chapter is to provide details of methodologies that were employed in multiple studies within this thesis. Methodologies that were exclusive to a particular study can be found in the relevant Chapter.*

### 3.1 Ethical approval and location of testing

Ethical approval for all studies in this thesis was granted by the Wales Research Ethics Committee, UK (REC approval number: 17/WA/0228), the Ethics Committee of Liverpool John Moores University (ethics number: M18SPS037) and the Nottingham Trent University non-invasive ethics committee. All players were informed that they could withdraw from participation at any stage throughout these studies and provided written informed consent to participate in the study after verbal and written information was provided.

All anthropometric assessments (stature and body mass), DXA scans, RMR assessments, phlebotomy, blood pressure, heart rate and questionnaire distribution were collected in the Tom Reilly Building, Liverpool John Moores University (Figure 9). Blood analysis was conducted in the Duncan Building, Royal Liverpool University Hospital. Training load and match load data occurred on the grass pitches at Finch Farm Training Complex, Halewood, Liverpool (Figure 10). Risk assessments were conducted and approved for all testing locations.

**Figure 9.** The Tom Reilly Building (TRB), Liverpool john Moores University. Facilities within the TRB were used for data collection in chapters 5, 6 and 7.

**Figure 10.** Finch Farm Training Complex. These facilities were used during training and match load data collection in Chapters 5, 6 and 7

## 3.2 Participant characteristics

Female soccer players from WSL 1 clubs volunteered to participate in these studies. A total of 80 players participated in the four studies; 20 of whom were affiliated to one WSL 1 club. A summary of participant characteristics from all four studies can be seen in Table 8. Participant characteristics in each study are included in the relevant chapter.

**Table 8.** Summary of participant characteristics from all four studies, including age, maturity stature and body mass. Data are presented as mean ± SD.

| Study | n | Age (yrs) | Stature (cm) | Body mass (kg) |
|---|---|---|---|---|
| 1<br>(Chapter 4) | 75 | 24.0 ± 3.6 | 168.3 ± 5.7 | 62.1 ± 5.5 |
| 2<br>(Chapter 5) | 15 | 22.1 ± 1.7 | 166.5 ± 4.9 | 60.4 ± 6.1 |
| 3*<br>(Chapter 6) | 20 | 21.8 ± 2.4 | 166.4 ± 3.8 | 59.8 ± 5.8 |
| 4*<br>(Chapter 7) | 1 | 23.3 ± 0.0 | 161.0 ± 0.0 | 55.4 ± 0.0 |

* Participant characteristics at the start of data collection (beginning of 2017/18 season).

## 3.3 Arthrometric assessments

### *3.3.1 Stature and body mass*

Participants arrived in the laboratory within 2 hours of waking. They removed all jewellery and wore only shorts and a sports bra for measures of stature and body mass. Participants stature (SECA, model-217, Hamburg, Germany) and body mass (SECA, model-875, Hamburg, Germany) were measured to the nearest 0.1cm and 0.1kg respectively, according to the International Society for the Advancement of Kinanthropometry (ISAK) guidelines (Marfell-Jones, 2006). Free-standing stature was recorded once participants were stood with feet placed parallel and touching, the calcanei positioned against the back of the height instrument whilst adopting an upright posture. The head was positioned in the Frankfurt plane and the measurement was taken when the height gauge was lowered to the vertex of the cranium. Two measurements were taken for each, with a third being taken if they differed by more than 2%. The mean was recorded if two measures were used, if a third measure was taken the median was recorded.

### *3.3.2 Dual-energy X-ray absorptiometry*

In studies 2 (Chapter 5) and 3 (Chapter 6), each participant underwent a whole body, unilateral hip (left hip) and lumbar spine (L1 – L4) whole beam DXA (Hologic QDR Series, Discovery A, Bedford, MA, USA) scan. The scans were performed by a practitioner certified under the Ionising Radiation (Medical Exposure) Regulations. The effective radiation dose for each scan was 0.001 mSv per person, which is considered a safe and ethical radiation dose (COMARE, 2019). On the morning, before each time point, calibration was carried out using an anthropometric spine and step phantom followed by radiographic uniformity scan according to the manufacturer’s guidelines.

Players presented for their DXA scans under the following protocol: ≥8 hours overnight fast, ≥12 hours after exercise and within 2 hours of waking. Following stature and body mass measurements players were asked to lie in a supine position with inverted feet secured with micropore tape (Nexcare, UK). Arms were positioned along the side of the body with custom made foam positioning aids used to ensure standardised positioning between the arms and the body. Secondly, their left foot was affixed with Velcro to a Perspex (Leeds, UK) triangular platform to invert the head of the left femur to measure hip bone density. Lastly, a box was placed under the popliteal crease of both knees, so the participants knees were at $90^0$ to assess lumbar bone density. All scans were performed and analysed by the same trained operator in accordance with the best practice guidelines (Nana et al., 2016). After ensuring each region was correctly segmented each scan was automatically analysed via the QDR software (version 12.4.3). Data analysed included whole-body and regional FFM and FM, total BMD, Z-score for BMD and hip and lumbar spine BMD. The measures were reported as a subtotal i.e., whole body minus the head. The test-retest reliability of the DXA scanner used for this study has been published previously (Langan-Evans et al., 2021). Laboratory technical error of measurement and CV for whole body FM, FFM and per cent body fat was 1.9% and 0.37kg, 1.0% and 0.44kg and 1.9% and 0.41% respectively, and for BMD measures, CV is <1.5%.

## 3.4 Resting metabolic rate assessments

### *3.4.1 Moxus Modular $VO_2$ indirect calorimeter*

In Study 2 (Chapter 5) RMR was measured via the Moxus Modular $VO_2$ indirect calorimeter (AEI Technologies, Naperville, IL). The Moxus was calibrated before each participant using a certified 3-L calibration syringe. Calibration was deemed to be achieved when measured stroke volume at 60L/min, 90L/min and 120L/min was within ±1.5% of syringe volume. Secondly, oxygen and carbon dioxide gas analysers were calibrated using known gas concentrations of 15.10% $O_2$ and 4.03% $CO_2$. Calibration was complete when measures of oxygen and carbon dioxide concentrations were within ±0.2% and ±0.8% of expected values respectively. Following calibration, participants relaxed in a supine position for 10 minutes with a Hans-Rudolph mouthpiece and nose clip on in a quiet, dark, thermoneutral room (21.5 ± 1°). Subsequently, data was collected continuously for 20 minutes, in which the last 10 minutes was used to calculate RMR. During data collection, participants were asked to breath normally, limit movement, remain awake and avoid talking. $VO_2$ and $VCO_2$ were collected continuously, and mean minute values were provided. The data was stored and processed using the MAX II Metabolic System software (version 1.2.14, Physio-Dyne Instrument Corp, Quoque, NY). $VO_2$ and $VCO_2$ were converted to RMR using the Weir equation (Weir, 1949).

$$Energy\ expenditure\ (kcal \cdot day_{=^{-1}}) = [(\dot{V}O_2 \times 3.941) + (\dot{V}CO_2 \times 1.11)] \times 1440.$$

To ascertain players with low RMR, the ratio between measured RMR and predicted RMR was calculated using the Ten Haaf method (Ten Haaf and Weijs, 2014), with an RMR ratio of <0.90 considered low (De Souza et al., 2008).

### *3.4.2 GEM Open Circuit Indirect Calorimeter*

In studies 4 (Chapter 6) and 5 (Chapter 7) RMR was measured via open-circuit indirect calorimeter (GEM Open Circuit Indirect Calorimeter; GEMNutrition Ltd., Warrington, UK). Players presented for their RMR assessment under standardised conditions: ≥8 hours overnight fast and ≥12 hours after exercise (Compher et al., 2006). The calorimeter was calibrated against known gas concentrations: 'zero' (0.0% $O_2$ and 0.0% $CO_2$) and 'span' (20.0% $O_2$ and 1.0% $CO_2$) gases (BOC, Guildford, UK) and an ethanol burn, to confirm an established respiratory exchange ratio of 0.67. Following calibration participants relaxed for 10 minutes under a transparent ventilated hood in a supine position in a dark, quiet, thermoneutral room (21.5 ± 1°). Subsequently, data was collected continuously for 20 minutes, in which the last 10 minutes was used to calculate RMR. During data collection, participants were asked to breath normally, limit movement, remain awake and avoid talking. $VO_2$ and $VCO_2$ were measured continuously and mean one-minute values provided throughout, with steady state conditions accepted if the CV for $VO_2$ and $VCO_2$ were <10% (Compher et al., 2006). $VO_2$ and $VCO_2$ were determined using the Haldane transformation (Haldane, 1918) and energy expenditure (kcal·day$^{-1}$) using the modified Weir equation (Weir, 1949):

$$\textit{Energy expenditure (kcal·day}^{-1}\textit{)} = [(\dot{V}O_2 \times 3.941) + (\dot{V}CO_2 \times 1.11)] \times 1440.$$

To ascertain players with low RMR, the ratio between measured RMR and predicted RMR calculated using Ten Haaf (Ten Haaf and Weijs, 2014). An RMR ratio of <0.90 was considered as low (De Souza et al., 2008).

## 3.5 Blood pressure

Following measurement of RMR, blood pressure (mmHg) was measured in the supine position on the left arm using an automated blood pressure device (GE DINAMAP ProCare 100-400 Series, Hertfordshire, UK). Measurements were taken 3 times to ensure reliable data and if the mean was not within 5%, a fourth measurement was carried out.

## 3.6 Biomarkers

In studies 2 (Chapter 5) 3 (Chapter 6) and 4 (Chapter 7) all venous blood samples were obtained by an accredited phlebotomist with players in a rested and fasted state. All samples were sent to a United Kingdom Accredited Services (UKAS) pathology laboratory for analysis. Blood was taken from the antecubital vein in the anterior crease of the forearm. 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 (FBC)) were centrifuged immediately on receipt and separated. Aliquots were made for IGF-1, Zinc, testosterone, vitamin D and insulin and stored at -20 degrees until analysis. All other tests including FBC were analysed on the day of measurement. Samples for sodium, potassium, chloride, bicarbonate, urea, creatinine, albumin, protein, alkaline phosphatase, bilirubin, alanine transaminase, gamma-glutamyl transferase, adjusted calcium, phosphate, total cholesterol, High-density lipoprotein cholesterol, triglycerides, magnesium, glucose, creatine kinase, iron, ferritin, transferrin, and C-reactive protein were analysed on the Roche Cobas c701/702 modular analysers (Roche Diagnostics Ltd., Burgess Hill, UK). Samples for cortisol, luteinizing hormone, follicle-stimulating hormone, oestradiol, sex hormone-binding globulin, insulin, thyroid-stimulating hormone, thyroxine, triiodothyronine, prolactin, progesterone, vitamin B12, and folate were analysed on the Roche Cobas e601/602 modular analysers (Roche Diagnostics Ltd., Burgess Hill, UK). Insulin-like growth factor-1 (IGF-1) was analysed on the IDS iSYS analyser (Immunodiagnostics Systems, Boldon, UK). Zinc was analysed by inductively coupled plasma mass spectrometry. Testosterone and Vitamin D were analysed by liquid chromatography-tandem mass spectrometry. The reference values used for analysis were provided by the UKAS laboratory and included females of various age ranges.

### 3.7 Low energy availability, menstrual cycle, and hormonal contraceptive questionnaires

In studies 2 (Chapter 5), 3 (Chapter 6) and 4 (Chapter 7) risk of low energy availability was assessed using the LEAF-Q. This 25-item questionnaire is comprised of questions relating to injury history and gastrointestinal and reproductive function. The LEAF-Q has produced acceptable sensitivity (78%) and specificity (90%) in order to correctly classify EA/reproductive function/bone health (Melin et al., 2014). A score of ≥8 was used to identify participants at long-term risk of low EA (Melin et al., 2014).

To assess menses or HC use, data from the LEAF-Q was used. The questionnaire provided insight to menarche, typical menstrual cycle, previous cycle, bleed length, previous menstrual dysfunction and any previous issues around increase exercise and menstrual cycle. In addition, at each testing time point the research team asked the players when their most recent period was, using day one of the bleed as the definition for this. For HC users the LEAF-Q provided the reasons for use and duration. In addition, at each testing time point the research team asked the HC users which form of HC they were on.

### 3.8 Assessment of dietary intake

In studies 2 (Chapter 5), 3 (Chapter 6) and 4 (Chapter 7) participants electronically tracked dietary intake using the RFPM, which has been previously validated (Costello et al., 2017) and used by our laboratory when assessing adult soccer players (Anderson et al., 2017). While the RFPM method offers several advantages, such as improved accuracy in portion size estimation and higher participant compliance compared to traditional self-report methods, it is not without limitations. For this method the participants provided a picture of their food and drink before and after consumption, which were time stamped, alongside a description of food/drink (including quantities, brand, and cooking methods) and further questions asked by researchers when needed. However, variations in camera angles, lighting, and the lack of standardised objects for scale can lead to inaccuracies in portion size estimation (Stables, Kasper, Sparks, Morton, and Close, 2021). To address this, participants underwent an induction where the RFPM method was explained in detail and a familiarisation day completed. Participant compliance can also be inconsistent, with the potential for missed food items (Stables et al., 2021). To mitigate this each player completed at least one 24-hour dietary recall every other day (using the triple pass method) in an attempt to ensure the player did not omit food/drinks and to cross check the dietary intake information (Capling et al., 2017). These were taken first thing in the morning when the players arrived at the training ground (Figure 10) to ensure maximum accuracy. Additionally, the awareness of being monitored might alter participants' eating habits (Hawthorne effect), leading to atypical eating patterns that do not reflect their usual diet (Stables et al., 2021). Participants were therefore strongly encouraged to consume their typical diet throughout the monitoring period. Dietary intake was analysed by a British Dietetic Association (BDA) and Sports and Exercise Nutrition register (SENr) accredited Dietitian using dietary analysis software (Nutritics, v5, Ireland), which produced energy and macronutrient breakdowns of each day. To account for subjectivity in interpretation, a second SENr accredited nutritionist individually analysed the players dietary intake (Nutritics, v5, Ireland). Estimated dietary intake was reported in kilocalories per day (Kcal.day$^{-1}$) and macronutrient intakes were reported in grams (g) and grams per kilogram of body mass (g.kg$^{-1}$). These were calculated per day and additionally averaged per week when appropriate. In order to determine under or over-reporters the Goldberg cut off (Black, 2000) was undertaken. Cut off values were applied according to age and physical activity level (strenuous). Resting metabolic rate using the Ten Haaf equation was used to calculate the ratio (Ten Haaf and Weijs, 2014). The recommendation to highlight any players outside these but still to include them to avoid bias in small sample sizes was followed (Black, 2000). Although RFPM has its limitations by addressing these issues through rigorous training, standardised protocols and complementary assessment tools enhanced the validity and reliability of dietary intake assessment via this method.

### 3.9 Quantification of training load, match load and energy expenditure during exercise

In studies 2 (Chapter 5), 3 (Chapter 6) and 4 (Chapter 7) during the dietary intake assessment period, training load was monitored using global positioning system (GPS) technology (Apex, STATSports, Newry, Northern Ireland). The portable GPS unit (30 x 80mm, 48grams) sampled (positioning and time, thus velocity and distance) at 10 Hz and has shown valid and reliable estimates of distance and velocity during team sport movement activities (Beato, Coratella, Stiff, and Iacono, 2018). The unit was placed in a custom-made vest that position the unit on the upper back, allowing for optimal exposure for the GPS antennae to gain clear satellite exposure. Global positioning system units were turned on and left outside 30 minutes before use to obtain a satellite lock. After each session, data was cropped from the start of training until the end of the last drill or full time on the manufacture's software (Apex 10 Hz version 2.0.2.4; STATSports). The external load variables selected for analysis were duration (min), total distance covered (km), high speed running (>19.8 km.h$^{-1}$) (m) and explosive distance (accelerations and decelerations above 25.5 W/kg) (m). For energy expenditure during field sessions estimated EEE was derived from GPS units. Individual information was inserted into the software and estimated EEE measured using the manufactures software.

### 3.10 Energy availability

In studies 2 (Chapter 5), 3 (Chapter 6) and 4 (Chapter 7) during the 3-day data collection, energy availability was calculated from the dietary intake record (RFPM), training and match load, and estimated FFM (DXA). To ensure that energy availability was not underestimated, energy expended for RMR during exercise was subtracted from EEE (Loucks, 2014). The established thresholds of optimal (> 45 kcal·kg FFM$^{-1}$·day$^{-1}$), reduced (30-45 kcal·kg FFM$^{-1}$·day$^{-1}$), and low (<30 kcal·kg FFM$^{-1}$·day$^{-1}$) were used as the reference levels for comparing the players energy availability (Loucks et al., 2011; Mountjoy et al., 2014).

### 3.11 Statistical analysis

Statistical analyses for Studies 1 and 2 (Chapter 4 and 5) were completed using SPSS version 28.0 (SPSS, Inc., Chicago, IL, USA) and statistical analyses for Study 3 (Chapter 6) were completed using Jamovi Version 2.3 (The Jamovi project, https://www.jamovi.org). All data were initially assessed for normality of distribution using the Shapiro–Wilk's test. Data are reported as means ± SD, and $P \leq 0.05$ was considered statistically significant. Figures were produced in GraphPad Prism version 10.0 software (GraphPad, San Diego, CA). In Study 1 (Chapter 4) independent samples t-tests were used to compare HC and non-HC users. In Studies 3 and 4 (Chapters 5 and 6) statistical comparisons between positions or training days and variables were performed using a one way between groups analysis of variance (ANOVA) with either Tukey's HSD post hoc (parametric) or Kruskal-Wallis test (non-parametric) undertaken to locate the significant difference for position and training days. In Study 4 (Chapter 6) linear mixed model was conducted to assess differences between position, time points, training days and the variables. A Holm-Bonferroni post hoc test was carried out to locate significant differences between position, training, and time points.

# CHAPTER 4

## An audit of hormonal contraceptive use in Women's Super League soccer players; implications on symptomology.

The aim of this Chapter was to audit hormonal contraceptive and non-hormonal contraceptive use, determine the reasons for initiation and discontinuation of hormonal contraceptives, and report the symptoms experienced by hormonal contraceptives users and non-users, in Women's Super League 1 players.

***This study was published in Science and Medicine in Football in 2021.***

Parker L.J, Elliott-Sale K.J, Hannon M.P, Morton J.P, Close G.L. (2021). An audit of hormonal contraceptive use in Women's Super League soccer players; implications on symptomology. *Science and Medicine in Football*. 6 (2), 153–158.

## 4.1 Abstract

**Purpose:** To audit hormonal contraceptive use and associated symptomology in elite female soccer in England.

**Methods:** 75 elite female soccer players from the Women's Super League completed a questionnaire to assess: HC use or non-use, reasons for initiation and discontinuation and the symptoms experienced by HC and non-HC users.

**Results:** 28% reported current HC use, with 43% having used HCs previously. Combined HCs accounted for 62% of total usage, with progestin-only HCs making up the remainder. 86% pre-empted negative symptoms before commencing HCs, with 38% experiencing adverse symptoms. Negative symptoms were most common in progestin-only HC users (63%). 86% reported benefits associated with HC usage including pain management and the ability to predict or control their cycles. Six non-HC users reported amenorrhea, with one medically diagnosed. Negative menstrual cycle related symptoms were reported by 74%, with 4% unable to train due to these symptoms. Unfavorable symptoms typically occurred during the first days of menstruation (59%).

**Conclusion:** Most WSL players do not currently use HCs (72%). Most HC users reported benefits of HC usage, whilst most non-HC users reported negative symptoms especially around menstruation. Practitioners should track players menstrual cycle to help minimise discomfort and maximise performance.

## 4.2 Introduction

In 2018 the WSL 1 became the first professional female soccer league in England. Since then, female soccer has grown in popularity and participation. The increased number of players and competitions is driving the need for researchers and practitioners to understand the potential impact of ovarian steroid hormones on training and performance. To capitalise on previous research in this field (Julian, Hecksteden, Fullagar, and Meyer, 2017) and to underpin future research, there is a need to define the menstrual characteristics (*i.e.*, hormonal contraceptive use and non-use) of elite female soccer players.

A greater number of athletes use HCs (49.5%) compared to the general population (30%) (Cea-Soriano et al., 2014; Martin et al., 2018). HCs are exogenous steroid hormones that inhibit ovulation and result in consistently low concentrations of endogenous oestrogen and progesterone (Elliott-Sale and Hicks, 2018). Data from Schaumberg et al. (2018) showed that athletes use HC for perceived performance benefits, however data from a recent review indicated that HCs may impair performance in some sportswomen (Elliott-Sale et al., 2020). It is crucial to establish how many WSL players use a HC, their reason for usage and any side-effects experienced, in order to ascertain their potential implications for players (i.e., this approach will help to minimise any potential negative effects on performance and maximise any potential positive effects on adverse menstrual cycle symptoms (Oxfeldt, Dalgaard, Jørgensen, and Hansen, 2020), thus ensuring that players can perform to the best of their ability on any given day, regardless of ovarian hormone profile).

In the absence of menstrual irregularities, non-HC users typically have an idealised 28-day menstrual cycle, although cycles can vary between 21 and 35 days (Lamina et al., 2013). Abnormalities of the menstrual cycle occur in 6-79% of sportswomen, with the prevalence of menstrual dysfunction varying with athletic discipline and level of competition (Warren and Perlroth, 2001). Whilst menstrual function data has been collected in many types of sportswomen (Cobb et al., 2003; Fahrenholtz et al., 2018; Hoch et al., 2009; Hoch et al., 2011; Melin et al., 2015; Torstveit and Sundgot-Borgen, 2005a), there are limited data in female soccer (Prather et al., 2016; Reed et al., 2013; Sundgot-Borgen and Torstveit, 2007) and currently no data on players in England since soccer turned professional. It is important to determine the menstrual characteristics of non-HC users in order to understand their potential consequences for players.

The aim of this study was to audit HC and non-HC use, determine the reasons for initiation and discontinuation of HCs, and report the symptoms experience by HC users and non-users, in WSL players. These data will build upon previous information gathered in 2015-2016 by Martin et al. (2018) in a diverse range of sportswomen, by providing soccer-specific information relevant to the professional game. These findings can be used by practitioners to benchmark their own players and implement strategies to support players with their reproductive health and associated conditions.

## 4.3 Methods

### *4.3.1 Participants*

Between 2017 and 2018, players were recruited from the WSL. All ten WSL clubs were contacted, and six clubs participated. Players were contacted through support staff, who took written informed consent and administered the questionnaires. All players were recruited using the same procedures; *i.e.*, no player or type of player was specifically targeted for recruitment. Each player returned their own questionnaire in individual, sealed envelopes. Athletes had to be >18 years old, contracted to a WSL club and have played at least one game in the WSL. 75 players out of 194 in the WSL completed the questionnaire (Figure 11). All players were nulliparous. The study was approved by the non-invasive ethics committee at Nottingham Trent University. Written informed consent was obtained from each player with the acknowledgement that their data would be used for publishing and fully anonymised.

### *4.3.2 Questionnaire*

Data were collected using a modified version of a previously designed questionnaire (Martin et al., 2018). The questionnaire was adapted to remove questions relating to categorising the sport involved. It was designed to identify the self-reported: (1) period prevalence and characteristics of HC and non-HC users; (2) reasons for initiation and discontinuation of HCs; and (3) perceived symptoms associated with HC use and non-use. As such, all data reported in this study are 'self-reported' and 'perceived'. Participants were required to record demographic information, age of menarche, competitive level, time competing at this level, and weekly training frequency and duration (Table 9). Participants were required to complete different sections of the questionnaire depending on HC use or non-use. The final section of the questionnaire required all participants to provide details on previous HC use, including preparation, duration of use and reason for discontinuation.

### *4.3.3 Data analysis*

Data were analysed using Microsoft Excel. To prevent duplicate data, date-of-births and identical values were visually checked. HC and non-HC user characteristics were compared using independent samples t-tests; the level of significance was set at $P \leq 0.05$. All data were handled in the same way. For qualitative questions, content analysis was conducted separately by two researchers to code responses and identify key themes. These codes were then refined into broader categories. Next, a thematic analysis was performed to identify patterns and construct narratives, using direct quotes to illustrate key findings. Differences between researchers at each stage were discussed until a consensus was reached. Data are represented as means (±SD), frequencies and percentages.

**Table 9.** Characteristics for the Football Associations Women's Super League players; hormonal contraceptive and non-hormonal contraceptive users.

| Demographic information | HC users | Non-HC users | P value | Total |
|---|---|---|---|---|
| Number of participants | 21 | 54 | | 75 |
| Age (y) | 23.7 ± 3.2 | 24.1 ± 3.8 | 0.643 | 24.0 ± 3.6 |
| Height (m) | 1.69 ± 4.7 | 1.67 ± 6.1 | 0.405 | 1.68 ± 5.7 |
| Body Mass (kg) | 63.3 ± 3.6 | 61.7 ± 6.1 | 0.248 | 62.1 ± 5.5 |
| Body mass index ($kg{\cdot}m^2$) | 22.3 ± 1.0 | 22.1 ± 2.0 | 0.589 | 22.1 ± 1.8 |
| Age at menarche (y) | 13.5 ± 1.3 | 14.0 ± 1.7 | 0.215 | 13.9 ± 1.6 |
| Duration competing at current level (y) | 6.2 ± 3.4 | 5.9 ± 3.9 | 0.820 | 6.1 ± 3.8 |
| Number of training sessions per week | 6.2 ± 1.7 | 6.3 ± 1.3 | 0.794 | 6.3 ± 1.4 |
| Average training session duration (mins) | 88.1 ± 12.7 | 93.4 ± 19.3 | 0.245 | 92.3 ± 17.9 |
| Total weekly training duration (mins) | 555.1 ± 183.1 | 598.8 ± 205.8 | 0.357 | 587.2 ± 198.9 |

All data expressed as mean (SD). No significant difference existed between groups for any variable. Significant difference was set at $P < 0.05$. HC, hormonal contraceptive

## 4.4 Results

### *4.4.1 Period prevalence and characteristics of hormonal contraceptive users*

The period prevalence and descriptive characteristics of HC users are presented in Figure 11 and Table 9. The average length of HC usage was 3.1 ± 3.1 years. The main reasons for choosing a particular HC method were: General Practitioner advice (38%), easiest form (20%), and friends' recommendation (14%). Only 55% of players discussed their HC use with their coach or team doctor.

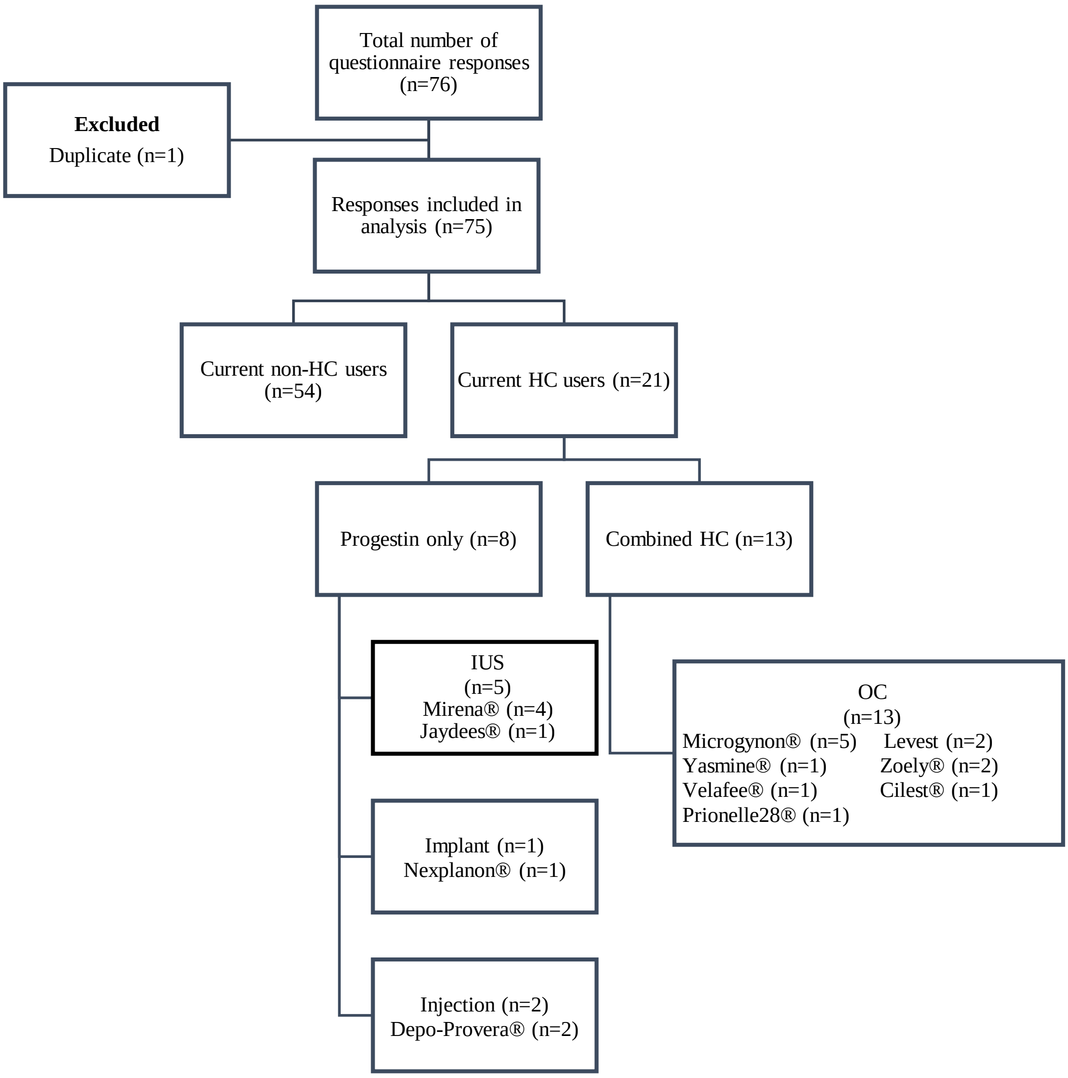


**Figure 11**. The prevalence and characteristics of hormonal contraceptive users and non-users. IUS, intrauterine system; OC, oral contraceptive; HC, hormonal contraceptive

### *4.4.2 Previous hormonal contraceptive use*

Of the players that did not currently use HCs, 20% reported previous usage. Of the players with current HC use, 19% had previously used a different HC formulation. The reasons for discontinuing or switching HC formulation are shown in Figure 12C.

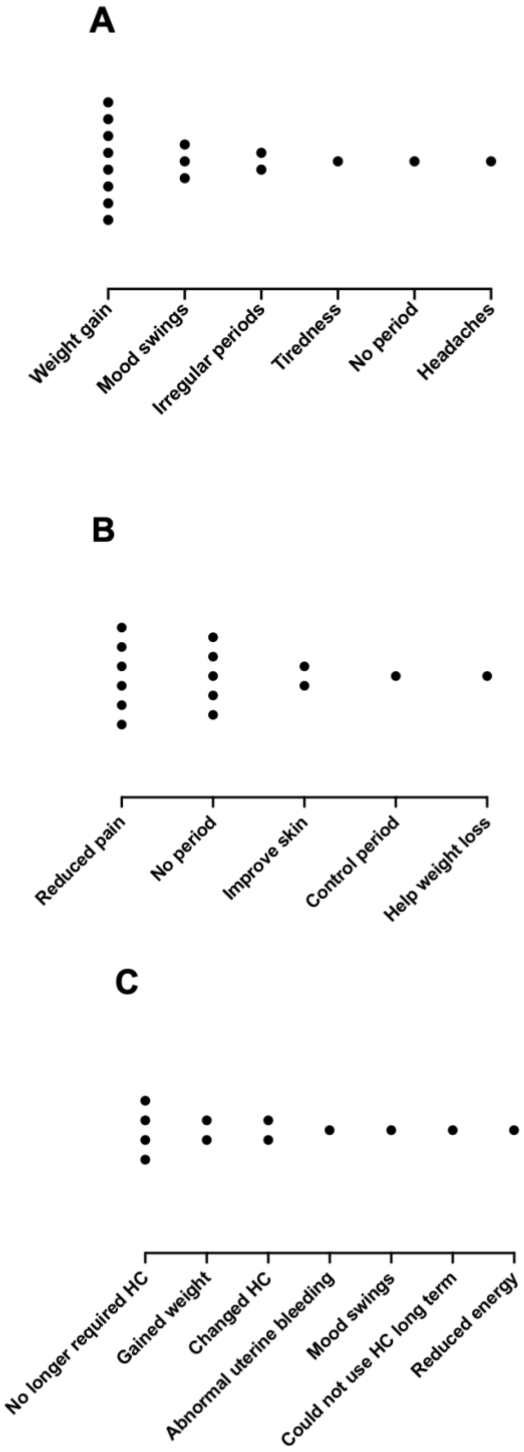


**Figure 12.** Anticipated negative symptoms before commencing hormonal contraceptives (A), anticipated positive symptoms before commencing hormonal contraceptives (B), reasons for discontinuing or switching hormonal contraceptives (C). Please note that each dot represents an individual person.

### 4.4.3 Symptomology of hormonal contraceptive users

The frequency and prevalence of symptoms experienced by players using HCs are presented in Table 10. Figure 12A and 12B shows the anticipated positive and negative side-effects before players commenced HCs. Negative symptoms were more common in progestin-only HCs (63%) compared with combined HCs (23%).

**Table 10.** Sample frequency (n = 21) and prevalence of self-reported perceived negative and positive symptoms experienced by hormonal contraceptive users.

| Negative effects | Sample frequency | Prevalence (%) | Positive effects | Sample frequency | Prevalence (%) |
|---|---|---|---|---|---|
| Mood swings | 2 | 10% | Regular period | 6 | 29% |
| Still got period | 2 | 10% | No period | 6 | 29% |
| Weight gain | 1 | 5% | Less pain | 5 | 24% |
| Acne | 1 | 5% | "Continue day" | 2 | 10% |
| Feeling sick | 1 | 5% | Skin | 1 | 5% |
| Headache | 1 | 5% | "Feel faster" | 1 | 5% |
|  |  |  | Lighter flow | 1 | 5% |

"Continue day" and "feel faster" are direct quotes taken from questionnaire responses therefore we are unable to interpret the exact meanings.

### 4.4.4 Period prevalence and characteristics of non-hormonal contraceptive users

The period prevalence and descriptive characteristics of non- HC use are presented in Figure 11 and Table 9. None of the non-HC users used a non-hormonal IUD. Mean cycle length was 31±10 days, with 67% reporting that their cycle varied in length (cycle length varied by 3–63 days). Six players reported being amenorrhoeic and eight players reported being oligomenorrheic, although only one case of amenorrhea was diagnosed by a clinician.

### 4.4.5 Symptomology of non-hormonal contraceptive users

The frequency and prevalence of adverse symptoms for non-HC users are presented in Table 11. Adverse menstrual cycle related symptoms were reported by 74% of players, with 4% stating that these symptoms had prevented them from training. Reasons for having to miss training included 'heavy bleed', 'back pain,' and 'being sick'. These issues mainly occurred during the first 2-3 days of menstruation (59%) and in the days leading up to menstruation (17%). Medication was used by 19% of the players to deal with menstrual-related issues, with 13% using nonsteroidal anti-inflammatory drugs (NSAIDs) and 6% taking paracetamol.

**Table 11.** Sample frequency (n = 54) and prevalence of negative self-reported perceived symptoms during the menstrual cycle for non-hormonal contraceptive users.

| Symptom | Sample frequency | Prevalence (%) |
|---|---|---|
| Cramps | 38 | 70% |
| Sore back | 8 | 15% |
| Tiredness | 7 | 13% |
| Loss of appetite | 2 | 4% |
| Sore legs | 2 | 4% |
| Vomiting/feeling sick | 2 | 4% |
| Mood swings | 2 | 4% |
| Bloating | 1 | 2% |
| Sore breasts | 1 | 2% |

### 4.5 Discussion

The aim of the present study was to audit HC and non-HC use in the WSL and to describe the associated symptomology. This study provides novel data on the reproductive characteristics of elite female soccer players. Less than a third of players currently use a form of HCs. From the players using HCs, 86% reported benefits and 38% reporting negative symptoms. From the non-HC using players, 74% reported negative symptoms.

In the non-HC users, all players without menstrual irregularities fulfilled the eumenorrheic criterion for cycle length (Lamina et al., 2013). Six players reported amenorrhea, although only one player was diagnosed by a clinician. Eight players reported oligomenorrhea, none of which were diagnosed by a clinician. Although, our questionnaire did not explore the reasons behind this observation, it highlights that WSL players are not discussing their menstrual characteristics with a healthcare professional. This observation is supported by recent data (Findlay, Macrae, Whyte, Easton, and Forrest, 2020; Larsen, Morris, Quinn, Osborne, and Minahan, 2020), which suggests that: (i) sportswomen have little/no medical support; (ii) sportswomen do not feel confident enough to disclose their menstrual issues with support staff;

(iii) support staff often do not have a good understanding of the best practice to deal with menstrual cycle/HC symptoms; and (iv) sportswomen do not have a full understanding about amenorrhea and their own menstrual function. As such, we recommend that players and support staff receive suitable training/education such that they have a good understanding of menstrual function and dysfunction, from both a health and performance perspective; *e.g.*, as a potential indicator of RED-S. As menstrual irregularities were self-reported in this questionnaire, future research should involve medical screening/diagnosis to provide data on how prevalent menstrual irregularities are in WSL.

Nine negative symptoms were reported by non-HC users (Table 11), with 74% of players reporting at least one symptom. This is similar with the general population (Ju, Jones, and Mishra, 2014) and other elite athletes (Martin et al., 2018), although a study in elite female rugby players reported that 93% of players had negative menstrual cycle related symptoms (Findlay et al., 2020). A limitation of the present study was that it did not specifically ask if these symptoms could have been affected by factors other than the menstrual cycle (*e.g.*, a medical condition, such as endometriosis), however, data collected on current medication usage did not infer any of the players having a medical condition that would have significantly influenced the reported symptoms. Symptoms were mainly experienced in the initial days of menstruation (59%), but also occurred in days leading up to menstruation (17%). In the present study, only two players reported that they missed training as a result of their menstrual cycle symptoms, thus supporting previous research in elite athletes (Martin et al., 2018). Despite non-HC users reporting symptoms interfering with their training, there was no statistical difference between groups in number of training sessions per week, average training session duration, or total weekly training duration (Table 9). A limitation of the present study was that we did not establish the severity of the symptoms experienced, the frequency of missed training sessions or if players trained despite feeling unable to do so. Findlay et al. (2020) reported that some women’s rugby players, no matter the severity, did not feel that their menstrual related symptoms were an acceptable reason to take rest or abstain from training. With 74% of players in the current study suffering from symptoms, further research is required to understand the frequency, severity, duration, and impact of these on training, and if ‘soccer’ allows players to adapt their training without negative repercussions. Ideally, practitioners need to allow flexibility in training sessions to accommodate the most severe symptoms.

The prevalence of HC use in elite female soccer players (28%) is similar to that of the general British (30%) (Cea-Soriano et al., 2014) and American (27.6%) public (Daniels, Daugherty, and Jones, 2014), but less than in other elite female sports (47.1% and 49.5%) (Larsen et al., 2020; Martin et al., 2018). This lower prevalence might be accounted for by the infancy of the professional soccer game. Previous research indicates that athletes often manipulate and control their menstrual cycle by using HCs (Schaumberg et al., 2018), and that elite (43%) versus sub-elite (22.5%) athletes are more likely to do this (Martin et al., 2018). In addition, this lower prevalence might be accounted for less severe symptoms in the non-HC group, meaning that they did not feel the need to use a HC in response to their symptoms. It will be interesting to track if more WSL players switch to using HCs to manipulate their menstrual cycle in the coming years as the sport becomes more professional.

Most players anticipated negative symptoms before taking HCs (76%), with weight gain being the most common (33%) concern. However, only 38% experienced negative symptoms; with mood swings and still having a “period” the most common issues (both 10%), and only one player reporting weight gain. Negative symptoms were most common in progestin only-HC users compared to combined HC users, which is a similar to previous findings (Martin et al., 2018). It is important to note that each pill formulation carries its own unique concentration of synthetic hormones which needs to be considered when interpreting data. These pre-conceived ideas and lived experienced can be used to guide players to make informed HC choices.

### 4.6 Conclusion

This study is the first to audit HC and non-HC use in the WSL. Less than one third of WSL players use a form of HC. Although most HC users reported benefits of HC use, it is not the intention of this paper to endorse or denounce HC use, as we do not yet fully understand if the symptomatic benefits of HC result in any adverse health or performance-related consequences. Almost three quarters of players reported negatives symptoms during their menstrual cycle, with 4% of players abstaining from training due to these unfavourable effects. These data can be used to inform support staff about the hormonal profile of female soccer players and to consider interventions in line with the demands and features of their sport.

With 26% of the player self-reporting a form of menstrual dysfunction (amenorrhea or oligomenorrhea) and only one player being diagnosed by a clinician, it is crucial to better understand the prevalence of this issue. One major cause of menstrual dysfunction in female athletes is LEA (Mountjoy et al., 2014; Roupas and Georgopoulos, 2011). Currently, there is no data on the prevalence of, or factors affecting, LEA in WSL 1 soccer players. Therefore, the next chapter will assess the EI, EE, body composition, menstrual function, and blood biomarker profiles of a single squad during pre-season. In this way, the next Chapter will aim to assess the prevalence and factors affecting LEA among WSL 1 players during pre-season.

# CHAPTER 5

## Observational study assessing nutritional intake, energy expenditure, energy availability, body composition and blood biomarkers among elite female soccer players in Women’s Super League

## 5.1 Abstract

**Purpose:** To examine the prevalence and factors affecting LEA including nutritional intake, body composition, and biomarker profiles among elite female soccer players during pre-season in WSL 1.

**Methods:** Fifteen players underwent assessments using GPS, RFPM, DXA, RMR and blood analysis during pre-season, incorporating a match day, training day, and rest day to capture variations influenced by competitive demands.

**Results:** Just under half (40%) of the squad were classified at risk of LEA, however energy availability was optimal for over half of the players (53%). No significant differences were shown between training days for total EI (P = 0.28), all three macronutrient intakes (carbohydrates (P = 0.44); protein (P = 0.18); and fat (P = 0.98)) or macronutrient intakes relative to body mass (carbohydrate/kg (P = 0.54); protein/kg (P = 0.19); and fat/kg (P = 0.98)). None of the players reported consuming the recommended 7g·kg-1·d$^{-1}$ of carbohydrates on match day with 47% consuming less than 3 g·kg-1·d$^{-1}$. Over the three days, 33% of players consumed <3g·kg-1·d$^{-1}$ of carbohydrate. There was a significant difference in EEE between the light training day and match day (P = 0.001) but no significant different in EEE between player position (P = 0.100).

**Conclusion:** None of the squad members exhibited LEA when measured over a 3-day period. Players did not significantly adjust their energy or carbohydrate intake based on the intensity of their training sessions with almost half of the squad (47%) consuming less than 3g·kg-1·d$^{-1}$of carbohydrates on match day.

## 5.2 Introduction

After conducting an audit of HC usage among WSL soccer players in Study 1 (Chapter 4) and considering the rapid transition of female soccer players from amateur to professional levels, it is crucial for practitioners to gain further understanding of female physiology and sport specific nutritional needs. Within soccer, the support staff bear the responsibility of safeguarding players' health and well-being whilst optimising their performance. While research within men's soccer is well-established, the field of female soccer is still in a developmental stage. Given the inherent physiological disparities between the two sexes, it is inaccurate to directly apply all insights from men's soccer to female players, without first testing for sex-specific responses. In men's soccer, teams commonly undergo extensive testing during pre-season to gain a comprehensive understanding of players' physiological states to improve performance and reduce injury risk. However, due to the infancy of professionalism and funding in female soccer, achieving the same level of testing in female soccer is currently unfeasible. Research that has published testing data on female soccer players has often focused on a few isolated markers, such as dietary intake and body composition (Clark et al., 2003), menstrual function and bone health (Sundgot-Borgen and Torstveit, 2007), or energy availability (Reed et al., 2013). However, this research has previously not included elite WSL 1 players or been conducted on the same squad.

Body composition testing is valuable for coaches and practitioners to help inform training, talent development, and nutrition support. Traditionally, skinfold calipers were employed for such assessments. However, many currently consider the gold standard method to be DXA due to its ability to provide consistent measurements of FM, FFM, and estimates of BMD (Randell et al., 2021). By incorporating these additional measurements, DXA contributes to fostering a more positive atmosphere around body composition evaluation by eliminating environments that focus solely on body fat. Due to cost and availability, DXA body composition testing is still relatively inaccessible in female soccer. To date, it has only been carried out in United States colligate female soccer players (Minett et al., 2017; Stanforth et al., 2014). Therefore, this research will aim to be the first to report DXA measured body composition of professional female players in WSL 1 to provide norms within this population.

Energy availability is one of the three inter-relating components of the Female Athlete Triad, along with menstrual function and BMD (Nattiv et al., 2007). When energy availability is reduced, known as LEA, it can lead to detrimental health and performance outcomes, such as decreased metabolic rate, menstrual irregularities, and compromised bone health (De Souza, Hontscharuk, Olmsted, Kerr, and Williams, 2007; Loucks, 2003). LEA is generally defined as being below 30 $\text{kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$ (Ihle and Loucks, 2004; Loucks and Thuma, 2003). To date, research on energy availability among female soccer players is limited. A study conducted on Division 1 collegiate female players showed that 29% of players experienced LEA at some point during the season, with 26% of players exhibiting LEA specifically during pre-season (Reed et al., 2013). During mid-season, 67% of the players reporting menstrual dysfunction exhibited an average energy availability for the season below 30 $\text{kcal}\cdot\text{kg FFM}^{-1}\cdot\text{day}^{-1}$. These data suggests that there is a LEA issue amongst female collegiate soccer players and warrants further investigation among players in the WSL 1.

Energy intake is a pivotal aspect of energy availability and prior research has highlighted that insufficient EI was the main contributor to LEA in female soccer players (Reed et al., 2013). Accurately measuring EI is crucial not only for assessing energy availability but also for evaluating macronutrient intake in relation to performance. Historically, recommended amounts of carbohydrates for female soccer players have come from male soccer data (Krustrup et al., 2006) but insights from female specific glycogen storage physiology need to be taken into account (Thomas et al., 2016). Glycogen stores can be lower during the mid-luteal phase of the menstrual cycle compared to the mid-follicular phase (McLay et al., 2007). However, studies have demonstrated that female athletes can effectively increase muscle glycogen stores and diminish the difference in glycogen storage between the two phases if dietary intake of carbohydrate is adequate (>8g $g \cdot kg^{-1} \cdot d^{-1}$) (McLay et al., 2007; Tarnopolsky et al., 2001). It is therefore considered best practice for female soccer players to consume 5-7 $g \cdot kg^{-1} \cdot d^{-1}$ on moderate training days, increasing to >7$g \cdot kg^{-1} \cdot d^{-1}$around match days (Collins et al., 2021; Maughan and Shirreffs, 2007). Previous investigations involving female soccer players reported daily carbohydrate intakes ranging from 4.1–4.7$g \cdot kg^{-1} \cdot day^{-1}$ (Martin et al., 2006; Mullinix et al., 2003). However, with these quantities, it is unlikely that female players will adequately increase liver and muscle glycogen stores around match days. Notably, these studies (Martin et al., 2006; Mullinix et al., 2003) were conducted with international teams and currently, no data are available for WSL 1 players.

Dietary protein plays a crucial role in stimulating MPS as protein provides the building blocks to help remodel and rebuild muscle post exercise. Despite the importance of protein to support recovery, there are currently no studies that have assessed protein requirements in female soccer players. Recent evidence has emerged indicating that females engaging in intermittent exercise may benefit from protein intake at approximately 1.7$g \cdot kg^{-1} \cdot day^{-1}$ (Wooding et al., 2017). Furthermore, while not directly related to soccer, a study involving female dancers demonstrated that a protein intake of 1.8 $g \cdot kg^{-1} \cdot day^{-1}$ as opposed to 1.3$g \cdot kg^{-1} \cdot day^{-1}$ accelerated the recovery of muscle function following intermittent sprint exercise (Brown et al., 2018). Based on limited current knowledge, there seems to be no need to alter this protein recommendation according to the menstrual cycle or hormonal contraceptive use (Wooding et al., 2017). Previous protein intake in US based female soccer players has reported intakes of 1.2$g \cdot kg^{-1} \cdot day^{-1}$ (Martin et al., 2006) and 1.3$g \cdot kg^{-1} \cdot day^{-1}$ (Mullinix et al., 2003), both of which do not align with the recommendations of Wooding et al. (2017). Research is required to assess the protein intake of WSL 1 players to inform if further education and adjustment is needed.

Dietary fat is important for female soccer players as fat serves as an energy source and a vehicle for uptake and absorption of fat-soluble vitamins and essential fatty acids. As with previous macronutrients, there are currently no specific fat recommendations for female soccer players, with the only previous reported intakes being $0.9g \cdot kg^{-1} \cdot day^{-1}$ (Martin et al., 2006; Mullinix et al., 2003). A reduction in fat intake could potentially lead to an overall caloric deficit, increasing the risk of LEA and subsequent health and performance issues. Therefore, this research will assess fat, carbohydrate, and protein intake, to add to the literature and provide a depiction of current intakes in WSL 1 players.

Biomarker assessment during pre-season is an objective method for evaluating overall player health. A variety of measures can be taken that will provide insight into stress, energy availability, dietary intake, bone health, and immune function. Of particular interest is the iron profile of players. Iron is used by the body for several processes such as oxygen transport and energy production (Beard, 2001). A female soccer player's iron requirements are likely to be higher than the general population due to foot strike haemolysis, gastrointestinal bleeding, anti-inflammatory drug use, and exercise-induced inflammation (Pedlar, Brugnara, Bruinvels, and Burden, 2018). To date, no data on elite female soccer players in WSL 1 has been published. During pre-season this insight would be invaluable for practitioners to identify potential issues and correct nutritional deficiencies before the season starts.

Resting metabolic rate contributes to TEE and helps inform nutritional strategies. Moreover, existing evidence indicates that athletes with LEA suffer with a suppressed RMR (De Souza et al., 2007; Koehler et al., 2016; Kosmiski, Schmiege, Mascolo, Gaudiani, and Mehler, 2014). To date, no research has measured RMR using indirect calorimetry in professional female soccer players. These data would be useful to obtain more accurate measures of TEE, but also aid as a potential screening tool for LEA (Melin et al., 2015).

Elite female soccer players typically cover ~10km per match, with 22-28% of this being HSR and sprinting (Vescovi and Favero, 2014). However, research on EEE in elite female soccer players during both matches and training remains limited. Initial findings from Brewer (1994) indicates EEE during a professional match to be ~1100kcal. More recent research by Mara et al. (2015) indicates that EEE during match play was ~644kcal and training ~607kcal. It is worth noting that the match play in their study involved a simulated friendly game consisting of 3 periods of 20 minutes each, which does not accurately represent a typical 90-minute match.

Therefore, measuring training loads and EEE in WSL 1 players will help practitioners more effectively prescribe dietary interventions and recommendations, and contribute to the broader understanding of energy availability in WSL 1 players.

In 2016 the WSL 1 in England transitioned to a winter calendar for the first time since its inception in 2011. With the league becoming more commercialised there is a demand for increased performance and decreases in injuries therefore gaining a better physiological and nutritional understanding of these players is crucial. Therefore, this study was designed to provide much needed data on the nutritional and physical characteristics of elite female soccer players in the WSL 1. The aims of this study were to: (1) measure the energy availability of female professional soccer players in WSL 1 across a rest day, light training day, and match day; (2) assess the macronutrient intake of the players across a rest day, light training day, and match day; (3) measure the players body composition and bone health (4) analyse players biomarkers; (5) measure players RMR and EEE and (6) monitor their training load over a three day period.

## 5.3 Methods

### *5.3.1 Participants*

Fifteen professional female soccer players (Table 12) consisting of 13 outfield players and 2 goalkeepers from the same team were recruited. Players were undertaking a pre-season before competing in the top division in England (WSL 1). On training days (approximately four days per week), the club provided a self-selected buffet breakfast and lunch. Ethics was approved for the study as reported in Chapter 3 (3.1)

### *5.3.2 Experimental design*

All players undertook a 3-day monitoring period to measure dietary intake (DI) and energy expenditure during exercise to calculate energy availability. This assessment took place within the first three weeks of pre-season (August 2017) and consisted of a pre-season match day (day 1), a day off (day 2) and a light training day (day 3). As this study aimed to monitor players in free-living conditions, no interventions were in place to alter any aspect of the players habitual routine or diet.

Within a week of the 3-day monitoring period players attended Liverpool John Moores University (section 3.1). They arrived between 7.30 and 10.30 h having fasted from midnight.

They completed the following measurements in the specified order: body composition, RMR, blood sample, blood pressure and a questionnaire assessing energy availability (Chapter 3). Ten players blood samples were taken with five players choosing not to participate in this aspect of the study.

### *5.3.3 Procedures*

During the 3-day monitoring period, players electronically tracked their DI using the RFPM, which was then assessed by a nutritionist accredited by both the BDA and SENr using Nutritics software (Nutritics, v5, Ireland). To identify potential under or over-reporters, the Goldberg cutoff method (Black, 2000) was employed, with an EI:RMR ratio outside the range of 1.16 to 2.78 classified as under or over-reporting (refer to section 3.8). During DI assessment, training, and match loads, as well as EEE were monitored using GPS technology (Apex, STATSports, Newry, Northern Ireland) (refer to section 3.9). Energy availability was then calculated using the DI records (RFPM), training and match data, and estimated FFM (DXA). Optimal (> 45 kcal·kg FFM$^{-1}$·day$^{-1}$), reduced (30-45 kcal·kg FFM$^{-1}$·day$^{-1}$), and low (<30 kcal·kg FFM$^{-1}$·day$^{-1}$) thresholds established by Loucks et al. (2011) and Mountjoy et al. (2014) were used as reference levels for comparing players' energy availability (refer to section 3.10).

On the testing day at Liverpool John Moores University, players arrived within 2 hours of waking, where their height and body mass were measured (refer to section 3.3.1). Players then underwent a DXA scan in a supine position to assess whole-body composition, as well as unilateral hip and lumbar spine scans (refer to section 3.3.2). Following the scans, players underwent RMR assessment. Calibration of the Moxus Modular $VO_2$ indirect calorimeter (AEI Technologies, Naperville, IL) was performed before each measurement (refer to section 3.4.1). After calibration, players rested supine for 10 minutes before data collection commenced for 20 minutes, with the final 10 minutes used to calculate RMR (refer to section 3.4.1). To identify players with low RMR, the ratio between measured RMR ($_m$RMR) and predicted RMR ($_p$RMR) was calculated using the Ten Haaf method (Ten Haaf and Weijs, 2014), with an RMR ratio of <0.90 considered low (De Souza et al., 2008).

Following the RMR measurement, blood pressure was measured in the supine position (refer to section 3.5). Blood samples were then collected from the antecubital vein in the anterior crease of the forearm and sent to a UKAS lab for analysis (refer to section 3.6). Finally, players completed two questionnaires: the LEAF-Q to assess risk of LEA (refer to section 3.7) and a questionnaire providing self-reported insights into HC use or menstrual function (refer to section 3.7).

### *5.3.4 Statistical analysis*

Statistical analysis was completed using SPSS version 28.0 (SPSS, Inc., Chicago, IL, USA). Data are reported as means ± standard deviations (SD), and $P \leq 0.05$ was considered statistically significant. Figures were produced in GraphPad Prism version 10.0 software (GraphPad, San Diego, CA). All variables were tested for normality using the Shapiro-wilk test of normality with all being normally distributed apart from fat mass and hip Z scores. A one way between groups ANOVA was conducted to assess differences between positions and stature, mass, body fat percent, fat free mass, whole body Z score, lumbar Z scores training duration, distance, HSR, ED, and EEE. To investigate which positions were significantly different a Tukey's HSD post hoc test were undertaken. For non-parametric data (fat mass and hip Z score) a Kruskal-Wallis test was undertaken. A one-way repeated measures ANOVA was conducted to assess differences between training days and EI, macronutrient intake, energy availability, EEE, training duration, distance, HSR and ED. To investigate which days were significantly different a Tukey's HSD post hoc test were undertaken. For non-parametric data (fat mass and hip Z score) a Kruskal-Wallis test was undertaken. Pearson's bivariate correlations were used to test any relationship between variables.

## 5.4 Results:

### *5.4.1 Anthropometry*

Table 12 provides an overview of body composition and bone health. All players had whole body, hip, and lumbar Z-scores >-1.0. There was a significant correlation between whole body Z scores and lumbar spine Z scores ($r = 0.71$, $P = 0.003$). The only body composition measure that was statistically significant by position was stature; between goalkeepers and midfielders ($P = 0.045$).

**Table 12.** Body composition, risk of low energy availability, resting metabolic rate, blood pressure and heart rate for professional female soccer players. Body composition and risk of low energy availability also reported by position.

| **Variable** | **Value (mean ± SD)** | **Range** | **Goalkeeper (n = 2)** | **Defender (n = 5)** | **Midfielder n = 4)** | **Forward (n = 4)** |
|---|---|---|---|---|---|---|
| Stature (m) | 1.67 ± 4.9 | 1.60 – 1.73 | 1.71 ± 0.0* | 1.67 ± 0.1 | 1.62 ± 0.0* | 1.69 ± 0.0 |
| Body mass (kg) | 60.4 ± 6.1 | 50 - 70.8 | 68.1 ± 3.8 | 59.0 ± 6.9 | 56.3 ± 5.3 | 62.3 ± 2.4 |
| Percent body fat (%) | 22.8 ± 3.1 | 18.6 - 30.9 | 26.7 ± 5.7 | 22.7 ± 3.0 | 22.4 ± 1.9 | 21.2 ± 2.2 |
| Total fat mass (kg) | 13.1 ± 2.6 | 9.5 - 20.9 | 17.6 ± 4.7 | 12.6 ± 1.7 | 12.0 ± 2.1 | 12.6 ± 0.9 |
| Total lean mass (kg) | 42.2 ± 4.5 | 35.0 - 50.5 | 45.3 ± 1.1 | 41.3 ± 5.8 | 39.2 ± 3.2 | 44.7 ± 3.0 |
| Whole body Z score | 1.7+ 0.8 | 0.5 - 3 | 2.3 ± 0.6 | 1.3 ± 0.9 | 1.7 ± 0.5 | 1.9 ± 0.8 |
| Hip Z score | 1.7 ± 0.7 | 1.0 - 3.9 | 2.9 ± 1.5 | 1.3 ± 0.2 | 1.8 ± 0.6 | 1.4 ± 0.3 |
| Lumbar spine Z score | 0.8 ± 0.7 | −0.3 - 2.2 | 1.1 ± 0.0 | 0.2 ± 0.4 | 1.2 ± 0.8 | 0.9 ± 0.6 |
| LEAF-Q | 5.9 ± 3.0 | 2.0-12.0 | 4.0 ± 1.4 | 5.6 ± 3.0 | 8.3 ± 2.9 | 5.0 ± 2.9 |
| RMR (kcal·d$^{-1}$)** | 1729 ± 159 | 1423 - 1994 | | | | |
| Blood pressure systolic (mmHg) | 116 ± 8 | 106 - 131 | | | | |
| Blood pressure diastolic (mmHg) | 70 ± 6 | 62 - 83 | | | | |
| Heart rate (beats per minute) | 58 ± 7 | 45 - 70 | | | | |

Values are mean ± SD for all variables. RMR = resting metabolic rate. LEAF-Q = Low Energy Availability in Females Questionnaire. * indicates a significant difference (P <0.05). **Data invalid, further information to be found in results and discussion.

### *5.4.2 The Low Energy Availability in Females Questionnaire (LEAF-Q)*

Table 12 and Figure 13 provides the squad's mean global score and positional breakdown. Using a global score of ≥8 (Melin et al., 2014), 40% of the squad was classified at risk of LEA. None of the non-HC players self-reported as having menstrual dysfunction.

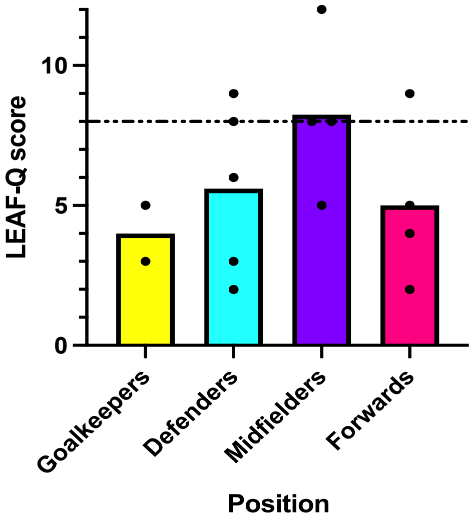


**Figure 13.** LEAF-Q scores per position. Dashed line (≥8) and above represents when the athlete is 'at risk' of low energy availability. LEAF-Q = low energy availability in females questionnaire. Each bar is the mean for position, with each dot being an individual player.

### *5.4.3 Energy availability*

Mean energy availability was optimal for over half of the players (53%) over the three-day measurement period (Table 13). Mean energy availability on the light training day was reduced (44 Kcal·kg $FFM^{-1}\cdot day^{-1}$) compared to the other two days which were optimal. On the rest day one player had LEA compared to none on the other three days (Figure 14). There was no significant difference in energy availability over the three days ($P = 0.437$). Over the three days energy availability ranged from 26 - 74 Kcal·kg $FFM^{-1}\cdot day^{-1}$ (Figure 14).

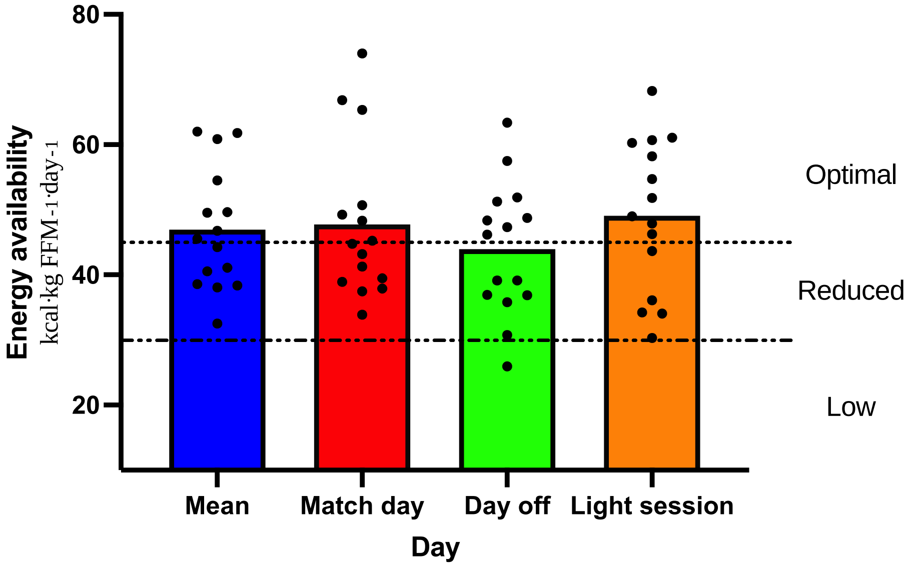


**Figure 14.** Energy availability scores over a three-day period where LEA is <30Kcal·kg FFM$^{-1}$·day$^{-1}$ reduced is 30-45 Kcal·kg FFM$^{-1}$·day$^{-1}$ and optimal is >45 Kcal·kg FFM$^{-1}$·day$^{-1}$. Each dot represents an individual player.

### *5.4.4 Dietary intake validity*

Twelve of the players (80%) were within the ratio (1.16 – 2.78) using the Goldberg cut-off equation (Black, 2000) with three players (20%) being classified as potential under-reporters (EI:RMR <1.16). As reported earlier, the recommendation not to remove these players from further analysis due to a small sample size was adhered to (Black, 2000). The coefficient of variation between the lead researcher and the second SENr nutritionists' food diary analysis were 4.61 ± 2.12%, 5.16 ± 3.21%, 3.23 ± 1.16% and 3.23 ± 2.13% for energy intake (kcal), carbohydrate (g), protein (g), and fat (g), respectively.

### *5.4.5 Dietary intake*

No significant differences were shown between training days for total EI (P = 0.28), all three macronutrient intakes (carbohydrates (P = 0.44); protein (P = 0.18); and fat (P = 0.98)) or macronutrient intakes relative to body mass (carbohydrate/kg (P = 0.54); protein/kg (P = 0.19); and fat/kg (P = 0.98)) (Table 13). There was a significant correlation between EI and carbohydrate intake (r = 0.85, P<0.001). None of the players reported consuming the recommended 7g·kg$^{-1}$·d$^{-1}$of carbohydrates on match day with 47% consuming less than 3 g·kg$^{-1}$·d$^{-1}$ (Figure 15). Over the three days, 33% of players consumed <3g·kg$^{-1}$·d$^{-1}$ of carbohydrate. Mean protein intake was 2 g·kg$^{-1}$·d$^{-1}$ with 87% of players consuming above the recommended

$1.7 g \cdot kg^{-1} \cdot d^{-1}$ and the lowest intake being $1.4 g \cdot kg^{-1} \cdot d^{-1}$. Fat intake remained constant over the three days. The biggest range of fat intake was on match day ($0.46 – 1.92 g \cdot kg^{-1} \cdot d^{-1}$), with the mean range over the three days being $0.80 – 1.63 g \cdot kg^{-1} \cdot d^{-1}$.

**Table 13.** Dietary intake, energy availability and exercise energy expenditure as total amounts and relative to body mass over three consecutive days.

| | Mean | Match day | Rest day | Light training |
|---|---|---|---|---|
| Energy intake (kcal) | 1961 ± 53 | 1983 ± 382 | 1845 ± 344 | 2055 ± 449 |
| Energy ($g \cdot kg^{-1}$ FFM) | 47 ± 1 | 48 ± 12 | 43.9 ± 11.7 | 49.2 ± 11.7 |
| Carbohydrate (g) | 218.0 ± 10.3 | 215.9 ± 82.0 | 202.1 ± 61.4 | 235.9 ± 72.0 |
| Carbohydrate ($g \cdot kg^{-1}$) | 3.7 ± 0.2 | 3.7 ± 1.5 | 3.4 ± 1.2 | 4.0 ± 1.3 |
| Protein (g) | 118.2 ± 5.1 | 125.3 ± 21.8 | 107.2 ± 29.2 | 122.3 ± 31.4 |
| Protein ($g \cdot kg^{-1}$) | 2.0 ± 0.1 | 2.1 ± 0.5 | 1.8 ± 0.5 | 2.0 ± 0.5 |
| Fat (g) | 68.5 ± 2.2 | 68.7 ± 22.0 | 67.6 ± 20.9 | 69.1 ± 18.1 |
| Fat ($g \cdot kg^{-1}$) | 1.1 ± 0.1 | 1.2 ± 0.4 | 1.1 ± 0.4 | 1.2 ± 0.4 |
| | | | | |
| Energy availability ($Kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$) | 47 ± 9 | 49 ± 12 | 48 ± 12 | 44 ± 10 |
| Estimated exercise energy expenditure | | 966 ± 269* | 0 | 481 ± 203* |
| Energy availability classification | Optimal | Optimal | Optimal | Reduced |
| Percentage of players with low EA | 0 | 0 | 7 | 0 |
| Percentage of players with reduced EA | 47 | 47 | 40 | 33 |
| Percentage of players with optimal EA | 50 | 53 | 53 | 67 |

Values are mean ± SD for energy intake, carbohydrate, protein, fat, energy availability and estimated exercise energy expenditure for all players. FFM = fat free mass, EA = energy availability. * Indicates a significant difference ($P < 0.05$).

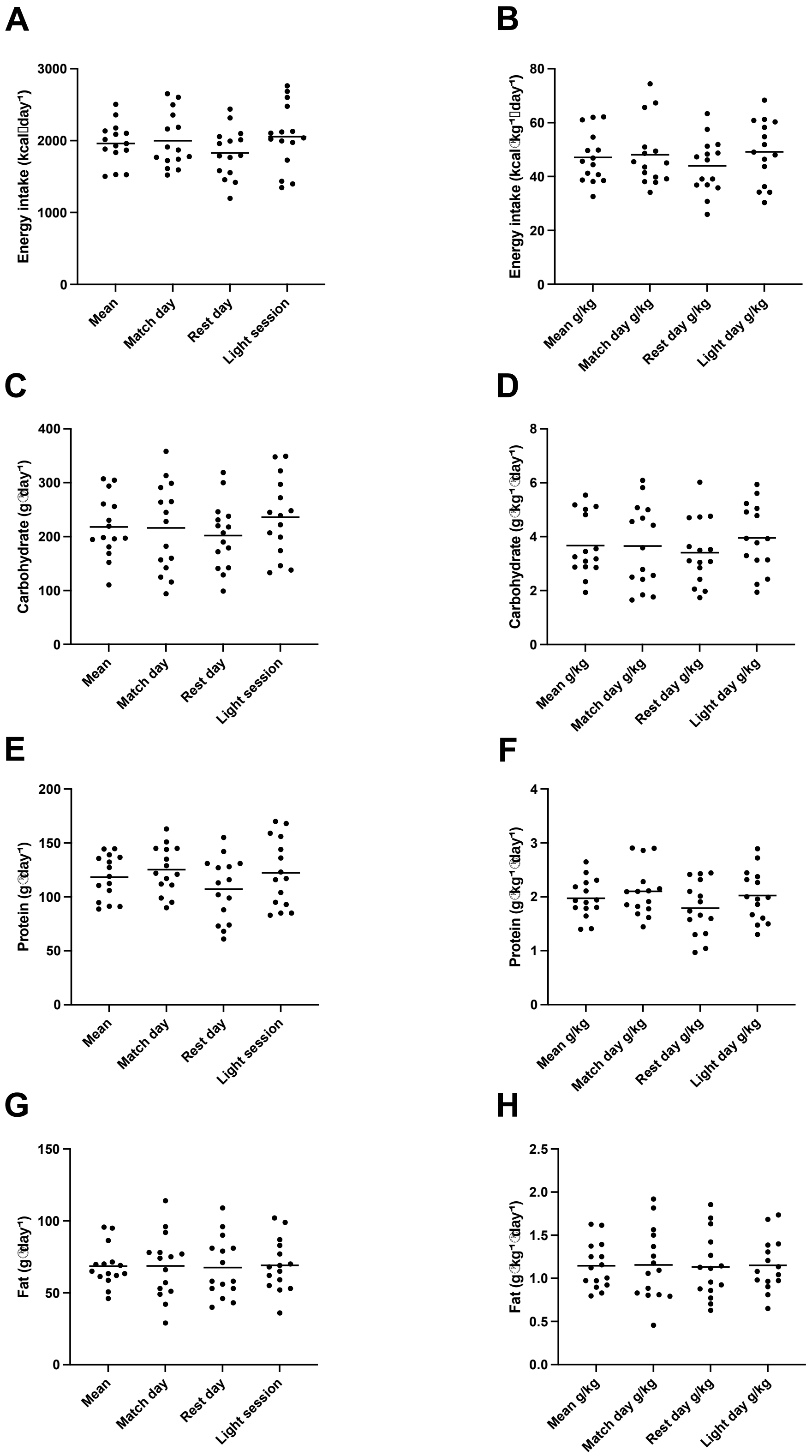


**Figure 15.** Energy intake (kcal) (A), energy intake relative to fat free mass (B), carbohydrate intake (C), carbohydrate intake relative to body mass (kg) (D), protein intake (g) (E), protein intake relative to fat free mass (kg) (F), fat intake (g) (G), fat intake relative to fat free mass (kg) (H) over three consecutive days and as a mean of all three. Solid line is the mean for each day, with each dot being an individual player.

### *5.4.6 Biomarkers*

Most micronutrients were within the recommended range for players. Within the iron profile the squad were predominantly within range; serum iron (80%) transferrin (90%), TIBC (100%), ferritin (100%), % transferrin saturation (80%). Relating to iron measurements, hemoglobin (40%) and hematocrit (30%) were out of range for over half the squad. Other markers that were out of range include prolactin (50%), cortisol (50%), as well as other female hormone markers such as LH (20%), FSH (10%), oestradiol (20%) and SHBG (20%). Full biomarker profiles can be found in Table 14.

**Table 14.** Micronutrient and biochemical profiles of fifteen female professional soccer players.

| Variable | Value (mean ± SD) | Range | Clinical range* | Players within range |
|---|---|---|---|---|
| Luteinising hormone U·L$^{-1}$ | 5.8 ± 2.8 | 2.2 - 9.9 | 3 - 80** | 80 (8) |
| Follicle stimulating hormone U·L$^{-1}$ | 4.1 ± 2.3 | 0.5 - 7.3 | 1 - 16** | 90 (9) |
| 17 beta oestradiol pmol·L$^{-1}$ | 345 ± 221 | <50 - 763 | 45 - 1200** | 80 (8) |
| Sex hormone binding globulin nmol·L $^{-1}$ | 96.0 ± 45.1 | 23 - 163 | 25 - 155 | 80 (8) |
| Insulin growth factor 1 nmol·L$^{-1}$ | 33.2 ± 4.3 | 29 - 39 | 11.2 - 54.5 | 100 (10) |
| Prolactin mU·L$^{-1}$ | 522.5 ± 228.0 | 245 - 987 | <500 | 50 (5) |
| Progesterone nmol·L$^{-1}$ | 14.4 ± 12.6 | 1.2 - 33.1 | <1 - 70** | 100 (10) |
| Ferritin ug·L$^{-1}$ | 64.1 ± 21.6 | 36 - 98 | 13 - 150 | 100 (10) |
| Iron umol·L$^{-1}$ | 13.9 ± 7.2 | 5.1 - 13 | 13 - 32 | 50 (5) |
| Transferrin g·L$^{-1}$ | 2.51 ± 0.34 | 2.14 - 3.34 | 2.2 - 3.6 | 90 (9) |
| Total iron binding capacity umol·L$^{-1}$ | 57 ± 7.7 | 49 - 76 | 41 - 77 | 100 (10) |
| % transferrin saturation | 23.6 ± 8.8 | 8.0 - 42 | 20 - 55 | 80 (8) |
| Haemoglobin g·L$^{-1}$ | 110.4 ± 20.0 | 77 - 140 | 118 - 148 | 40 (4) |
| Haematocrit L·L$^{-1}$ | 0.32 ± 0.06 | 0.22 - 0.4 | 0.36 - 0.44 | 30 (3) |
| Thyroid stimulating hormone mU·L$^{-1}$ | 2.52 ± 0.68 | 1.6 - 3.6 | 0.3 - 6.0 | 100 (10) |
| Free thyroxine pmol·L$^{-1}$ | 16.4 ± 2.2 | 13.1 - 20.8 | 10--22 | 100 (10) |
| Free triiodothyronine pmol·L$^{-1}$ | 4.89 ± 0.49 | 4.2 - 5.5 | 3.6 - 6.4 | 100 (10) |
| Total testosterone nmol·L$^{-1}$ | 0.9 ± 0.3 | 0.5 - 1.4 | <1.9 | 100 (10) |
| Insulin pmol·L$^{-1}$ | 47 ± 14.1 | 31 - 71 | <174 | 100 (10) |
| Cortisol nmol·L$^{-1}$ | 545 ± 246 | 341 - 1195 | 140 - 500 | 50 (5) |
| Vitamin B12 ng·L$^{-1}$ | 428.9 ± 117.4 | 198 - 609 | 160 - 800 | 100 (10) |
| Folate ug·L$^{-1}$ | 8.62 ± 2.50 | 5.1 - 13 | 3.9 - 20 | 100 (10) |
| Zinc umol·L$^{-1}$ | 14.6 ± 1.2 | 13 - 17 | 10.7 - 24.5 | 100 (10) |
| Vitamin D nmol·L$^{-1}$ | 89.9 ± 16.9 | 54 - 110 | 50 - 200 | 100 (10) |
| Magnesium nmol·L$^{-1}$ | 0.83 ± 0.05 | 0.75 - 0.92 | 0.7 - 1.0 | 100 (10) |

*Values are indicative of internal laboratory references for females aged 18-60; **Values are whole ranges and depend on the stage on the menstrual cycle.

### *5.4.7 Resting metabolic rate**

On analysis of the RMR data it became apparent that there was a serious fault with the Moxus indirect calorimeter. When investigated further, although measures such as $FiO_2$, $FiCO_2$ and $MixCO^2$ value ($FeCO_2$) were normal, many of the players macronutrient utilisation breakdown was at 140% - e.g. one player was utilising 140% of their energy from carbohydrate. On further discussion with the technicians, it was agreed that there was a serious fault with the machine and so all results were deemed void. There was no other technology or time available to re-measure the players, so an estimated RMR was calculated using the Ten Haaf equation (Ten Haaf and Weijs, 2014). Due to this, analysis regarding measured RMR and predicted RMR was not conducted. These data have been included in the table but have not been analysed or used for the rest of this study.

### *5.4.8 Training and match profiles*

Between the light training day and match day there was a significant difference in duration (P <0.001), distance (P <0.001), ED (P = 0.015), and HSR (P = 0.038). When analysed by position there was a significant difference in mean ED between goalkeepers and defenders (P = 0.004), midfielders (P = 0.016) and forwards (P <0.001) and mean HSR between goalkeepers and defenders (P = 0.019 95%) and forwards (P = 0.013) but not midfielders (P = 0.094) (Table 15). There was no significant difference between positions for mean duration (P = 0.907) or mean distance (P = 0.109).

### *5.4.9 Estimated energy expenditure during exercise*

There was a significant difference in EEE between the light training day and match day (P = 0.001) but no significant different in EEE between player position (P = 0.100) (Table 13).

**Table 15.** Overview of the absolute training and match load during pre-season as a squad mean and broken down into means by position.

| Variable | Mean | Match day | Light training | Goalkeeper (n = 2) | Defender (n = 5) | Midfielder n = 4) | Forward (n = 4) |
|---|---|---|---|---|---|---|---|
| Duration (mins) | 83 ± 9 | 70 ± 17* | 97 ± 0* | 71 ± 0 | 85 ± 9 | 84 ± 10 | 87 ± 5 |
| Distance (m) | 6905 ± 1849 | 9075 ± 2437* | 4736 ± 2066* | 3721 ± 654 | 7031 ± 1282 | 7270 ± 2064 | 7975 ± 937 |
| HSR (m) | 156 ± 116 | 225 ± 239 | 86 ± 66 | 0 ± 0$^{AB}$ | 162 ± 41$^{A}$ | 106 ± 23 | 276 ± 145$^{B}$ |
| ED (m) | 788 ± 325 | 958 ± 430* | 619 ± 262* | 172 ± 34$^{ABD}$ | 870 ± 159$^{A}$ | 785 ± 341$^{B}$ | 999 ± 134$^{D}$ |

**Abbreviations:** HSR = high speed running; ED = explosive distance. * Significant difference between match day and light training. Positions that share a letter indicates a significant difference from each other (P <0.05)

### *5.4.10 Relationship between energy availability and associated risk factors*

There were significant correlations between energy availability; EI (r =0.83, P<0.001), carbohydrate (r = 0.75, P<0.001) and FFM (r = -0.65, P<0.001) (Figure 16).

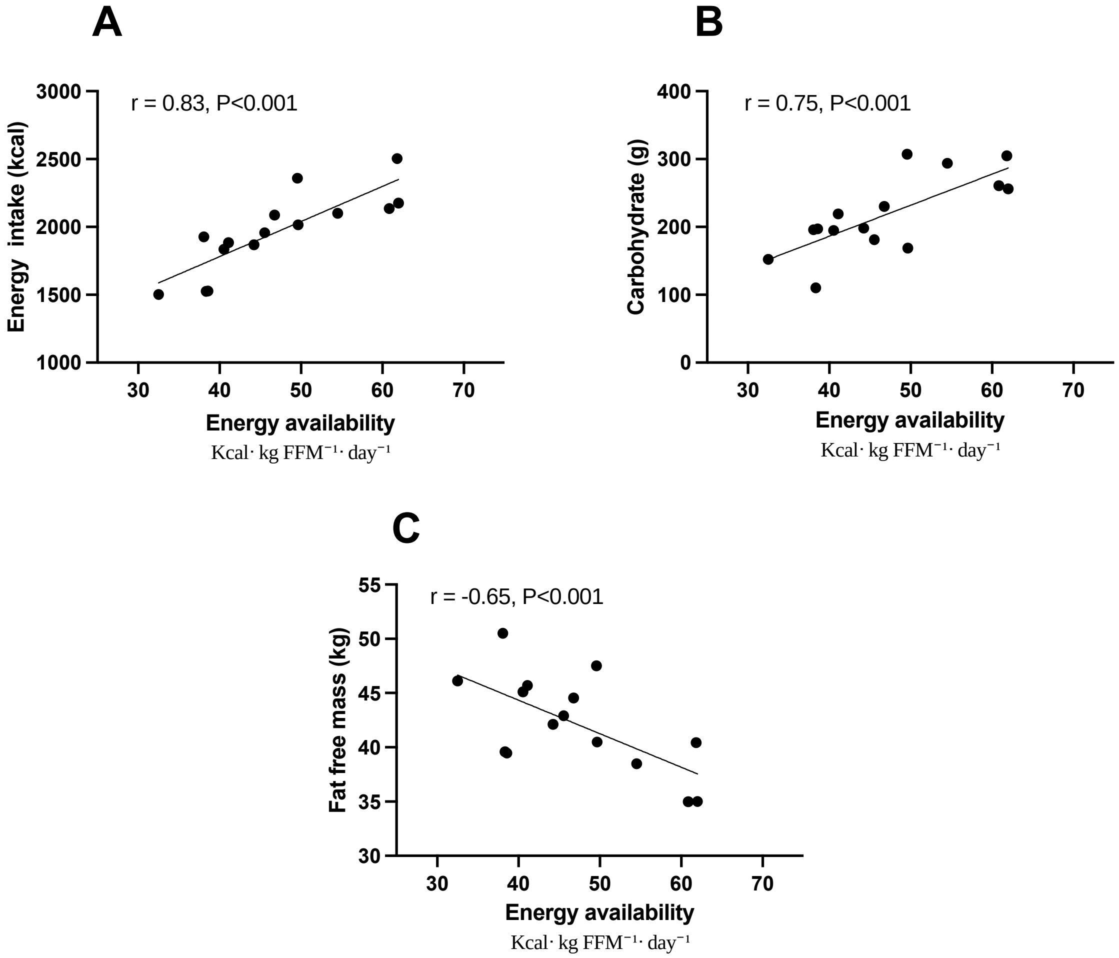


**Figure 16.** Correlation between energy availability (Kcal·kg FFM$^{-1}$·day$^{-1}$) and associated risk factors (A) energy intake, (B) carbohydrate intake and (C) fat free mass.

### *5.4.11 Menstrual cycle and hormonal contraceptive questionnaires*

Descriptive characteristics of both non-HC and HC users are presented in Figure 17.

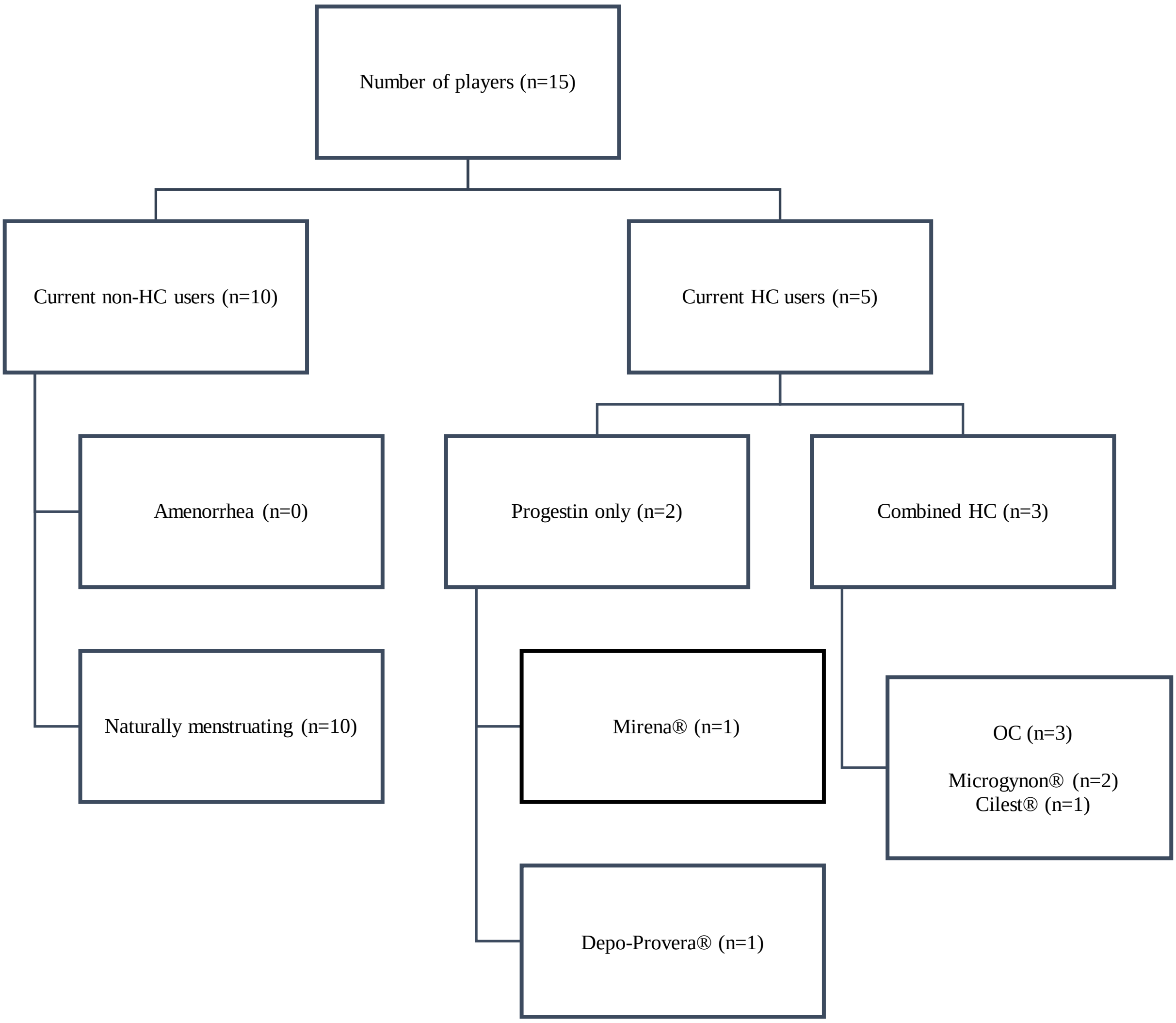


**Figure 17.** The prevalence and characteristics of hormonal contraceptive users and non-users. IUS, intrauterine system; OC, oral contraceptive; HC, hormonal contraceptive.

## 5.5 Discussion

The aim of this research was to assess the prevalence and factors affecting LEA in elite female soccer players during pre-season in a WSL 1 team. This involved assessing EEE, EI, and training loads over a three-day pre-season period, alongside measuring body composition, blood biomarkers and RMR. The main findings of this study were that despite 40% of the squad being classified as at risk of LEA according to the LEAF-Q, only one player had LEA on one of the 3 days measured, 0% of the squad having LEA averaged over the three days and none of the players self-reporting any menstrual dysfunction. Coupled with this, none of the biomarker results indicated LEA. Over this period 53% of the squad were considered to have ‘optimal’ energy availability (>45 Kcal•kg FFM$^{-1}$•day$^{-1}$) and, as a squad, only on the light training day were they not considered ‘optimal’ (44 Kcal•kg FFM$^{-1}$•day$^{-1}$).

At the time of data collection, this was the first study to investigate body composition, EI, energy availability and blood markers in a WSL 1 team. Since this study was designed and undertaken a similar study by Moss et al. (2020) has been published. Due to the similarities of the studies this paper will be included in the discussion to help provide direct comparisons, in a similar population, to this study.

### *5.5.1 Energy availability*

Applying the criteria established by Loucks et al. (2011), 53% of the squad consumed sufficient energy to meet the demands of training relative to their FFM. The remaining players (47%) were categorised as having reduced energy availability, with none of them meeting the criteria for LEA. These findings are in contrast to Reed et al. (2013) who reported that 33.3% of female soccer players in US colleges experienced LEA during the season. In contrast, in WSL 1 players Moss et al. (2020) reported the following distribution: 15% with ‘optimal’ energy availability, 62% with ‘reduced’ energy availability and 23% with ‘low’ energy availability. One major difference between Moss et al. (2020) and this study was the timing of data collection. The current study collected data during pre-season, when players might increase their energy intake to meet heightened training demands. Similar behavior has been noted among US collegiate female soccer players (Reed et al., 2013). Compared to this Moss et al. (2020) collected data at the end of the season (May), where it is possible that league positions were decided, matches were less competitive, and players may have been more likely to under fuel. The results of this study suggest that LEA could be less prevalent during pre-season. Moreover, while Moss et al. (2020) report that only 15% of players exhibit ‘optimal’ energy availability, they only note one player experiencing symptoms or markers of LEA. Several factors could contribute to this finding. Firstly, there may be under-reporting of EI among players, with one player being identified using the Goldberg cut-off equation. Secondly, players categorised within the 'reduced' energy availability threshold (62%) might still be functioning adequately at this level of energy availability. Finally, the study's limitation of measuring only at one time point during the season might not provide a comprehensive understanding of players' menstrual health. Despite 53% of the squad consuming sufficient energy, there is room for improvement through enhanced education. Both the current study and Moss et al. (2020) examined energy availability at singular time points in a season. To gain a deeper understanding of energy availability patterns among WSL players throughout a season, a longitudinal study would offer valuable insights.

### *5.5.2 The Low Energy Availability in Females Questionnaire (LEAF-Q)*

The data from the LEAF-Q indicated that 40% of players were 'at risk' of LEA, with 75% of the midfielders being 'at risk'. When measured 53% of the squad had 'optimal' energy availability, 47% of squad 'reduced' energy availability and no players reported amenorrhea. Although there appears to be some over reporting of LEA risk, the players with 'reduced' energy availability could be 'at risk' of LEA symptoms if this level of energy availability continues over a long period of time. However, there are some issues with using the LEAF-Q in this population. The LEAF-Q was designed as a screening tool for female endurance runners (Melin et al., 2014) and has not been validated in female soccer players. Players can score up to 7 points due to injuries (total and time lost), and with soccer being a contact sport, injuries are potentially more likely than in endurance sports, thus contributing to a possible over sensitivity of the scoring. However, with no other validated questionnaire, and with it being quick and cheap the LEAF-Q could still be a useful screening tool for LEA. These results indicate that practitioners need to be cautious when administrating and analysing the questionnaire to not cause unnecessary concern to the player and multi-disciplinary team.

### *5.5.3 Body composition*

Mean FM was 13.1kg ± 2.6 with FFM being 42.2 ± 4.5 kg. Comparing this to previous studies utilising DXA, Emmonds et al. (2019) reported FM and FFM values 2% lower and 9% higher respectively, with Moss et al. (2020) reporting FM and FFM values 13% lower and 16% higher. A possible explanation for this is the testing time points. In comparison to the present study, which was conducted in early pre-season, Emmonds et al. (2019) measured at the end of pre-season and Moss et al. (2020) towards the end of the season. For future, a longitudinal study measuring body composition over a season would be useful to allow practitioners to get a better insight as to how much body composition can be manipulated over a season and what changes are possible in this population.

Regarding bone health, 100% of the squad had Z-scores above the recommended threshold (>-1.0) (Mountjoy et al., 2014; Nattiv et al., 2007) for whole body (Z-scores: 0.5–3.0), hip (Z-scores: 1.0–3.9) and lumbar spine (Z-scores: -0.3–2.2), which aligns with findings from Moss et al. (2020) for whole body scores (Z-scores: 1.1–3.8). Bone health can be affected by factors such as nutrition (LEA), exercise and medications (Goolsby and Boniquit, 2017). Therefore, given that the squad did not experience symptoms of LEA, it was expected that their bone markers would remain above the recommended threshold. Furthermore, the weightbearing activity and mechanical loading involved in soccer training and matches are believed to have beneficial and protective effects on bone health (Bellver, Del Rio, Jovell, Drobnic, and Trilla, 2019). These findings underscore that female soccer players can maintain optimal bone health through training loads and adequate energy availability.

### *5.5.4 Dietary intake*

This study measured EI over a three-day period and showed no significant differences among the three days, although the lowest calorie consumption occurred on the rest day. This suggests that players may have adjusted their intake based on their activity levels. The mean intake over the three-day period was 1961 ± 291 $kcal \cdot d^{-1}$, which aligns with similar findings in WSL 1 players (2124 ± 444 $kcal \cdot d^{-1}$; P = 0.255) (Moss et al., 2020). In comparison, other comparative studies reported significantly higher dietary intakes of 2226 ± 368 $kcal \cdot d^{-1}$ ($P < 0.05$) in young German players (Braun et al., 2018) and 2387 ± 177 $kcal \cdot d^{-1}$ ($P < 0.001$) in US colligate players (Reed et al., 2013). These discrepancies in energy intake could stem from the inherent challenges in accurately measuring intake among free-living athletes (Burke et al., 2018). Moreover, the varying methods employed to assess intake, such as self-reported food diaries and dietary recalls, each come with their own limitations and make comparisons between studies difficult. Complicating matters, the previous studies were conducted at different time points within a season, introducing further complexity to the comparison. As earlier indicated, three players (20%) fell into the potential under-reporters category (EI:RMR <1.16) as determined by the Goldberg cut-off equation (Black, 2000). This classification might account for the slightly lower energy intakes compared to the studies by Braun et al. (2018) and Reed et al. (2013), although this is difficult to compare with these studies not employing under reporting measures. To mitigate potential under-reporting when employing RFPM in future studies, it is recommended to involve multiple researchers working with each player to ensure comprehensive data collection. This becomes particularly relevant for larger squads, where managing data collection for all 15 players simultaneously can be challenging.

The study showed the carbohydrate intake patterns of soccer players across different types of days, namely rest days, light training days, and match days. Of particular concern in this study was the carbohydrate intake among players on match days. It was noted that none of the squad consumed the recommended $7 g \cdot kg^{-1} \cdot d^{-1}$ of carbohydrates (Maughan and Shirreffs, 2007; McLay et al., 2007), while 47% of players consumed less than $3 g \cdot kg^{-1} \cdot d^{-1}$, and 20% consumed less than $2 g \cdot kg^{-1} \cdot d^{-1}$. Although there are currently no female soccer specific carbohydrate guidelines, it is highly unlikely that $2\text{-}3 g \cdot kg^{-1} \cdot d^{-1}$ would be sufficient to optimally fuel for games but also recovery post-match. The results of this study ($3.7 g \cdot kg^{-1} \cdot d^{-1}$) are similar to Moss et al. (2020) who reported $3.5 g \cdot kg^{-1} \cdot d^{-1}$. This study accentuates the necessity for future research involving muscle biopsies in female soccer players to ensure that the current recommendations are accurate. In the meantime, there is a likely need for targeted nutritional education to improve players' understanding of the critical role of proper carbohydrate intake in optimising game-day performance. While the current research provides insightful data, it acknowledges the need for future investigations spanning various points in a season, involving the same squad, to ascertain whether the identified trends persist consistently over time.

Average protein intake was $2 g \cdot kg^{-1} \cdot d^{-1}$ with most (87%) players consuming $>1.7 g \cdot kg^{-1} \cdot d^{-1}$. While the established Recommended Daily Allowance (RDA) for protein in Europe stands at $0.8 g \cdot kg^{-1} \cdot d^{-1}$ body weight per day (EFSA Panel on Dietetic Products, 2023), it has been advised that female athletes may benefit from higher intake ($>1.7 g \cdot kg^{-1} \cdot d^{-1}$) to optimise their training and enhance recovery (Morton et al., 2018; Wooding et al., 2017). Players in the study were not routinely prescribed protein supplementation, but supplementation was made available if the player wished to consume it after training. These findings not only corroborate other investigations into protein intake among female soccer players, such as the $1.83 g \cdot kg^{-1} \cdot d^{-1}$ reported by Moss et al. (2020) but they also highlight the adequacy of protein intake within this specific demographic. This suggests that the current dietary practices among these players align well with the recommended protein intake, contributing positively to their training and recovery efforts.

This study examined the average relative fat intake of female soccer players across three days, revealing a mean fat intake of $1.1 \pm 0.1 g \cdot kg^{-1} \cdot day^{-1}$. Comparatively, the current findings are lower than those reported by Moss et al. (2020) ($1.33 \pm 0.43 g \cdot kg^{-1} \cdot day^{-1}$). The significance of fat intake is important for two key reasons: its role as a calorie source and its contribution to facilitating the uptake of essential nutrients. The current findings indicate that the observed level of fat intake does not raise concerns regarding energy intake or the absorption of essential nutrients, as summarised in Table 4. To further validate these findings, future research could delve into fat intake patterns throughout an entire soccer season, while concurrently monitoring energy availability and relevant biomarkers. This comprehensive investigation would offer additional evidence supporting the conclusion that their current fat intake adequately meets the needs of female soccer players.

### *5.5.5 Biomarkers*

Half of the squad exhibited elevated cortisol levels, a hormone linked with periods of LEA and heightened stress, such as during pre-season training in female soccer players (Meeusen et al., 2013; Schorr, Lawson, Dichtel, Klibanski, and Miller, 2015). Even though measurements were taken after a day of rest, it is important to note that cortisol can remain high days after intense training periods, potentially impacting its reliability as an LEA indicator. To support this, the thyroid hormone $T_3$ was within range for the whole squad. This hormone is a reliable marker for energy availability and serves as a strong indicator of potential LEA in athletes (Loucks and Callister, 1993; Loucks and Heath, 1994). Additionally, 50% of the players had elevated prolactin concentrations, a hormone that, when elevated, can suppress oestrogen (Suay et al., 1999). This elevation has potential consequences for players' well-being, including decreased fertility, bone mineral density, and an increased risk of osteoporosis (Levine and Muneyyirci-Delale, 2018). The concurrence of heightened prolactin and cortisol levels and normal $T_3$ and other thyroid hormones within the squad suggests a possible connection to increased training loads and stress experienced during pre-season (Mastorakos, Pavlatou, Diamanti-Kandarakis, and Chrousos, 2005) rather than LEA. Due to this, future studies should look at these markers over a season to ensure that the correct hypothesis is attributing the results to intensified training rather than any underlying issues.

The squad's micronutrient levels, including magnesium, zinc, Vitamin B12, and folate, were all found to be within the normal range. Similarly, Vitamin D levels of the players were also within the normal range. This was likely due to the location of the players in the weeks and months prior to data collection (i.e., a combination of time spent on vacation at Mediterranean destinations, and also outside during British summer) with the major source of vitamin D being casual exposure of the skin to solar ultraviolet B radiation (Chen et al., 2007). This pattern has been observed in elite male soccer players, where there was a significant decrease in Vitamin D concentrations from August to December (Morton et al., 2012). For future studies, it would be useful to replicate this study across a season to assess the outcome in female soccer players at the same latitude, helping to provide an insight to supplement protocols needed in this population.

There were mixed results for the hematological markers evaluated in this study. While iron, ferritin, transferrin, TIBC, and % transferrin saturation was within clinical ranges for most players (80% or more), hemoglobin and hematocrit levels were outside the norm for a significant portion (60% for hemoglobin, 70% for hematocrit). One player exhibited low levels of multiple markers: iron, % transferrin saturation, hemoglobin, and hematocrit. Notably, their hemoglobin was alarmingly low (77 g/L), falling below the World Health Organization's anemia threshold of 120 g/L (Bermejo and García-López, 2009). In relation to sport, Peeling et al. (2007) proposed the following categories to define the varying levels of iron deficiency in athletes.

- Stage 1 – iron deficiency: ferritin < 35 μg/L, Hb > 115 g/L, transferrin saturation > 16%
- Stage 2 – iron-deficient non-anaemia (IDNA): ferritin < 20 μg/L, Hb > 115 g/L, transferrin saturation < 16%
- Stage 3 – iron-deficient anaemia (IDA): ferritin < 12 μg/L, Hb < 115 g/L, transferrin saturation < 16%

The player's haemoglobin (77 g/L) and transferrin saturation (8%) met IDA criteria, but their ferritin (66 ug·L$^{-1}$) remained within the normal range. Thus, they might not meet the threshold for even stage 1 iron deficiency. There are a number of limitations associated when using ferritin as a marker of iron status, due to its role as an acute phase protein (Bermejo and García-López, 2009). Ferritin levels are increased during periods of inflammation and after intense exercise, by as much as 27% (Voss et al., 2014). Therefore, during an intense pre-season, ferritin could be reported as falsely high and thus mis-diagnosed this player. Bermejo and García-López (2009) recommend using thresholds of Hb <120g/L and serum ferritin <30 ng/L and/or % transferrin saturation < 20%. Using this definition, the player would be classified with IDA. This individual's case provides a great example how a snapshot of biomarkers can only provide limited information and context. In addition, there are questions around the applicability of clinical ranges from non-sporting populations and the need for individualised reference ranges. Measuring these markers over numerous times in a season would allow greater analysis and provide each player with an individual biomarker passport to better determine when a player is not only outside of standard clinical ranges but within their 'normal' range.

### *5.5.6 Training and match profiles*

During match play, outfield players in this study covered 9.7 ± 1.8km compared to 10.3 ± 0.9 km (internationally) (Datson et al., 2017) and 9.7 ± 1.4km (domestically) (Andersson et al., 2010). Intensity was lower in the current study 0.3 ± 0.3km compared to 0.6 ± 0.2 km (internationally) (Datson et al., 2017) and 1.3 ± 0.9km (domestically) (Andersson et al., 2010). The disparity in HSR intensity could be attributed to data collection during the pre-season, potentially reducing match intensity, and different populations measured (international vs domestic). Notably, this study utilised GPS technology, considered the gold standard, while previous studies employed time motion analysis. Additionally, HSR thresholds varied, with the current study and Datson et al. (2017) using >19.8 $km \cdot h^{-1}$ with Andersson et al. (2010) adopting 18 $km \cdot h^{-1}$. It has been suggested that using the same threshold as male soccer players (HSR >19.8 $km \cdot h^{-1}$) will under-estimate female soccer demands (Bradley and Vescovi, 2015). However, Datson et al. (2017) argue the proposed female-specific thresholds (HSR >15 $km \cdot h^{-1}$) were derived from a small sample size of non-elite players (Bradley and Vescovi, 2015) and may not be representative of elite female soccer players. Therefore, similar to Datson et al. (2017) our HSR threshold likely contributes to similar distances covered yet differing reported intensities. Measurements over a season in this population would provide more context to determine if the lower intensity found in this study is due to pre-season and the physical state of status of the players, or if other factors such as tactics, opposition, and physical capability are also contributing factors.

### *5.5.7 Estimated energy expenditure during exercise*

There was a significant difference between EEE on light training days compared to match day (P = 0.001), but this did not vary by position. In comparison to Moss et al. (2020) the current study observed a significantly higher calorie expenditure on light training days (481 ± 203kcal) than the Moss study (299 ± 78kcal) (P<0.05). This variance likely stems from the timing of assessments and the potential impact of pre-season training at a higher intensity. The EEE on match days was similar between both studies 966 ± 269kcal v 881 ± 473kcal (Moss et al., 2020) (P = 0.557). This provides valuable insight for practitioners to provide dietary interventions around match days to ensure players are optimally fueled and re-fueled. The limitation of this study was that on the rest day, no activity diary was kept. Although it is highly unlikely players undertook any strenuous exercise on this day – particularly during pre-season when training volumes are higher than normal – in future studies training logs should be kept to better analyse any non-club related exercise.

## 5.6 Limitations

A major limitation of this observational study is the data only being a ‘snapshot’ during pre-season. This limitation becomes evident when considering measurements like energy availability, which might exhibit considerable differences over time. A longitudinal study design would offer a clearer context, enabling us to observe how these measurements evolve throughout the entire duration of the season. Measurements such as EI are notoriously difficult to measure, partly due to potential behavioral changes in players during data collection. To enhance data quality, it is suggested that future studies adopt a team approach (consisting of one player and two research staff) during RFPM, as like with this study a single person might struggle to collect data from an entire squad simultaneously.

Regarding EEE, time spent away from the club was not accounted for. While this external activity's impact might be minimal, there is a chance that some non-club EEE was not considered due to lack of measurement. To address this, it is advisable for future studies to maintain logs of any activities performed outside of the club. These records could then be factored into calculations for both EEE and energy availability, providing a more comprehensive picture.

The absence of measured data for RMR is a limitation in this study, a factor beyond the researcher's control. Had this error been identified earlier, the option to conduct measurements using an alternate device could have been pursued. Regrettably, this opportunity did not arise in this instance.

## 5.7 Practical applications

1. Low energy availability appears to be less of a concern in elite female soccer players than previous described.
2. The LEA-Q appears to overestimate players at risk of LEA, therefore, caution needs to be applied when administrating and analysing the questionnaire to not cause unnecessary concern to the player and multi-disciplinary team.
3. Female soccer players can maintain optimal bone health through training loads and adequate energy availability.

4. There is a need for targeted nutritional education to improve players' understanding of the critical role of proper carbohydrate intake in optimising game-day performance.
5. Current protein and fat intakes appear to be sufficient.
6. There is a significant difference in EEE in different intensity sessions, therefore, players to periodise their nutrition accordingly.

## 5.8 Conclusion

The current study's findings indicate that none of the squad members exhibited LEA when measured over a 3-day period. This, along with the players' hormonal profiles ($T_3$) and responses to menstrual cycle questionnaires, suggests that LEA or menstrual dysfunction were not present during the initial weeks of pre-season training. However, players did not significantly adjust their energy or carbohydrate intake based on the intensity of their training sessions. Of note, almost half of the squad (47%) consumed less than $3g \cdot kg^{-1} \cdot d^{-1}$of carbohydrates on match day. Given the established performance benefits of consuming sufficient carbohydrates around match days, this highlights a potential gap in nutritional knowledge among female soccer players. To ensure the suggested increase does not affect the players body composition it would suggested that players periodise their carbohydrate more, getting closer to consuming $7g \cdot kg^{-1} \cdot d^{-1}$ on match days (McLay et al., 2007) while reducing intake on lower intensity days. Most players in the squad were within normal range for micronutrient, hormone, and iron markers, suggesting that players DI was providing sufficient nutrients. However, for certain markers that deviated from the standard clinical ranges, it is likely that these discrepancies were a result of the players' increased training load and the stress experienced during rigorous pre-season training. During the pre-season match, the players covered 9.7km, which aligns with distances covered in previous studies (9.9km and 9.7km) (Andersson et al., 2010; Datson et al., 2014).

In summary, LEA does not appear to be a concern among these WSL 1 soccer players based on the pre-season measurements. However, since this was only a snapshot and data from Study 1 (Chapter 4) suggests that a quarter of WSL 1 players suffer from menstrual dysfunction, a more comprehensive assessment of energy availability and other nutritional markers is required throughout a season. Therefore, the next study will assess EI, EE, body composition, menstrual function and blood biomarker profiles at four evenly spaced intervals during the season. This will help evaluate changes in body composition and further elucidate the prevalence and factors affecting LEA among WSL 1 players throughout the season.

# CHAPTER 6

# Longitudinal observation study assessing nutritional intake, energy expenditure, energy availability, body composition and blood biomarkers among elite female soccer players in Women’s Super League

## 6.1 Abstract

**Purpose:** To examine longitudinal changes on the prevalence and factors affecting LEA including nutritional intake, body composition, and biomarker profiles among elite female soccer players over a 10-month season in WSL 1.

**Methods:** Nineteen players underwent assessments using GPS, RFPM, DXA, RMR and blood analysis at four time points throughout the season, incorporating match days, training days, and rest days to capture variations influenced by competitive demands.

**Results:** Significant reductions in FM ($P = 0.039$) and BF% ($P = 0.008$), along with increases in FFM ($P = 0.046$), were observed during the season. Thirty-two percent of players were classified as at risk of LEA by the LEA-Q, with 78% of the players maintaining energy availability of >45 kcal·kg FFM$^{-1}$·day$^{-1}$ throughout the season. Two players self-reported menstrual dysfunction, with one player exhibiting ‘reduced’ energy availability (34 kcal·kg FFM$^{-1}$·day$^{-1}$) decreased BM, FM, BF% and RMR ratio, suggesting likely LEA. Carbohydrate intake on match days ($4.6 \pm 1.1$g·kg$^{-1}$·d$^{-1}$) consistently fell below recommended levels (>7g·kg$^{-1}$·d$^{-1}$). Vitamin D concentrations significantly decreased mid-season ($P < 0.001$), with one player clinical deficient (<50nmol·L$^{-1}$). Hematological indices fluctuated, with 50% classified as stage 1 iron deficient at some point during the season.

**Conclusion:** Over the season, LEA was likely experienced by only one player. Most players consistently consumed insufficient carbohydrates on match days. Vitamin D levels significantly declined throughout the season, and at one point, half of the squad was classified as iron deficient. These findings underscore the importance of personalised nutrition to enhance performance and health in elite female soccer players.

## 6.2 Introduction

In this chapter, we aimed to assess the longitudinal changes in the nutritional intake, body composition, and biomarker profiles of elite female soccer players, building upon the findings of Study 2 (Chapter 5). Throughout a playing season, female soccer players face varying training and match demands influenced by factors such as environmental conditions, playing position, opponent quality, style of play, and team tactics. Given the dynamic nature of these factors and the cumulative impact of both match play and training on physiological function and biochemical markers (Andersson et al., 2008; Gravina, Ruiz, Lekue, Irazusta, and Gil, 2011; Krustrup, Zebis, Jensen, and Mohr, 2010), it becomes crucial to comprehensively assess the nutrition intake, energy availability, body composition, and blood biomarker profiles over the course of a season rather than focus upon a snapshot at a single timepoint during the season. This observation will contribute to a more thorough understanding of any potential changes and the health and performance implications associated with them.

Body composition testing is valuable to help inform training, talent development and nutrition support. To date, only Reed et al. (2013) have measured body composition over a season using DXA. Their study focused on US collegiate female soccer players, revealing no significant differences in weight, FM, or FFM between pre-season, mid-season, and post-season (Reed et al., 2013). Unfortunately, bone-related measures were not reported in their study. It is important to highlight that the study focused on collegiate players with a season spanning a 4-month period, whereas in the UK, the season lasts for 10 months. Therefore, our research aims to be the first to employ DXA for observing body composition and assessing bone health in professional female players in WSL 1 over the course of a 10-month season. This will provide valuable insights into potential changes within this specific population.

As previously stated, experiencing LEA can significantly negatively impact both the health and performance of athletes. Low energy availability is at the heart of the Female Athlete Triad (Nattiv et al., 2007) and Relative Energy Deficiency in Sport (RED-S) (Mountjoy et al., 2014). Chronic LEA can lead to the suppression of metabolic (Melin et al., 2015) and reproductive hormones, menstrual dysfunction (Loucks, 2003) and compromised bone health (De Souza et al., 2008). In previous studies (Study 2, Chapter 5), LEA was not a prevalent issue among the squad measured. Reed et al. (2013), conducted a study on US collegiate female soccer players, measuring energy availability at three different time points during the season. While the mean energy availability remained above the LEA threshold of 30 kcal·kg $\text{FFM}^{-1}\cdot\text{day}^{-1}$ at each time point, they observed that 29% of players experienced LEA either at the pre-season or mid-season assessment. Additionally, they noted a 19% decline in energy availability from pre-season to mid-season, followed by a 35% improvement at post-season, primarily attributed to a decrease in EEE. The study identified low EI as the main contributor to LEA, often linked to unintentional under-eating during periods of increased exercise (Reed et al., 2013). As previously stated, this study focused on collegiate players with a season spanning a 4-month period, compared to the WSL season lasting 10 months. In the current study, our aim is to replicate these dataset, specifically focusing on professional female soccer players in WSL 1 over a 10-month season.

It is considered best practice for female soccer players to consume 5-7g·kg$^{-1}$·d$^{-1}$ of carbohydrate on moderate training days, increasing to >7g·kg$^{-1}$·d$^{-1}$ around match days (Collins et al., 2021; Maughan and Shirreffs, 2007). These carbohydrate levels are advised to optimise performance during both training and matches (Williams and Rollo, 2015). Previous studies on female soccer players have reported daily carbohydrate intakes within the range of 4.1–4.7g·kg·day$^{-1}$ (Martin et al., 2006; Mullinix et al., 2003). Notably, Reed et al. (2014) found carbohydrate intakes of 7 ± 1g·kg$^{-1}$·d$^{-1}$, 5 ± 1g·kg$^{-1}$·d$^{-1}$ and 5 ± 1g·kg$^{-1}$·d$^{-1}$ during pre-season, mid-season, and post-season, respectively. In Study 2 (Chapter 5), WSL 1 players were observed to consume 3.7 ± 0.2g·kg$^{-1}$·d$^{-1}$ of carbohydrates in pre-season. These quantities suggest a potential challenge in adequately replenishing and maintaining glycogen reserves. Given the importance of glycogen for optimal performance, there arises a need for further investigation to determine if this trend persists throughout the season.

Dietary protein plays a crucial role in the remodeling and rebuilding of muscles after exercise. Recent evidence suggests that females engaging in intermittent exercise may benefit from a protein intake of approximately 1.7g·kg·day$^{-1}$ (Wooding et al., 2017). Previous studies on protein intake in female soccer players based in the US have reported varying amounts, ranging from 1.2g·kg·day$^{-1}$ (Martin et al., 2006) to 1.3g·kg·day$^{-1}$ (Mullinix et al., 2003) and 2g·kg·day$^{-1}$, 2g·kg·day$^{-1}$ and 1g·kg·day$^{-1}$ pre-season, mid-season, post-season respectively (Reed et al., 2014). In our own investigation (Study 2, Chapter 5), protein intake in WSL 1 players does not seem to be a concern, as 87% of players were observed to consume more than 1.7g·kg·day$^{-1}$. It is worth noting that Reed et al. (2014) observed a statistically significant decline in protein intake towards the end of the season. Therefore, obtaining longitudinal data in WSL 1 players is necessary to determine if a similar trend occurs in this specific population.

Dietary fat is important for female soccer players, serving as an energy source and a vehicle for uptake and absorption of fat-soluble vitamins and essential fatty acids. Reported intakes in US collegiate female soccer players have ranged from 0.9g·kg·day$^{-1}$ (Martin et al., 2006; Mullinix et al., 2003) to 2g·kg·day$^{-1}$, 2g·kg·day$^{-1}$ and 1g·kg·day$^{-1}$ in pre-season, mid-season and post-season respectively (Reed et al., 2014). Similar intakes were seen in WSL 1 players (Chapter 5) with an intake of 1.1 ± 0.1g·kg·day$^{-1}$. It is crucial to note that any reduction in fat intake could potentially lead to a caloric deficit, thereby increasing the risk of LEA and subsequently posing health and performance issues (Reed et al., 2011).

While biomarker assessment offers objective data to evaluate overall player health, periodic assessments during a competitive season are essential for a more accurate measure of athlete health and physiological state (Bessa et al., 2016). The absence of female soccer-specific reference values poses a challenge, diminishing the accuracy and interpretation of biomarkers. In addition, single measurements do not allow for a precise determination of a player's health status (Hecksteden et al., 2017; Lee et al., 2017; Meyer and Meister, 2011). Certain biomarkers, such as Vitamin D, are known to change seasonally, while others may vary due to training load (e.g., iron profiles) or the menstrual cycle (e.g., Oestrogen). Notably, while biomarker concentrations in conjunction with exercise have been previously reported in male athletes, such information is lacking for female soccer players. Therefore, this study aims to assess biomarkers at four different time points throughout the season. The objective is twofold: firstly, to quantify the changes occurring in female soccer players over time, and secondly, to interpret each player's biomarkers in reference to their own historical data. This personalised approach enhances the precision and reliability of result interpretation providing practitioners with a deeper understanding of potential fluctuations over a season in this population.

Since Study 2 (Chapter 5) did not include RMR data, there is still a lack of measured RMR data for elite female soccer players in WSL 1. Consequently, measuring RMR at three different points in a season will offer more accurate insights into TEE at various stages. This approach not only serves as a potential screening tool for LEA but also provides valuable insights into RMR changes for players over the course of a season (Melin et al., 2015).

In Study 2 (Chapter 5), EEE on light training and match days was recorded as 481 ± 203kcal and 966 ± 269kcal respectively. However, since these measurements were taken during pre-season, they are likely to be higher due to increased training demands. To gain a better understand of how EEE changes over the season and to test the hypothesis that light training is likely to be lower and match expenditure higher during the season, this study will measure EEE on days with similar training load profiles (light training and match days) at three additional time points. This expanded data set will offer more comprehensive insights, allowing practitioners to better understand the fueling requirements for performance. Additionally, to address previous limitations identified in Study 2 (Chapter 5), this study will maintain training logs for EEE on non-training days to quantify non-club related exercise more accurately.

The primary objective of this chapter was to provide longitudinal data on the prevalence and factors affecting LEA among elite female soccer players throughout a season in WSL 1. Across four designated time points, the study aimed to:

(1) assess energy availability of female professional soccer players in WSL 1
(2) evaluate the macronutrient intake of the players on rest days, training days, and match days
(3) measure the players' body composition and bone health
(4) analyse common hormonal and micronutrient blood biomarkers
(5) measure players' RMR and EEE
(6) monitor their training load.

## 6.3 Methods

### *6.3.1 Participants*

Nineteen professional female soccer players (Table 16 and 17) provided written informed consent after receiving verbal and written information. The study was approved by the Wales Research Ethics Committee [approval number: 17/WA/0228] (section 3.1). The participant cohort comprised 17 outfield players and 2 goalkeepers, all representing the same team. Thirteen players completed the entire testing battery (excluding biomarker testing), four completed 75% of the testing battery, and the remaining one completed at least 50% of the testing. Biomarker analysis included results from ten players who provided samples for at least 75% of the designated time points, while ten players chose not to participate in this aspect of the study. Data from one player were excluded from the analysis due to non-compliance across multiple measures and testing sessions.

All participants competed in the top division of English women's soccer (WSL 1). The club provided breakfast and lunch on training days, which occurred approximately four days per week. Vitamin D supplementation was offered during lunch, with a recommended dose of 2000 IU administered from November 2017 to April 2018. The supplementation was available on a counter during each meal, and players were responsible for self-administration. Compliance with the supplementation protocol was not explicitly measured.

### *6.3.2 Experimental design*

During pre (3 consecutive days in August), early mid (3 consecutive days in November) late mid (3 consecutive days in February) and end of (3 consecutive days in May) season, repeated measures of EI, EEE, energy availability, body composition, RMR, blood sample, BP and LEAF-Q were obtained. Each assessment consisted of a match day (day 1), a day off (day 2) and a light training day (day 3). As this study was aiming to monitor players in free-living conditions, no interventions were in place to alter any aspect of the players habitual routine or diet (Chapter 3).

As with Study 2 (Chapter 5), players attended Liverpool John Moores University between 7.30 and 10.30 h after an overnight fast from midnight and more than 12 hours after exercise to complete the following measures: body composition, RMR, blood sample, blood pressure and the LEAF-Q (section 3.3 - 3.6).

### *6.3.3 Procedures*

During each 3-day monitoring period players electronically tracked DI using the RFPM which were then assessed by a BDA and SENr accredited nutritionist using Nutritics (Nutritics, v5, Ireland). In order to determine under or over-reporters the Goldberg cut off (Black, 2000) was undertaken, with an EI:RMR ratio outside 1.16 – 2.78 classified as under or over-reporters (section 3.8). During DI assessment, training, match load and EEE was monitored using GPS technology (Apex, STATSports, Newry, Northern Ireland) (section 3.9). For reporting match loads only outfield players who played a minimum of 75 minutes were included in the analysis. The day off in November 2017, February 2018 and May 2018 training logs were completed (including activity, exercise duration and rest periods) for any non-club based sessions and assigned a metabolic equivalent value from the compendium of physical activities (Ainsworth et al., 2011). Values were corrected, with energy expended from measured RMR for the duration of the exercise subtracted to provide EEE (Kozey, Lyden, Staudenmayer, and Freedson, 2010). Energy availability was then calculated using the DI record (RFPM), training and match data and estimated FFM (DXA). The established thresholds of optimal (> 45 kcal·kg FFM$^{-1}$·day$^{-1}$), reduced (30-45 kcal·kg FFM$^{-1}$·day$^{-1}$), and low (<30 kcal·kg FFM$^{-1}$·day$^{-1}$) were used as the reference levels for comparing the players energy availability (Loucks et al., 2011; Mountjoy et al., 2014).

On the testing days at Liverpool John Moores players attended within 2 hours of waking, where their stature and body mass were taken (section 3.3.1). Players were then asked to lie in a supine position on the DXA scanner. Whole DXA was carried out for all four time points. Unilateral hip and lumbar spine were only carried out over three time points (August, November, and May). This was to prevent unnecessary radiation exposure (section 3.3.2). Once the scans were complete, players undertook an RMR assessment. A calibration was conducted before each measurement, after which, players relaxed in a supine position for 10 minutes, before data collection was carried out for 20 minutes, with the last 10 minutes used to calculate RMR (section 3.4.2). To ascertain players with low RMR, the ratio between measured RMR and predicted RMR was calculated using the Ten Haaf method (Ten Haaf and Weijs, 2014), with an RMR ratio of <0.90 considered low (De Souza et al., 2008). Following the RMR measurement, blood pressure was measured in the supine position (section 3.5). Blood samples were then collected from the antecubital vein in the anterior crease of the forearm and sent to a UKAS lab for analysis (section 3.6). Players finally completed the LEAF-Q to assess risk of LEA (section 3.7) provide a self-reported insight to the players HC use or menstrual function (section 3.7).

### *6.3.4 Statistical analysis*

Statistical analysis was completed using Jamovi Version 2.3 (The Jamovi project, https://www.jamovi.org). Data are reported as means ± standard deviations (SD), and $P \leq 0.05$ was considered statistically significant. Figures were produced in GraphPad Prism version 10.0 software (GraphPad, San Diego, CA). All variables were tested for normality using the Shapiro-wilk test of normality with all being normally distributed. To account for missing time points, a linear mixed model was conducted to assess differences between the fixed factors (position and time points) and the following random factors: BM, BF%, FFM, whole body Z score, lumbar Z scores training duration, distance, HSR, ED, and EEE. To investigate which positions and time points were significantly different a Holm-Bonferroni post hoc test was carried out. A linear mixed model was conducted to assess differences between the fixed factors (training days and time points) and the following random factors: EI, macronutrient intake, energy availability, EEE, training duration, distance, HSR and ED. To investigate which training days and time points were significantly different a Holm-Bonferroni post hoc test was carried out. Within-player observations are not independent of each other, while between-player observations would usually be treated as independent. However, in this case, they were not independent due to being from the same team and therefore exposed to the same training, tactics, and game play.

A one-way repeated measures ANOVA was conducted to assess differences between training days over the whole season for energy and macronutrient intake. To investigate which days were significantly different a Tukey's HSD post hoc test were undertaken.

## 6.4 Results:

### *6.4.1 Body composition*

Tables 16 and 17 provide an overview of body composition and bone health by position across the four time points. All players had hip and lumbar Z-scores >-1.0 (Nattiv et al., 2007) for all time points across the season. Fat mass (1.1 ± 0.2kg) ($P = 0.039$) and BF % (1.9 ± 0.2) ($P = 0.008$) significantly reduced between pre-season (August) and early season (November). Fat free mass significantly increased (2.2 ± 0.6kg) ($P = 0.046$) and BF % (1.8 ± 0.2kg) ($P = 0.012$) significant reduced between pre-season (August) and late season (February).

**Table 16.** Body composition, risk of low energy availability, resting metabolic rate, blood pressure and heart rate for professional female soccer players over a full season.

| Time point | August 2017 | November 2017 | February 2018 | May 2018 | Whole season |
|---|---|---|---|---|---|
| Stature (m) | 1.66 ± 0.1 | 1.67 ± 0.1 | 1.67 ± 0.1 | 1.66 ± 0.0 | 1.66 ± 0.0 |
| Body mass (kg) | 59.8 ± 5.8 | 59.8 ± 5.5 | 60.1 ± 5.4 | 59.5 ± 5.3 | 59.8 ± 5.4 |
| Total fat mass (kg) | 13.1 ± 2.7 | 12.0 ± 2.5[a] | 12.1 ± 2.5 | 12.4 ± 2.5 | 12.4 ± 2.5 |
| Total fat free mass (kg) | 41.6 ± 4.0 | 42.7 ± 3.6 | 42.8 ± 3.4[a] | 42 ± 3.2 | 42.3 ± 2.5 |
| Percent body fat (%) | 23 ± 3.1 | 21.1 ± 2.9[a] | 21.2 ± 2.9[a] | 21.8 ± 3.1 | 21.7 ± 3.0 |
| Hip Z score | 1.7 ± 0.8 | 2.0 ± 0.8 | - | 1.9 ± 0.9 | 1.8 ± 0.8 |
| Lumbar spine Z score | 0.8 ± 0.6 | 1.0 ± 0.7 | - | 1.2 ± 0.8 | 1.0 ± 0.7 |
| LEAF-Q | 5.8 ± 3.0 | 6.4 ± 2.4 | 5.9 ± 3.0 | 6.0 ± 2.9 | 6.0 ± 2.8 |
| RMR ($kcal \cdot d^{-1}$) | - | 1572 ± 151 | 1560 ± 148 | 1496 ± 121[b] | 1543 ± 73 |
| Predicted RMR ($kcal \cdot d^{-1}$) | 1432 ± 90 | 1456 ± 81 | 1458 ± 77[a] | 1440 ± 74 | 1443 ± 78 |
| RMR ratio | - | 1.08 ± 0.10 | 1.07 ± 0.08 | 1.04 ± 0.06 | 1.06 ± 0.08 |

Data are mean ± SD for all variables. RMR = resting metabolic rate. LEAF-Q = Low Energy Availability in Females Questionnaire. Predicted RMR based on Ten Haaf equation (Ten Haaf and Weijs, 2014), [a] indicates a significant difference from August 2017 ($P < 0.05$), [b] indicates a significant difference from November 2017 ($P < 0.05$).

**Table 17.** Body composition, risk of low energy availability, resting metabolic rate, blood pressure and heart rate for professional female soccer players over a full season broken down by position.

| Time point | Goalkeeper (n=2) | Defender (n=5) | Midfielder (n=5) | Forward (n=6) |
|---|---|---|---|---|
| Stature (m) | 1.71 ± 0.0 | 1.65 ± 0.0 | 1.63 ± 0.0 | 1.68 ± 0.0 |
| Body mass (kg) | 68.1 ± 2.1 | 57.7 ± 4.4 | 58.3 ± 5.6 | 60.2 ± 4.1 |
| Total fat mass (kg) | 16.9 ± 2.8 | 12.0 ± 2.0 | 12.2 ± 1.7 | 11.4 ± 1.9$^{a}$ |
| Total fat free mass (kg) | 45.7 1.7 | 40.6 ± 2.7 | 40.9 ± 3.7 | 43.8 ± 3.0 |
| Percent body fat (%) | 26.0 ± 3.8 | 22.0 ± 2.7 | 22.1 ± 1.3 | 19.8 ± 2.6 |
| Hip Z score | 3.0 ± 1.1 | 1.5 ± 0.5 | 1.9 ± 0.6 | 1.6 ± 0.7 |
| Lumbar spine Z score | 1.2 ± 0.2 | 0.5 ± 0.6 | 1.4 ± 0.7 | 1.0 ± 0.7 |
| LEAF-Q | 6.1 ± 1.7 | 4.8 ± 2.3 | 7.6 ± 2.5 | 5.7 ± 3.1 |
| RMR (kcal·d$^{-1}$) | 1552 ± 79 | 1532 ± 158 | 1516 ± 153 | 1572 ± 138 |
| Predicted RMR (kcal·d$^{-1}$) | 1526 ± 38 | 1408 ± 61 | 1417 ± 83 | 1481 ± 67 |
| RMR ratio | 1.01 ± 0.02 | 1.08 ± 0.09 | 1.06 ± 0.07 | 1.07 ± 0.10 |

Data are mean ± SD for all variables. RMR = resting metabolic rate. LEAF-Q = Low Energy Availability in Females Questionnaire. *Predicted RMR based on Ten Haaf education (Ten Haaf and Weijs, 2014), $^{a}$ indicates a significant difference from goalkeeper ($P < 0.05$).

### *6.4.2 The Low Energy Availability in Females Questionnaire*

Tables 16 and 17 and Figures 18 and 19 provide the squad's mean global LEAF-Q score over the season and positional breakdown. Using a global score of ≥8 (Melin et al., 2014) 36%, 35%, 28% and 29% of the squad were classified at risk of LEA in August 2017, November 2017, February 2018 and May 2018 respectively. Split positionally, 14%, 17%, 58% and 27% of the goalkeepers, defenders, midfielders, and forwards were classified at risk of LEA over the season, however, when compared positionally these were not significantly different. In February 2018, one player reported experiencing altered menstrual function, noting a lack of menstruation for approximately six weeks. However, by May 2018, this player reported a return to her normal menstrual cycle. Similarly, another player reported altered menstrual function in May 2018, stating it had been approximately eight weeks since her last period.

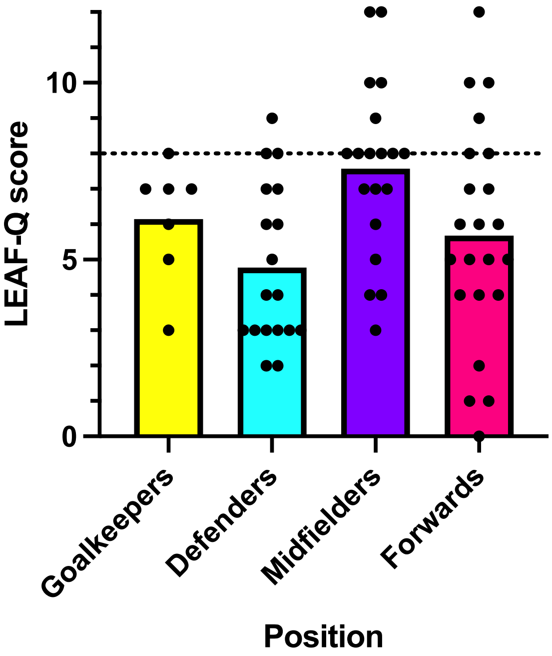


**Figure 18.** LEAF-Q scores per position. Dashed line (≥8) and above represents when the athlete is 'at risk' of low energy availability. LEAF-Q = low energy availability in female's questionnaire.

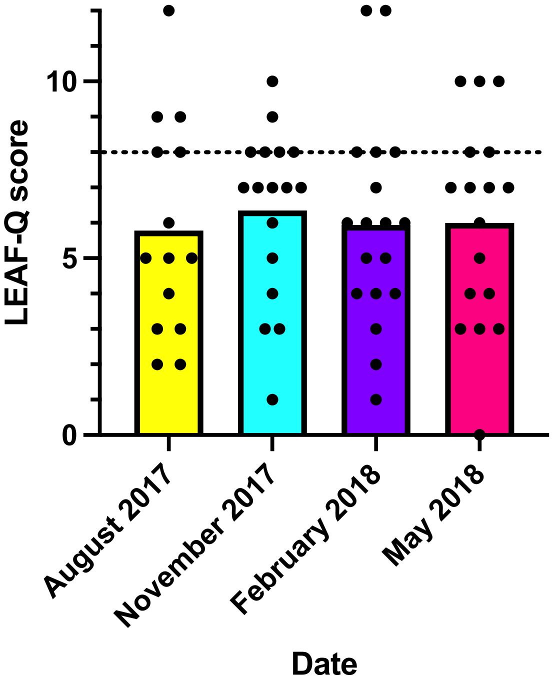


**Figure 19.** LEAF-Q scores for each time point over the season. Dashed line (≥8) and above represents when the athlete is 'at risk' of low energy availability. LEAF-Q = low energy availability in female's questionnaire.

### *6.4.3 Dietary intake validity*

Overall 84% of players were within the accepted threshold of under or over reporting of DI (1.16 – 2.78) using the Goldberg cut-off equation (Black, 2000). Over the season 80% (August 2017), 82% (November 2017), 78% (February 2018) and 94% (May 2018) were within ratio, with all players outside of the ratio being classified as potential under-reporters (EI: RMR = <1.16). As reported previously, the recommendation not to remove these players from further analysis due to a small sample size was adhered to (Black, 2000). The coefficient of variation between the lead researcher and the second SENr nutritionists' food diary analysis were 3.78 ± 1.92%, 4.16 ± 2.21%, 3.83 ± 2.16% and 4.23 ± 1.83% for energy intake (kcal), carbohydrate (g), protein (g) and fat (g), respectively.

### *6.4.4 Energy availability*

Mean energy availability observed throughout the entire season was deemed optimal for 78% of players. On average, no player exhibited LEA on training days (54 ± 10Kcal$\cdot$kg FFM$^{-1}\cdot$day$^{-1}$) or days off (47 ± 10Kcal$\cdot$kg FFM$^{-1}\cdot$day$^{-1}$), while 6% of players experienced LEA on match days (52 ± 8Kcal$\cdot$kg FFM$^{-1}\cdot$day$^{-1}$) (Table 19). Players were significantly more prone to have lower energy availability on days off compared to training days ($P < 0.001$) and match days ($P = 0.031$) (Table 19). No players mean energy availability was <30 Kcal$\cdot$kg FFM$^{-1}\cdot$day$^{-1}$ when classified by time point in the season (Figure 20) and position (Figure 21). The squad energy availability classification was optimal on each day measured barring the day off in February 2018 when it was reduced (42 Kcal$\cdot$kg FFM$^{-1}\cdot$day$^{-1}$).

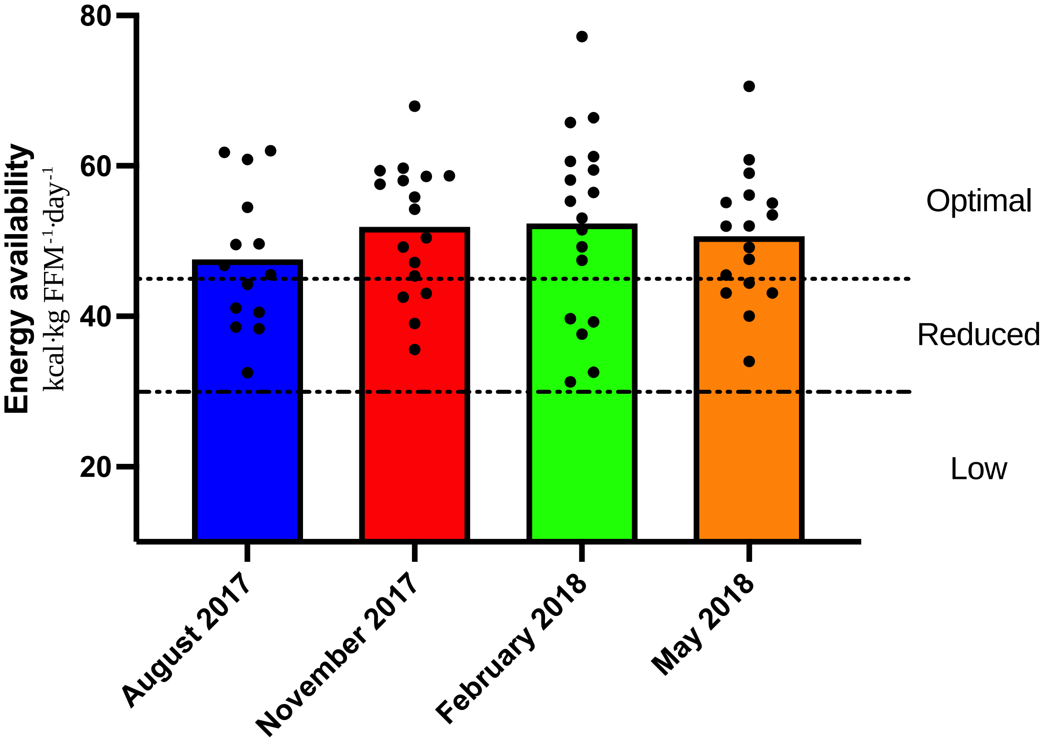


**Figure 20.** Energy availability scores for each time point over the season where LEA is <30Kcal·kg FFM$^{-1}$·day$^{-1}$ reduced is 30-45 Kcal·kg FFM$^{-1}$·day$^{-1}$ and optimal is >45 Kcal·kg FFM$^{-1}$·day$^{-1}$. Each dot represents an individual player.

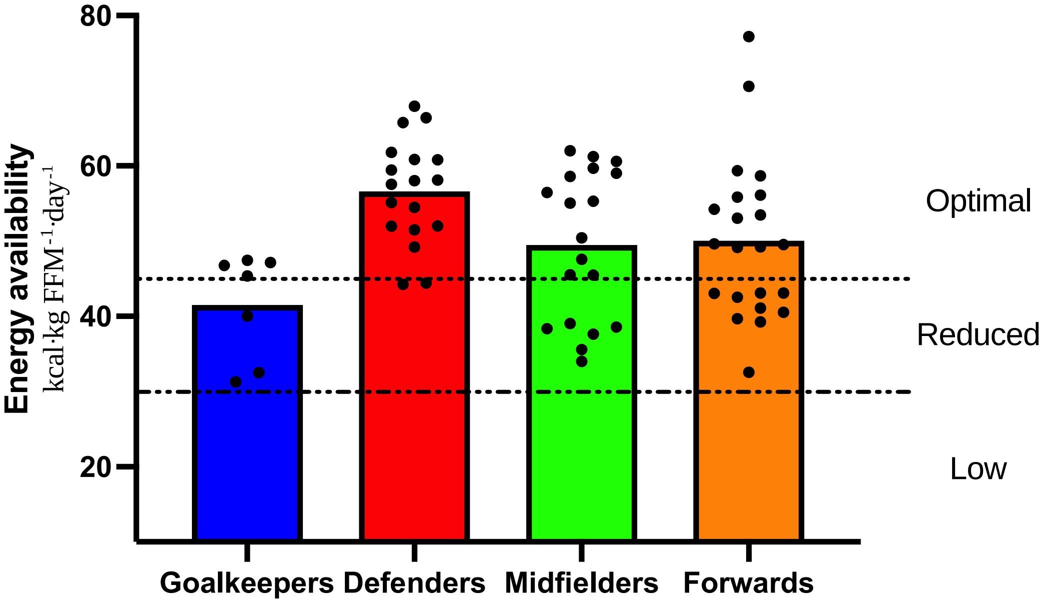


**Figure 21.** Energy availability scores per position over the season where LEA is <30Kcal·kg FFM$^{-1}$·day$^{-1}$ reduced is 30-45 Kcal·kg FFM$^{-1}$·day$^{-1}$ and optimal is >45 Kcal·kg FFM$^{-1}$·day$^{-1}$. Each dot represents an individual player.

### *6.4.5 Dietary intake*

Table 18 provides an overview of energy and macronutrient intake. Mean carbohydrate intake for match days was below $7g{\cdot}kg^{-1}{\cdot}d^{-1}$ throughout the season ($4.5 \pm 1.1g{\cdot}kg^{-1}{\cdot}d^{-1}$) with only two players consuming over $7g{\cdot}kg^{-1}{\cdot}d^{-1}$ on match days over the season (Figure 22). Energy intake and carbohydrate intake were significantly higher on light training ($P < 0.001$) and match days ($P = 0.028$) compared to rest days. Average protein intake over all three training days was $2.1 \pm 0.4g{\cdot}kg^{-1}{\cdot}d^{-1}$ (range of $1.9 – 2.3g{\cdot}kg^{-1}{\cdot}d^{-1}$), with mean fat intake over the season being $1.3 \pm 0.5g{\cdot}kg^{-1}{\cdot}d^{-1}$.

**Table 18.** Dietary and macronutrient intake as total amounts and relative to body mass over the season.

| | Time-point | Energy intake (kcal) | Energy (kcal/kg FFM) | Carbohydrate (g) | Carbohydrate ($g \cdot kg^{-1} \cdot d^{-1}$) | Protein (g) | Protein ($g \cdot kg^{-1} \cdot d^{-1}$) | Fat (g) | Fat ($g \cdot kg^{-1} \cdot d^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Overall mean | Aug 2017 | 1963 ± 302 | 47.7 ± 9.4 | 220 ± 59 | 3.7 ± 1.2 | 116 ± 20 | 2.0 ± 0.4 | 69 ± 14 | 1.2 ± 0.3 |
| | Oct-17 | 2210 ± 336 | 52.1 ± 8.8 | 259 ± 65[a] | 4.4 ± 1.2 | 124 ± 27 | 2.1 ± 0.4 | 73 ± 21 | 1.2 ± 0.4 |
| | Feb-18 | 2223 ± 471 | 52.5 ± 12.5 | 240 ± 45 | 4.0 ± 0.8 | 125 ± 32 | 2.1 ± 0.6 | 83 ± 27 | 1.4 ± 0.5 |
| | May-18 | 2122 ± 344 | 50.8 ± 8.8 | 225 ± 49 | 3.8 ± 0.9[b] | 118 ± 19 | 2.0 ± 0.3 | 81 ± 21 | 1.4 ± 0.7 |
| Match day | Aug 2017 | 1848 ± 349 | 44.9 ± 9.8 | 206 ± 61 | 3.5 ± 1.2 | 106 ± 30 | 1.8 ± 0.5 | 69 ± 21 | 1.2 ± 0.4 |
| | Oct-17 | 2358 ± 480[a] | 55.3 ± 10.3[a] | 307 ± 99[a] | 5.1 ± 1.6[a] | 130 ± 36 | 2.2 ± 0.5 | 63 ± 16 | 1.1 ± 0.3 |
| | Feb-18 | 2322 ± 546[a] | 55 ± 14.5[a] | 285 ± 81[a] | 4.8 ± 1.4 | 103 ± 46 | 1.7 ± 0.8 | 80 ± 28 | 1.4 ± 0.5 |
| | May-18 | 2117 ± 461 | 50.4 ± 9.7 | 253 ± 78 | 4.3 ± 1.3 | 117 ± 37 | 2.0 ± 0.5 | 65 ± 20 | 1.1 ± 0.3 |
| | Mean | 2188 ± 351[*] | 51.9 ± 8.5[*] | 268 ± 61[*] | 4.5 ± 1.1[*] | 114 ± 21[*] | 1.9 ± 0.4[*] | 70 ± 14[*] | 1.2 ± 0.3[*] |
| Day off | Aug 2017 | 2078 ± 456 | 50.2 ± 11.3 | 243 ± 68 | 4.1 ± 1.2 | 120 ± 31 | 2.0 ± 0.5 | 70 ± 19 | 1.2 ± 0.4 |
| | Oct-17 | 1993 ± 427 | 47.2 ± 11.9 | 216 ± 84 | 3.7 ± 1.6 | 110 ± 30 | 1.8 ± 0.5 | 73 ± 36 | 1.3 ± 0.7 |
| | Feb-18 | 1780 ± 465 | 42.1 ± 12.2[a] | 175 ± 41[a] | 2.9 ± 0.8[a] | 108 ± 37 | 1.8 ± 0.6 | 73 ± 32 | 1.2 ± 0.6 |
| | May-18 | 1993 ± 495 | 47.9 ± 13.0 | 179 ± 58[a] | 3.0 ± 1.1[a] | 113 ± 33 | 1.9 ± 0.6 | 89 ± 39 | 1.5 ± 0.7 |
| | Mean | 1960 ± 330[#*] | 46.7 ± 9.8[#*] | 198 ± 46[#*] | 3.3 ± 0.9[#*] | 114 ± 23[*] | 1.9 ± 0.4[*] | 78 ± 25 | 1.3 ± 0.5 |
| Training day | Aug 2017 | 1963 ± 370 | 48.0 ± 12.4 | 209 ± 80 | 3.6 ± 1.6 | 124 ± 22 | 2.1 ±0.5 | 68 ± 23 | 1.2 ± 0.4 |
| | Oct-17 | 2278 ± 494 | 53.7 ± 12.3 | 253 ± 51 | 4.3 ± 1.5 | 132 ± 32 | 2.2 ± 0.6 | 82 ± 22 | 1.4 ± 0.4 |
| | Feb-18 | 2567 ± 645[a] | 60.4 ± 16.1[a] | 260 ± 63 | 4.3 ± 1.1 | 163 ± 42[a] | 2.7 ± 0.7[ab] | 97 ± 39[a] | 1.6 ± 0.7[a] |
| | May-18 | 2255 ± 436 | 54.0 ± 11.2 | 242 ± 67 | 4.1 ± 1.3 | 123 ± 29[c] | 2.1 ± 0.5[c] | 89 ± 25 | 1.5 ± 0.4 |
| | Mean | 2306 ± 371 | 54.8 ± 10.3 | 243 ± 54 | 4.1 ± 1.1 | 138 ± 21 | 2.3 ± 0.4 | 87 ± 22 | 1.5 ± 0.4 |

Values are mean ± SD for energy intake, carbohydrate, protein, and fat for all players. FFM = fat free mass. [a] indicates a significant difference from August 2017 ($P < 0.05$), [b] indicates a significant difference from November 2017 ($P < 0.05$), [c] indicates a significant difference from February 2018 ($P < 0.05$). [*] Indicates a significant difference from mean training day ($P < 0.05$), [#] indicates a significant difference from mean match day ($P < 0.05$).

**Table 19.** Energy availability and exercise energy expenditure over the season.

| | Time-point | EA (Kcal·kg FFM$^{-1}$· day$^{-1}$) | Estimated exercise energy expenditure | EA classification | % of players with low EA | % of players with reduced EA | % of players with optimal EA |
|---|---|---|---|---|---|---|---|
| Overall mean | Aug 2017 | 48 ± 9 | 729 ± 199 | Optimal | 0 | 47 | 53 |
| | Oct-17 | 52 ± 9 | 712 ± 209 | Optimal | 0 | 24 | 76 |
| | Feb-18 | 52 ± 13 | 661 ± 151 | Optimal | 0 | 28 | 72 |
| | May-18 | 51 ± 9 | 574 ± 157 | Optimal | 0 | 29 | 71 |
| Match day | Aug 2017 | 45 ± 10 | 955 ± 275 | Optimal | 7 | 40 | 53 |
| | Oct-17 | 55 ± 10[a] | 986 ± 401 | Optimal | 0 | 18 | 82 |
| | Feb-18 | 55 ± 15[a] | 888 ± 275 | Optimal | 6 | 17 | 77 |
| | May-18 | 50 ± 10 | 810 ± 326 | Optimal | 0 | 29 | 71 |
| | Mean | 52 ± 8 | 912 ± 169[*] | Optimal | 6 | 6 | 88 |
| Day off | Aug 2017 | 50 ± 11 | - | Optimal | 0 | 33 | 67 |
| | Oct-17 | 47 ± 12 | 106 ± 70 | Optimal | 6 | 50 | 44 |
| | Feb-18 | 42 ± 12[a] | 62 ± 60 | Reduced | 22 | 22 | 56 |
| | May-18 | 48 ± 13 | 79 ± 64 | Optimal | 12 | 29 | 59 |
| | Mean | 47 ± 10[*#] | 81 ± 48[*#] | Optimal | 0 | 50 | 50 |
| Training day | Aug 2017 | 48 ± 12 | 503 ± 192 | Optimal | 0 | 47 | 53 |
| | Oct-17 | 54 ± 12 | 438 ± 72 | Optimal | 0 | 18 | 82 |
| | Feb-18 | 60 ± 16[a] | 434 ± 61 | Optimal | 0 | 17 | 83 |
| | May-18 | 54 ± 11 | 338 ± 40[abc] | Optimal | 0 | 29 | 71 |
| | Mean | 54 ± 10 | 420 ± 64 | Optimal | 0 | 28 | 72 |

Values are mean ± SD for energy availability and estimated exercise energy expenditure for all players. FFM = fat free mass, EA = energy availability. [a] indicates a significant difference from August 2017 ($P < 0.05$), [b] indicates a significant difference from November 2017 ($P < 0.05$), [c] indicates a significant difference from February 2018 ($P < 0.05$). [*] Indicates a significant difference from mean training day ($P < 0.05$), [#] indicates a significant difference from mean match day ($P < 0.05$).

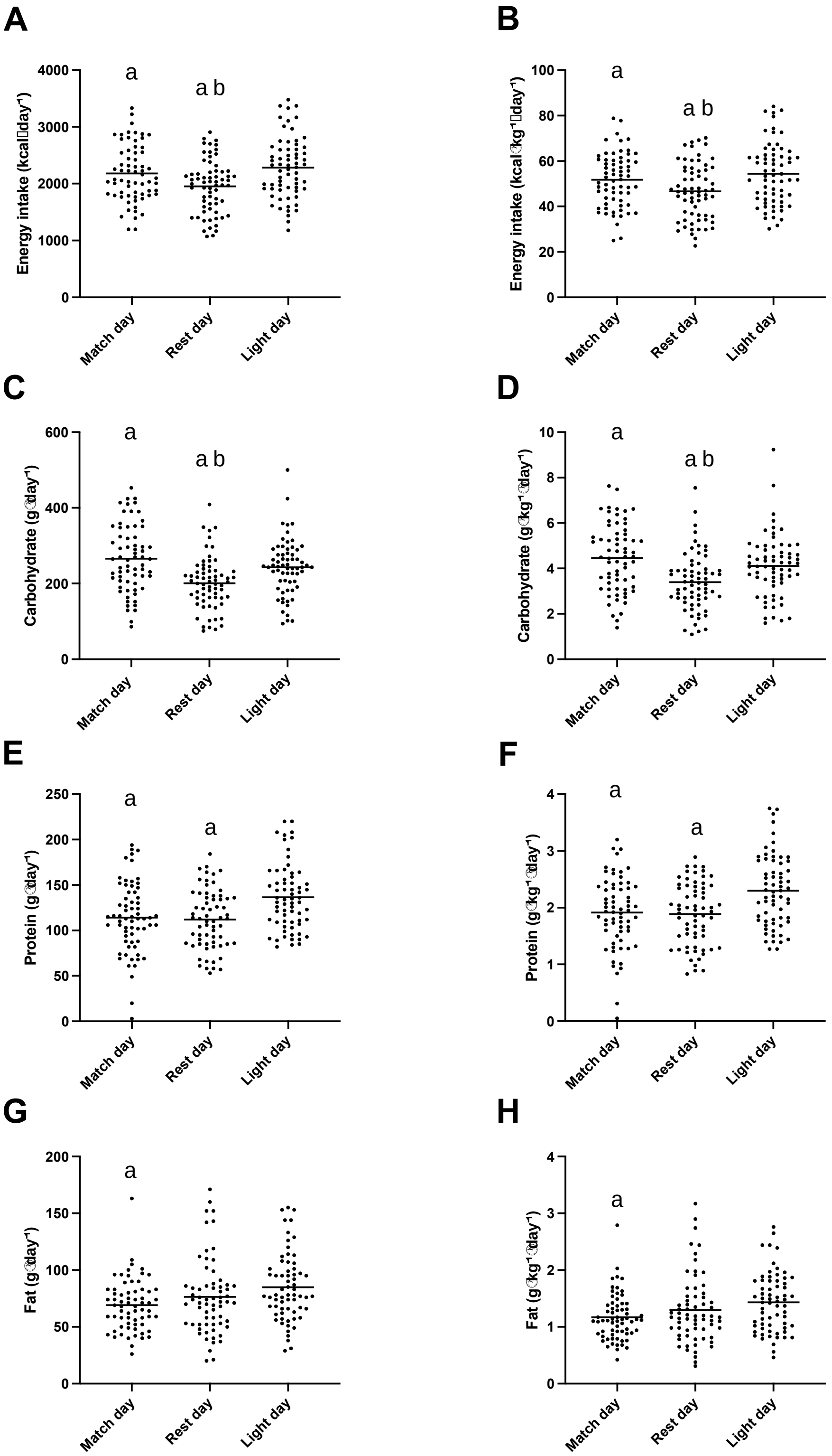


**Figure 22.** Energy intake (kcal) (A), energy intake relative to fat free mass (B), carbohydrate intake (C), carbohydrate intake relative to body mass (kg) (D), protein intake (g) (E), protein intake relative to fat free mass (kg) (F), fat intake (g) (G), fat intake relative to fat free mass (kg) (H) on three types of training days over four time points in a season (August 2017 – May 2018). $^{a}$ denotes significant difference from light day ($P < 0.05$). $^{b}$ denotes significant difference from match day ($P < 0.05$). ($P < 0.05$). Solid line is the mean for each day, with each dot being an individual player.

### *6.4.6 Biomarkers*

Full biomarker profiles can be seen in Table 20. Prolactin was significantly less during the season than in pre-season ($P = 0.003$). Vitamin D exhibited a significant reduction during the season (November 2017 and February 2018) in comparison to both the pre-season (August 2017) and end of season (May 2018) ($P <0.001$) However, vitamin D remained within clinical norms for all players throughout the season, except for one player in February 2018 where it dropped to 48 $\text{nmol}\cdot\text{L}^{-1}$. All other micronutrients were within clinical ranges for players throughout the season (Table 20). Haemoglobin ($P <0.001$) and hematocrit ($P <0.001$) concentrations were significantly lower during pre-season compared to the remainder of the season (Figure 23). Following pre-season, all players returned to concentrations within clinical norms.

**Table 20.** Micronutrient and biochemical profiles of ten female professional soccer players over four time points in the season.

| | **August 2017** | **November 2017** | **February 2018** | **May 2018** | |
|---|---|---|---|---|---|
| **Variable** | **Value (mean ± SD)** | **Value (mean ± SD)** | **Value (mean ± SD)** | **Value (mean ± SD)** | **Normal clinical range**** |
| Luteinising hormone U·L$^{-1}$ | 5.8 ± 2.8 | 8.66 ± 8.94 | 8.4 ± 4.6 | 8.9 ± 5.4 | 3 - 80** |
| Follicle stimulating hormone U·L$^{-1}$ | 4.1 ± 2.3 | 4.98 ± 2.4 | 5.1 ± 1.8 | 5.4 ± 1.7 | 1 - 16** |
| 17 beta oestradiol pmol·L$^{-1}$ | 345 ± 221 | 288 ± 238 | 382 ± 217 | 295 ± 215 | 45 - 1200** |
| Sex hormone binding globulin nmol·L$^{-1}$ | 96.0 ± 45.1 | 88 ± 39.8 | 84.1 ± 38.6 | 90.5 ± 46.2 | 25 - 155 |
| Insulin growth factor 1 nmol·L$^{-1}$ | 33.2 ± 4.3 | 38.6 ± 39.8 | 38.3 ± 7.7 | 37.3 ± 8.1 | 11.2 - 54.5 |
| Prolactin mU·L$^{-1}$ | 522.5 ± 228.0 | 282 ± 105[a] | 309 ± 152[a] | 358 ± 141[a] | <500 |
| Progesterone nmol·L$^{-1}$ | 14.4 ± 12.6 | 8.5 ± 14.3 | 19.6 ± 23.4 | 13.0 ± 17.4 | <1 - 70** |
| Ferritin ug·L$^{-1}$ | 64.1 ± 21.6 | 49.0 ± 24.4 | 45.6 ± 17.4 | 44.7 ± 15.2 | 13 - 150 |
| Iron umol·L$^{-1}$ | 13.9 ± 7.2 | 15.5 ± 7.6 | 18.0 ± 5.9 | 17.7 ± 3.8 | 13 - 32 |
| Transferrin g·L$^{-1}$ | 2.51 ± 0.34 | 2.84 ± 0.49[a] | 2.62 ± 0.37[b] | 2.57 ± 0.37[b] | 2.2 - 3.6 |
| Total iron binding capacity umol·L$^{-1}$ | 57 ± 7.7 | 64.8 ± 11.0[a] | 59.6 ± 8.4[b] | 58.4 ± 8.2[b] | 41 - 77 |
| % transferrin saturation | 23.6 ± 8.8 | 25.5 ± 4.8 | 31.3 ± 13.9 | 30.7 ± 6.3 | 16 - 55 |
| Haemoglobin g·L$^{-1}$ | 110.4 ± 20.0 | 137.0 ± 7.0[a] | 135 ± 6.3[a] | 135 ± 8.7[a] | 118 - 148 |
| Haematocrit L·L$^{-1}$ | 0.32 ± 0.06 | 0.40 ± 0.02[a] | 0.40 ± 0.02[a] | 0.40 ± 0.02[a] | 0.36 - 0.44 |
| Thyroid stimulating hormone mU·L$^{-1}$ | 2.52 ± 0.68 | 1.78 ± 0.52[a] | 1.97 ± 0.47 | 2.15 ± 0.67 | 0.3 - 6.0 |
| Free thyroxine pmol·L$^{-1}$ | 16.4 ± 2.2 | 16.2 ± 1.9 | 16.0 ± 2.0 | 17.1 ± 2.7 | 10 - 22 |
| Free triiodothyronine pmol·L$^{-1}$ | 4.89 ± 0.49 | 4.99 ± 0.49 | 4.89 ± 1.26 | 5.27 ± 0.67 | 3.6 - 6.4 |
| Total testosterone nmol·L$^{-1}$ | 0.9 ± 0.3 | 1.1 ± 0.4 | 1.1 ± 0.2 | 1.3 ± 0.4[a] | <1.9 |
| Insulin pmol·L$^{-1}$ | 47 ± 14.1 | 39.4 ± 19.6 | 54.1 ± 16.8 | 51.7 ± 20.4 | <174 |
| Cortisol nmol·L$^{-1}$ | 545 ± 246 | 437 ± 146 | 337 ± 120[a] | 423 ± 119 | 140 - 500 |
| Vitamin B12 ng·L$^{-1}$ | 428.9 ± 117.4 | 549 ± 228 | 459 ± 108 | 530 ± 254 | 160 - 800 |
| Folate ug·L$^{-1}$ | 8.62 ± 2.50 | 7.3 ± 4.5 | 5.6 ± 2.4 | 7.2 ± 3.0 | 3.9 - 20 |
| Zinc umol·L$^{-1}$ | 14.6 ± 1.2 | 24.5 ± 14.0[a] | 18.7 ± 1.9[ab] | 19.5 ± 3.2[ab] | 10.7 - 24.5 |
| Vitamin D nmol·L$^{-1}$ | 89.9 ± 16.9 | 75.8 ± 12.2[a] | 67 ± 13.8[a] | 91.8 ± 12.4[bc] | 50 - 200 |
| Magnesium nmol·L$^{-1}$ | 0.83 ± 0.05 | 0.84 ± 0.07 | 0.83 ± 0.06 | 0.85 ± 0.07 | 0.7 - 1.0 |

* Significant change from August 2017. [a] indicates a significant difference from August 2017 (P = <0.05), [b] indicates a significant difference from November 2017 (P = <0.05), [c] indicates a significant difference from February 2018 (P = <0.05). *Values are indicative of internal laboratory references for females of various age ranges; **Values are whole ranges and depend on the stage on the menstrual cycle.

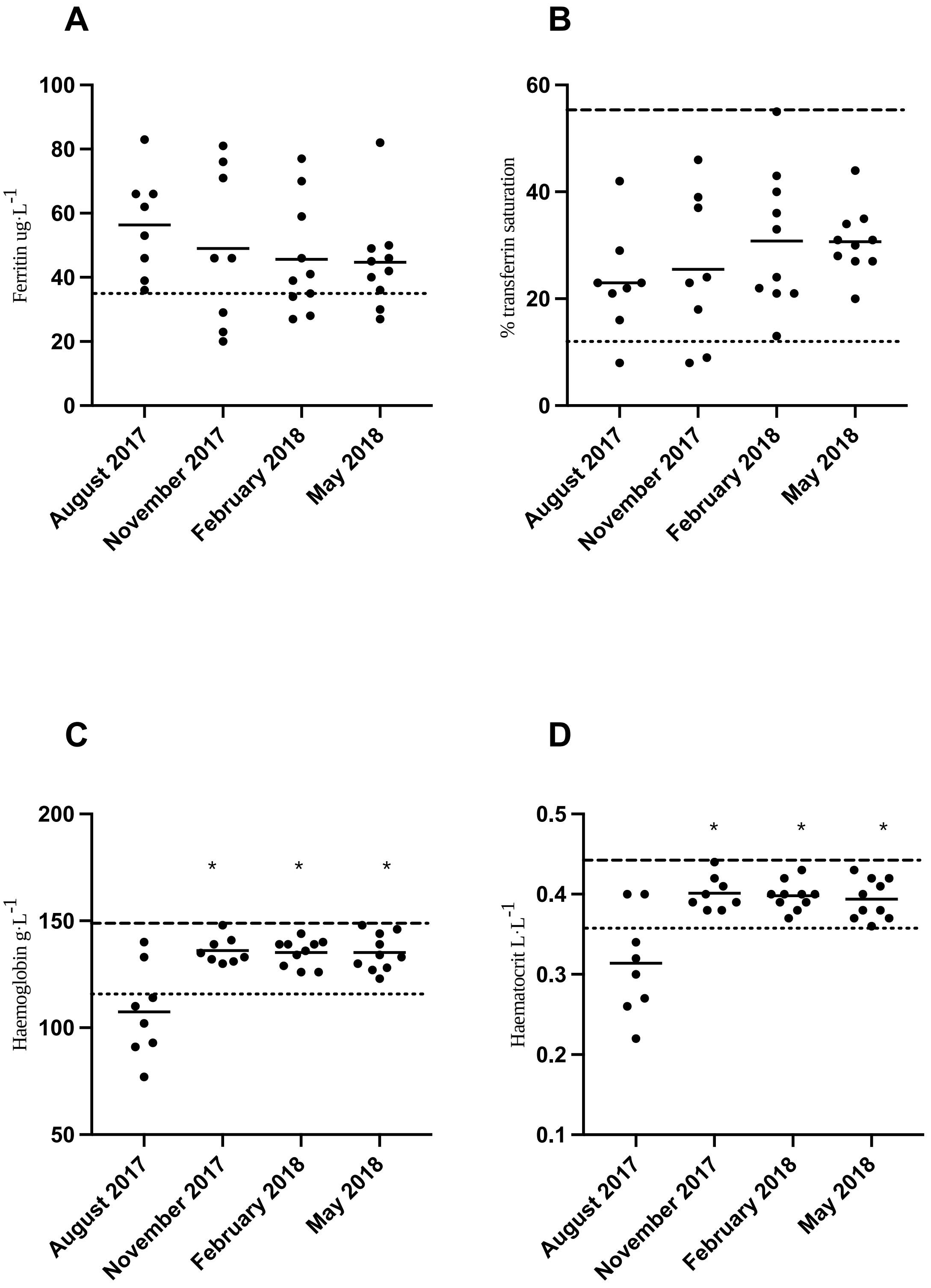


**Figure 23.** Ferritin (A), % transferrin saturation (B), haemoglobin (C) and haematocrit (D) over four time points in a season (August 2017 – May 2018). * denotes a significant different from August 2017 (P <0.001). Solid line is the mean for each day, dotted line the lower clinical range, dashed line the higher range with each dot being an individual player.

### *6.4.7 Resting metabolic rate*

Table 17 compares RMR, predicted RMR and RMR ratio over the season and by position. Measured RMR in November was significantly higher than at the end of season (May) (P = 0.042). Predicted RMR was significantly higher mid-season (February) compared to pre-season (August) (P = 0.046). There was no significant difference between mean RMR and mean predicted RMR when broken down by time point (P = 0.371) or position (P = 0.387). Predicted RMR underestimated measured RMR when broken down by time point and position, but not significantly (Table 17). Resting metabolic rate ratio was >0.90 for all the squad over the season (De Souza et al., 2008).

### *6.4.8 Training and match profiles*

Match days were significantly higher for distance (P <0.001), HSR (P <0.001) and ED (P <0.001) compared to light training days (Table 21). Broken down by position goalkeepers covered significantly less distance compared to defenders (P <0.001), midfielders (P <0.001) and forwards (P = 0.002). Goalkeepers covered less HSR than defenders (P = 0.005) and forwards (P <0.001) and less ED compared to defenders (P <0.001), midfielders (P <0.001) and forwards (P <0.001). There was no significant difference between positions for duration (P = 0.665).

**Table 21.** An overview of absolute training and match load during a season as a squad average and broken down into means by position.

| **Variable** | **Match day** | **Light training** | **Goalkeeper (n = 2)** | **Defender (n = 5)** | **Midfielder n = 5)** | **Forward (n = 6)** |
|---|---|---|---|---|---|---|
| Duration (mins) | 86 ± 12$^*$ | 86 ± 8 | 83 ± 9 | 81 ± 12 | 79 ± 16 | 77 ± 13 |
| Distance (m) | 10570 ± 1925$^*$ | 4017 ± 1178 | 3820 ± 1242 | 6693 ± 1414$^a$ | 6687 ± 1772$^a$ | 6298 ± 2015$^a$ |
| HSR (m) | 324 ± 213$^*$ | 54 ± 68 | 9 ± 8 | 209 ± 98$^a$ | 168 ± 108 | 210 ± 122$^a$ |
| ED (m) | 1184 ± 343$^*$ | 374 ± 216 | 212 ± 41 | 723 ± 174$^a$ | 699 ± 302$^a$ | 699 ± 301$^a$ |

**Abbreviations:** HSR = high speed running; ED = explosive distance. $^*$ Significant difference from light training day (P <0.05). $^a$ indicates a significant difference from goalkeeper (P <0.05).

### *6.4.9 Estimated energy expenditure during exercise.*

Estimated energy expenditure was significantly higher on match days compared to light training days (P = <0.001) and days off (P <0.001) over a season (Table 19). In May 2018, EEE on the light training day was significantly less than in August 2017 (P = <0.001), October 2017 (P = 0.011) and February 2018 (P = 0.011) (Table 4).

### *6.4.10 Menstrual cycle and hormonal contraceptive questionnaires*

Descriptive characteristics of both non-HC and HC users are presented in Figure 24.

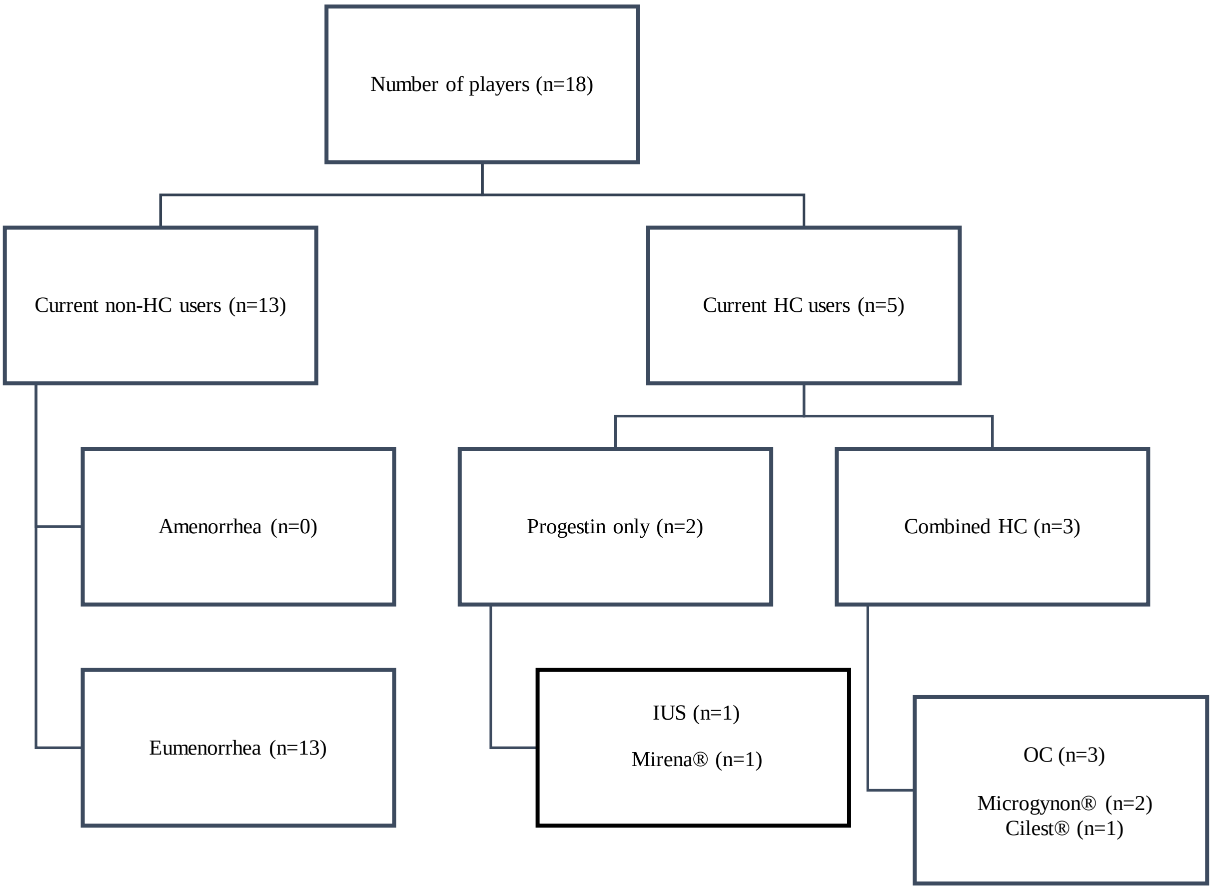


**Figure 24.** The prevalence and characteristics of hormonal contraceptive users and non-users. IUS, intrauterine system; OC, oral contraceptive; HC, hormonal contraceptive.

### 6.5 Discussion:

The aim of this research was to expand upon findings of the previous chapter by conducting a comprehensive assessment of the prevalence and factors affecting LEA among elite female soccer players participating in a WSL 1 team across an entire season. This was undertaken through measurements of body composition, EI, EEE, menstrual function, blood biomarkers, and RMR. Key findings of this study revealed that 78% of the squad-maintained energy availability >45 Kcal·kg FFM$^{-1}$ over the season. However, two players self-reported menstrual dysfunction on the LEAF-Q during the season. One player experienced no other symptoms related to her dysfunction (energy availability >45 Kcal·kg FFM$^{-1}$, biomarkers all within range, RMR ratio >0.90) and returned to her normal menstrual function at the next time point. The second player reported dysfunction during the last time point in the season. Alongside her self-reported dysfunction, a measured energy availability of 34 kcal·kg FFM$^{-1}$·day$^{-1}$ ('reduced' energy availability), a reduction in FM, BF%, BM and RMR ratio suggests that this player was likely suffering from LEA, caused by a decrease in EI. Furthermore, there was a notable squad reduction in FM and a simultaneous increase in FFM observed over the course of the season. Despite patterns of energy and carbohydrate intake generally aligning with training demands, players did not meet the recommended carbohydrate intake for match-play fuelling. Mean carbohydrate intake on match days was 4.5 ± 1.1g·kg$^{-1}$·d$^{-1}$, significantly lower than the recommended 7g·kg$^{-1}$·d$^{-1}$, with only two players achieving the recommended amount throughout the entire season. Overall, the majority of the squad did not suffer from LEA throughout a WSL 1 season. However, the presence of menstrual dysfunction in two players underscores the importance of individualised assessment and treatment.

### *6.5.1 Energy availability*

In line with findings in Chapter 5, this study revealed that over the entire season, energy availability was >45 Kcal·kg FFM$^{-1}$ for 78% of players. Notably, none of the players mean energy availability was classified as low when analysed by time point or playing position (Figures 20 and 21). This contrasts with Moss et al. (2020) where, at the end of the season, WSL 1 players were categorised as: 15% 'optimal' energy availability, 62% 'reduced' energy availability, and 23% 'low' energy availability. At the equivalent time point in our study (May), 71% of players were classified as having 'optimal' energy availability, with 29% categorised as 'reduced'. Significant changes in energy availability were not identified across the four designated time points in the season (Table 19). In contrast, Reed et al. (2013) reported a significant drop in energy availability among Division 1 collegiate athletes during the season, decreasing from 43 Kcal·kg FFM$^{-1}$·day$^{-1}$ to 35 Kcal·kg FFM$^{-1}$·day$^{-1}$ mid-season, before returning to pre-season levels. This drop was attributed to low EI during the season and a decrease in EEE towards the end of season. In contrast to Reed et al. (2013) the present study identified significant increases in EI on both training and match days from pre-season to mid-season. However, similarities were found with EEE, as training days exhibited a significant decrease at the end of the season compared to previous time points. This decrease is likely due to reduced training demands towards the end of the season, where sessions may focus less on physical conditioning (Oliveira et al., 2019). Analysis of training days throughout the season revealed that players demonstrated significantly lower energy availability on days off compared to both training and match days, despite EEE being significantly lower on days off (Table 19). Overall, this novel research indicates that, at a squad level, the majority of elite female soccer players measured did not suffer from LEA over a season.

### *6.5.2 Menstrual function*

During the season, two players reported menstrual dysfunction while completing the LEAF-Q. The first instance occurred in February 2018, where a player reported a lack of menstruation for six weeks. Upon analysis, her LEAF-Q score was 12, exceeding the normal range (≥8 = at risk of LEA), while all other markers remained within the normal range and consistent with previous assessments. By May 2018, her menstrual cycle had returned to its regular pattern. In another case, a player reported altered menstrual function in May 2018, indicating an absence of menstruation for approximately eight weeks. Her LEAF-Q score was 8, with measured energy availability values of 38 kcal·kg $FFM^{-1}\cdot day^{-1}$ in February 2018 and 34 kcal·kg $FFM^{-1}\cdot day^{-1}$ in May 2018, both of which are defined as ‘reduced’ energy availability. Additionally, she experienced a 2kg (17%) decrease in FM, a 2.8% (12%) decrease in BF%, and a 2.2kg (4%) decrease in BM throughout the season. Despite her RMR ratio remaining above the threshold of >0.90, it dropped from 1.05 to 0.95 over the course of the season. However, her hip and lumbar Z scores remained consistent throughout the season, all above 0. Unfortunately, this player did not undergo any blood biomarker assessments. Considering the symptoms and measured markers, it is likely that this player was experiencing LEA. Due to the timing of the testing being at the end of the season, further assessments and an intervention could not be conducted.

### *6.5.3 The Low Energy Availability in Females Questionnaire (LEAF-Q)*

Throughout the entire season, the LEAF-Q indicated that 32% of players were ‘at risk’ of LEA, with midfielders exhibiting the highest risk at 58%. When assessed over the season, 78% of players demonstrated 'optimal' energy availability, while 22% had 'reduced' energy availability. However, when breaking down energy availability by training days, some players experienced LEA on days off. For instance, in February 2018, 22% of the players had LEA on their day off. Unfortunately, the reasons for this were not identified, but it is possible that, with reduced EEE on this day, players consciously reduced EI. While overall energy availability remained optimal for most of the squad, some research suggests that within-day energy deficiency is associated with clinical markers of metabolic disturbances e.g. reduced RMR ratio, oestrogen and $T_3$ and raised cortisol (Fahrenholtz et al., 2018). Although these were measured, it was out of the scope of this thesis to assess within-day energy availability. As discussed in the previous chapter, the LEAF-Q, while widely used, has its limitations, particularly in accurately identifying players at risk of LEA in female soccer players. Recent research by Dasa, Friborg, Kristoffersen, Pettersen, Sagen, et al. (2023), highlights these limitations, indicating that the LEAF-Q does not effectively detect symptoms of the Female Athlete Triad and LEA in this population. Given the tendency for overestimation of LEA by the LEAF-Q in our study, our data supports the need for the development of a more tailored screening tool for energy availability in female soccer players. Such a tool should consider the unique demands and injury risks inherent in soccer compared to non-contact sports.

### *6.5.4 Body composition*

Mean FM over the season was 12.4 ± 2.5 kg, FFM 42.3 ± 2.6 kg and BF% 21.7 ± 3.0%. There is no significant difference ($P > 0.05$) in FM measurement with previous studies by Reed et al. (2013) (13.3kg), Emmonds et al. (2019) (12.9kg) and Moss et al. (2020) (11.5kg) while Minett et al. (2017) reported significantly higher ($P < 0.001$) FM at 14 kg. The FFM in the current study (42.3 ± 2.6 kg) is significantly lower than previous squads measured by Reed et al. (2013) (44.8kg, $P < 0.001$), Emmonds et al. (2019) (46.3kg, $P < 0.05$), Minett et al. (2017) (48kg, $P < 0.001$) and Moss et al. (2020) (49.5kg, $P < 0.001$). Despite the squad having a similar stature (166 cm) compared to other studies (ranging from 165 to 169 cm), there is a notable difference in the BM of the current squad (59.8 kg) in comparison to other squads measured (ranging from 61 to 64 kg). This difference in mass is predominantly attributed to variations in FFM. This difference in FFM is unlikely to be attributed to the age of the squad, as the mean age in the current study (21.8 ± 2.4) falls within the range of the other studies (19.0 – 25.4). Although all studies used DXA to measure FFM, different machines were used: this study and Minett et al. (2017) used Hologic, while Reed et al. (2013), Emmonds et al. (2019) and Moss et al. (2020) used Lunar. According to Park, Lim, Kim, and Kim (2021) the Hologic machine estimates significantly less FFM compared to the Lunar, which could account for the differences between the current study and the studies by Reed et al. (2013), Emmonds et al. (2019) and Moss et al. (2020). However, this does not explain the difference with for Minett et al. (2017), who also used Hologic. Another potential reason for differences in FFM is training history. Sutton, Scott, Wallace, and Reilly (2009) demonstrated that soccer players with a high training history have significantly more FFM than those with a low training history. Unfortunately, the training history of the players was not detailed in the previous studies, making this factor difficult to confirm. Finally, training methodology can impact players FFM. Lesinski, Prieske, Helm, and Granacher (2017) observed significant changes in FFM in female soccer players based on the type, amount, and duration of training. However, limited information regarding training methodologies in the studies by Reed et al. (2013), Emmonds et al. (2019), Minett et al. (2017) and Moss et al. (2020) makes it difficult to determine its impact. Overall, the differences in FFM are unlikely to be caused by age but could be due to the different DXA machines used, as well as variations in training history and methodologies between the squads.

As discussed in the previous chapter, it was hypothesised that players would reduce FM and increase FFM over the season. This hypothesis was substantiated, with FM significantly decreasing from pre-season (August 2017) to November 2017. This reduction remained relatively consistent between November 2017 and the end of season (May 2018) (0.1 – 0.3kg difference). A similar trend was observed for FFM, which significantly increased from pre-season to February 2018. These trends diverge from the findings of Reed et al. (2013), who reported that their players maintained consistent levels of both FM and FFM throughout the season. The current results also differ from Minett et al. (2017) who found that players experienced significant losses in FFM during the season. One likely reason for the disparities found between the current study and the other two is that the 'seasons' measured in the latter studies lasted only 3 - 4 months, while the playing squad in the present study was measured over a 10-month season. This extended duration allows for a more prolonged period for body composition changes to occur, coupled with potential differences in training philosophies. In addition, Minett et al. (2017) reported a reduction in strength training frequency from 3 days per week to once per week during the season. Due to the brevity of the season, the emphasis would likely have been on recovery and performance, whereas in a longer season, a more continuous strength programme would be adopted. In summary, novel data from this study provide evidence that significant body composition changes are possible over a season.

Regarding bone health, the entire squad maintained BMD Z-scores within the acceptable range throughout the entire season for both the hip and lumbar spine (>-1.0) (Nattiv et al., 2007). Consistent with previous research (Minett et al., 2017; Stanforth et al., 2014) no significant differences in Z-scores were detected over the course of the season. These findings, in conjunction with other markers in this study, suggest that LEA is not prevalent in these players. Consequently, the players are at decreased risk of bone related injuries or conditions such as fractures and osteoporosis.

### *6.5.5 Dietary intake*

Energy intake and carbohydrate intake over the season was significantly higher on training days (P <0.001) and match days (P = 0.028) compared to days off. This novel data over a season provides evidence for the first time in female soccer, that players are periodising both their calorie and carbohydrate intake around training demands. Unfortunately, what could not be ascertained is whether this is intentional or not, and if it is due to any nutritional education or a subconsciously learned behavior over time.

Mean EI over the season was 2138 ± 379 $kcal \cdot d^{-1}$, aligning with previous studies by Moss et al. (2020) (2124 ± 444 $kcal \cdot d^{-1}$), Braun et al. (2018) (2226 ± 368 $kcal \cdot d^{-1}$) and Reed et al. (2013) (2387 ± 177 $kcal \cdot d^{-1}$). As previously described, carbohydrate intake significantly differed between training days, match days, and days off. However, despite players periodising their carbohydrate intake, consuming more on match days than on days off, the average intake was 4.5 ± 1.1$g \cdot kg^{-1} \cdot d^{-1}$, well below the recommended 7$g \cdot kg^{-1} \cdot d^{-1}$ (Collins et al., 2021; Maughan and Shirreffs, 2007; McLay et al., 2007). Only two players over the whole season consumed the recommended 7$g \cdot kg^{-1} \cdot d^{-1}$, with 39% of players consuming less than 4$g \cdot kg^{-1} \cdot d^{-1}$ and 19% consuming less than 3$g \cdot kg^{-1} \cdot d^{-1}$. Although this represents an improvement from the snapshot in Chapter 5 (3.7 ± 1.5$g \cdot kg^{-1} \cdot d^{-1}$), with significant increases on match days from 3.5 ± 1.2$g \cdot kg^{-1} \cdot d^{-1}$ in August 2017 to 5.1 ± 1.6$g \cdot kg^{-1} \cdot d^{-1}$ in November 2017, this began to decline again to 4.3 ± 1.3$g \cdot kg^{-1} \cdot d^{-1}$ by May 2018. This novel data highlights the continued need for targeted nutrition education to improve players' understanding of the critical role of carbohydrates for fueling match day performance.

Average protein (2.0 ± 0.2$g \cdot kg^{-1} \cdot d^{-1}$) and fat (1.3 ± 0.2$g \cdot kg^{-1} \cdot d^{-1}$) intake continued to meet the required amounts for elite female soccer players (Morton et al., 2018; Wooding et al., 2017). These results support the findings from previous studies that female soccer players consume enough protein and fat throughout a season to positively impact their training and recovery efforts (Moss et al., 2020; Reed et al., 2014). However, we suggest adjustments could be made on match days. Although protein (1.9 ± 0.4$g \cdot kg^{-1} \cdot d^{-1}$) and fat (1.2 ± 0.3$g \cdot kg^{-1} \cdot d^{-1}$) intake were not excessive, reducing either on match days would allow for increased calorific content from carbohydrate intake, which we believe is necessary. This adjustment could then be made without affecting the players' BM and FM over a 10-month season.

### *6.5.6 Biomarkers*

After the pre-season, there was a significant decrease in prolactin concentrations ($P = 0.003$), returning them to within clinical norms for the whole squad. Additionally, during the season, 80% of the squad had cortisol concentrations within clinical norms, compared to only 50% during pre-season. This evidence supports the hypothesis from Study 2 (Chapter 5), suggesting that elevated levels of prolactin and cortisol in pre-season may have been related to increased training loads and stress, rather than energy deficiency (LEA) or another underlying issue.

As discussed in the previous chapter, thyroid hormones, particularly $T_3$, are influenced by energy balance and serve as strong indicators of potential LEA in athletes (Loucks and Callister, 1993; Loucks and Heath, 1994). Throughout the whole season, $T_3$ remained within the normal range for all squad members, aligning with the normal range of other thyroid hormones. This further reinforces the suggestion that the players did not experience LEA. The squad's micronutrient biomarkers, including magnesium, folate, and Vitamin B12, remained within the recommended range for most of the squad over the season. Although zinc concentration remained in range for most of the season, in November 2017, only 63% of the squad fell within clinical ranges. Notably, all players outside of the ranges had higher levels than normal. Clinically, this was not a concern, as marginally excess zinc tends to be excreted through the gastrointestinal tract (Plum, Rink, and Haase, 2010), and concentrations returned to within the clinical normal range for the rest of the season. A potential rationale for the elevated zinc levels could be a consequence of previous intense training sessions. With zinc bound in the red blood cell membrane, the breakdown of red blood cells during intense exercise causes an increase of zinc released into the blood (Soria et al., 2015).

Vitamin D concentrations significantly decreased during the mid-points of the season (November 2017 and February 2018) compared with both pre-season ($P < 0.001$) and the end of the season ($P < 0.001$). Although there was a significant decrease over these time points, only one player fell below the clinical deficiency concentrations of 50 $nmol \cdot L^{-1}$. There has been much debate around what levels of circulating serum 25-hydroxyvitamin D indicates sufficiency or deficiency (Owens et al., 2018). There generally appears to be a greater consistency around <50 $nmol \cdot L^{-1}$ being accepted as deficient, with >75 $nmol \cdot L^{-1}$ as sufficient

(Knechtle, Jastrzębski, Hill, and Nikolaidis, 2021; McClung, Gaffney-Stomberg, and Lee, 2014; Owens et al., 2018). The squad average dipped below 75 nmol·L$^{-1}$ during February 2018. Vitamin D concentrations below 75 nmol·L$^{-1}$ have been suggested to impair muscle regeneration (Owens et al., 2015) and increase risk of upper respiratory tract infections (Jung, Seo, Lee, Kim, and Song, 2018). At the individual level in February 2018, 70% of the squad were below the sufficient cut-off (75 nmol·L$^{-1}$), coinciding with reduced exposure to ultraviolet B radiation from sunlight (Gillie, 2010). Vitamin D supplementation was offered to players during club mealtimes with a generic recommendation of 2000 IU intake per day, although adherence was not recorded. Given the crucial role of vitamin D in bone health, muscle function, and protein synthesis (Lee et al., 2017), these results highlight the need for a more individualised supplement and education protocol to mitigate the decrease of vitamin D during the winter months.

There were fluctuations observed in the hematological markers assessed in this study. Abnormal iron markers can cause negative health and performance outcomes (McClung et al., 2014). Iron is essential to Hb synthesis and subsequently oxygen transport (Lee et al., 2017), with deficiencies causing fatigue, cognitive impairment and immune deficiencies (McClung et al., 2014). Haemoglobin and hematocrit, which experienced reductions during pre-season (60% for Hb, 70% for hematocrit), returned to within normal ranges for the entire squad for the remainder of the season. A plausible explanation for this phenomenon could be that low Hb and hematocrit levels were a result of an expansion of plasma volume in the hours and days following exercise (Cowell et al., 2003). According to Sawka, Convertino, Eichner, Schnieder, and Young (2000) plasma volume increases during the initial two weeks of exercise—corresponding to the pre-season in this study—accounting for all blood volume expansion. Once a new steady state is established, Hb and hematocrit return to pre-training values. It is important to ensure that Hb and hematocrit returned within clinical norms as they are involved in transporting oxygen from the lungs to the tissues and delivering metabolically produced carbon dioxide to the lungs for expiration (Mairbäurl, 2013). This reduction in oxygen transport to exercising muscle may place higher demands on anaerobic metabolism, which could negatively influence performance (Sim et al., 2019).

Ferritin concentrations remained within clinical ranges throughout the season. However, applying iron deficiency classifications proposed by Peeling et al. (2007), as discussed in the previous chapter, revealed 50% of the squad were classified with stage 1 iron deficiency at least once during the season. Among these players, one exhibited stage 1 iron deficiency for 75% of the season, another for 50%, and three for one time point (25% of season). Specifically, in November 2017, February 2018, and May 2018, 30%, 30%, and 20% of the squad tested were defined as having stage 1 iron deficiency, respectively. It is notable that stage 1 depleted iron stores have demonstrated minimal impact on performance (Burden et al., 2015; Rubeor et al., 2018). Nevertheless, early detection of such deficiencies is hypothesised to prevent progression to more severe stages (Peeling et al., 2007). However, our data demonstrated that without intervention ferritin concentrations increased sufficiently in three out of five players with iron deficiency, surpassing the iron deficiency threshold (>35 µg/L$^{-1}$) by the subsequent time point. For the other two players, ferritin levels remained between 20 – 35 µg/L$^{-1}$ with transferrin saturation (> 16%) and Hb (> 115 g/L$^{-1}$) also remaining above the threshold, indicating stage 1 iron deficiency persisted until the end of the season. Potential reasons for iron deficiency among players could include inadequate iron intake due to insufficient calorie intake (LEA), dietary restrictions (e.g., vegetarian or vegan), acute iron loss (e.g., sweating, blood loss from menses or gastrointestinal bleeding), or hormonal fluctuations impacting iron absorption (DellaValle, 2013; Telford et al., 2003). Low energy availability and vegetarian or vegan diets were ruled out among the players with iron deficiency. Further investigation into their iron intake over several weeks would be necessary to better diagnose the underlying reasons. Additionally, detailed examination of menstrual cycle characteristics, including bleed length and volume, would aid in diagnosis, given that none of the players were using hormonal contraceptives.

Overall, it is likely a combination of factors that negatively impacted players' iron stores. These individual cases underscore the importance of longitudinal data in understanding player health status. Continuous monitoring can facilitate early identification of issues before symptoms manifest, potentially preventing negative performance outcomes such as lethargy, fatigue, negative mood states, and compromised work capacity (Sim et al., 2019). Future assessments in subsequent seasons should involve comparisons to both clinical norms and the individual's historical data, enabling tailored education, supplementation, and guidance.

### *6.5.7 Resting metabolic rate*

The mean measured RMR over the season was 1543 ± 73kcal·d$^{-1}$ compared to predicted 1443 ± 78 kcal·d$^{-1}$. Measured RMR was significantly higher in November 2017 compared with May 2018, albeit only by 76kcal·d$^{-1}$. The mean RMR from the current study (1543 ± 73 kcal·d$^{-1}$) is similar to the findings of Moss et al. (2020) (1510 ± 186 $kcal\cdot d^{-1}$), although the mean RMR ratio was lower in Moss et al. (2020) (0.94 ± 0.08) compared to this study (1.06 ± 0.08). An RMR ratio >0.90 is a useful marker for energy deficiency due to previous research demonstrating that long-term energy restriction resulted in reduced RMR, coinciding with reduced BMD and amenorrhea (De Souza et al., 2007; De Souza et al., 2008; Kaufman et al., 2002). The current study did not have any players that were below the RMR threshold of 0.90, whereas Moss et al. (2020) reported 23% of their squad falling below this threshold. Potential reasons for this difference include the use of different prediction equations, with Moss et al. (2020) employing the Cunningham equation and the current study using Ten Haaf (Ten Haaf and Weijs, 2014). The rationale for using Ten Haaf was that it was conducted in both male and female individuals who were training around 5 times a week, aligning closely with our current population. Moreover, Ten Haff equation has been shown to be the best prediction equation in female rugby players, with 86% of players being within 10% of measured RMR (O'Neill et al., 2022). Our study found a 5% difference between mean measured and predicted RMR, with 81% of players predicted RMR being within 10% of measured. Given this accuracy our data confirms that the Ten Haaf equation appears to accurately calculate RMR in this population.

### *6.5.8 Training and match profiles*

Throughout the season, players covered an average distance of 10.6 ± 1.9 km during match play, compared to 9.7 ± 1.8 km in pre-season. The variability in game time among squad members during pre-season, with different players sharing minutes, contributed to this difference. The average distance covered over the season aligns more closely with the findings of Datson et al. (2017), who reported international players completed 10.3 ± 0.9 km. However, the intensity of match play remained constant throughout the season at 0.3 ± 0.2 km, which is still below the values reported by Datson et al. (2017) at international level (0.6 ± 0.2 km) and Andersson et al. (2010) at domestic level (1.3 ± 0.9km). As discussed in the previous chapter, the differences in intensities are likely attributed to varying measurement methodologies (GPS vs time motion analysis) and thresholds (18 $km\cdot h^{-1}$ vs 19.8 $km\cdot h^{-1}$). While the purpose of this study was not to undertake in-depth research into the match demands of female soccer players, but rather to understand the energy demands and compare them to the fueling being completed. The data from this study supports previous research on the TD elite female soccer players cover during matches. Furthermore, it contributes to the argument that standardised speed thresholds need to be agreed upon for elite female soccer players to better understand and compare studies on the intensity of training and matches.

### *6.5.9 Estimated energy expenditure during exercise.*

A significant difference in EEE was observed between training days, match days, and days off. As suggested in Chapter 5, EEE on light training days was higher in pre-season compared to the end of the season, with EEE recorded as 503 kcal in pre-season versus 438 kcal, 434 kcal, and 338 kcal throughout the season. On average, over the season, EEE on light training days was 420 ± 64 kcal. This contrasts with Moss et al. (2020) findings, which reported an EEE of 299 ± 78 kcal. However, when comparing similar time points in the season, the current squad significantly reduced EEE, with it recorded as 338 ± 40 kcal in May 2018, aligning more closely with the findings of Moss et al. (2020) (299 ± 78 kcal). Over the season, EEE on match days did not significantly change. Although some variability was observed throughout the season, this is expected given the dynamic nature of game demands, which can change depending on factors such as opposition, playing philosophy, and game situation (Hewitt et al., 2014; Trewin, Meylan, Varley, Cronin, and Ling, 2018). Overall, this research highlights the significant differences in energy demands based on the intensity of the training day, thus warranting distinct fueling regimes.

## 6.6 Limitations

The current study had some limitations that warrant acknowledgment. Firstly, the absence of bone biomarkers was a limitation as it could have offered more insight into bone resorption and formation, providing a more sensitive measure of bone health beyond DXA results (Hagman et al., 2021; Kuo and Chen, 2017). Potential markers, such as urinary N-terminal telopeptide (NTX), β- Carboxyl-terminal cross-linked telopeptide of type I collagen (β-CTX) and procollagen type 1 N-terminal propeptide (P1NP) with the ratio between the last two being used as a bone turnover ratio (Papageorgiou et al., 2017) have been shown to demonstrate the negative effects of LEA on bone turnover (Elliott-Sale et al., 2018). Secondly, for a more conclusive assessment of bone health, the utilisation of a HR-pQCT could have been employed to measure densitometry of the cortical and trabecular regions, along with the geometric properties of bone, providing a more nuanced indication of bone health (Ammann and Rizzoli, 2003; Minett et al., 2017). The use of a HR-pQCT scanner would have addressed some limitations associated with DXA. Unfortunately, due to cost constraints and equipment availability, neither tool was employed. However, for future investigations integrating bone biomarkers, DXA, and HR-pQCT would have provided a more comprehensive bone health profile.

Another limitation pertained to the monitoring of players' menstrual cycles throughout the season. Menstrual cycle-related questions were only administered by the research team at the four designated time points. This approach prevented continuous monitoring and a holistic view of players' menstrual cycle health, hindering a complete understanding of each player's 'normal' menstrual cycle. Continuous menstrual cycle tracking can help identify menstrual dysfunction that can indicate an underlying issue e.g. LEA. Significant interindividual variation in the menstrual cycle means that tracking is important to individualise support for each player (Parker, Elliott-Sale, Hannon, Morton, and Close, 2022).

Accurately measuring energy availability, and, particularly, EEE is challenging (Burke et al., 2018). The difficulty arises from the cost-effectiveness and non-intrusiveness required in practical field measurements. Wearable technology (Murakami et al., 2016) and accelerometers (Abel et al., 2008) tend to underestimate EEE, particularly at higher intensities. Although DLW is considered the gold standard for estimating TEE, it provides an average over a longer period rather than specific energy expenditure during exercise.

Additional measurements, such as performance-based testing markers, could have provided more context to the study findings. While these measurements were conducted by the performance team, the infrequency of testing (twice in a season) and logistical challenges in aligning testing time points limited their inclusion.

To gain a deeper understanding of eating attitudes and their underlying reasons, employing an eating attitudes questionnaire, like the Eating Disorder Inventory 2 (Garner and Olmsted, 1984) or the Eating Disorder Examination – Questionnaire (Fairburn and Beglin, 1994) would have been beneficial. Such questionnaires could have provided additional insights into the drivers behind food choices and whether any disordered eating or eating disorders were present in the group.

## 6.7 Conclusion

The LEAF-Q indicated that nearly a third of the squad were at risk of LEA, however, only one of the squad members presented with markers to indicate they were in LEA. Although only one player appeared to suffer from LEA at the time points measured, due to interindividual variation, continuous tracking of the MC could help early identification of any menstrual disturbances, which can then be investigated. Players significantly increased their EI and carbohydrate intake on training and match days compared to days off. However, mean carbohydrate consumption on match days was only $4.6g \cdot kg^{-1} \cdot d^{-1}$, with only two players consuming the recommended $7g \cdot kg^{-1} \cdot d^{-1}$ over the whole season. This highlights that further work is still required to fuel female soccer players for match performance. The current study provided evidence that body composition can be significantly manipulated over a season with FM significantly decreasing and FFM significantly increasing. Importantly, the squad's bone health scores fell within the normal range for the whole season indicating that bone health is not a concern within this population. Although supplementation was not monitored, vitamin D levels significantly decreased during the season. While significant, only one player fell below the clinical deficiency concentration of 50 $nmol \cdot L^{-1}$. However, with >75 $nmol \cdot L^{-1}$ seen as sufficient, 70% of the squad were below this in February 2018. Therefore, a more individualised supplement and education protocol is required to mitigate the decrease of vitamin D and the potential consequences on bone health, muscle function, and protein synthesis during the winter months. For hematological markers, although remaining within clinical norms, ferritin levels dropped in 50% of players at various time points in the season to classify them as stage 1 iron deficient according to Peeling et al. (2007). Over half the players ferritin concentrations returned to normal without an intervention, with two players remaining as iron deficient at the end of season. These individual cases underscore the importance of longitudinal data in understanding player health status with continuous monitoring allowing early identification of issues before symptoms manifest (Sim et al., 2019).

Overall, this novel data suggests that LEA may not be as prevalent as previously reported. However, the presence of menstrual dysfunction in two players, with one likely suffering from LEA, highlights the importance of individualised assessment and treatment for each player. To provide an example, at the end of the season, one player suffered a significant knee injury requiring surgery. In the following season, this player reported suffering from amenorrhea. Therefore, to help assess if this menstrual dysfunction was due to LEA, the following chapter will assess this soccer player’s two-year journey from eumenorrhea, through injury, to amenorrhea, detailing the challenges faced by both the player and the nutritionist.

# CHAPTER 7

## 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.

***This study was published in Science and Medicine in Football in 2022.***

Parker LJF, Elliott-Sale KJ, Hannon M, Morton JP, Close GL. (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*. 6 (5), 643-649.

## 7.1 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.

## 7.2 Introduction

Low energy availability is the cornerstone of both the Female Athlete Triad (Nattiv et al., 2007) and RED-S (Mountjoy et al., 2014) and results in a myriad of negative health and performance outcomes (Dipla, Kraemer, Constantini, and Hackney, 2021; Logue et al., 2020). Low energy availability is often identified and treated by nutritionists as they are largely responsible for an athlete's DI. 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?

## 7.3 Methods

### *7.3.1 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 WSL.

### *7.3.2 Research design*

Figure 25 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 lead 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] (Chapter 3).

### *7.3.3 Nutrition intervention*

The focus of the post injury nutrition intervention was to maintain the players FFM 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 ($<2.5 g \cdot kg^{-1}$) and higher protein ($2-2.5 g \cdot kg^{-1}$) (Close, Sale, Baar, and Bermon, 2019), ingesting ~25g 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 ($5 g \cdot d^{-1}$) (Johnston, Burke, MacNeil, and Candow, 2009) and collagen ($20 g \cdot d^{-1}$) (Shaw, Lee-Barthel, Ross, Wang, and Baar, 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.

## 7.4 Research protocol

### *7.4.1 Anthropometrical assessments*

Body mass was assessed in minimal clothing and without shoes (SECA, model-875, Hamburg, Germany) (section 3.3.1). Body composition was assessed using whole beam 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 (section 3.3.2).

### *7.4.2 Menstrual function*

Menstrual function and risk of LEA was assessed using the LEAF-Q (Melin et al., 2014). Menses was tracked (electronic calendar) and verbally confirmed by the athlete prior to DXA scanning. Ovulation was assessed using a urinary detection kit (Clearblue, Digital Ovulation Test, SPD, Development Company, Bedford, UK).

### *7.4.3 Resting metabolic rate*

Resting metabolic rate 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 x 10-minute duplicates). Data for the second 10 minute period were used to determine RMR and analysed as previously described (Hannon et al., 2020) (section 3.4.2). Based on the work done by O'Neill et al. (2022) the following equations were used to predict RMR: Cunningham (CRMR) (Cunningham, 1980), Ten Haaf (Ten Haaf and Weijs, 2014) and Watson (Watson et al., 2019). Ten Haaf (Ten Haaf and Weijs, 2014) was used as the primary comparative equation.

### *7.4.4 Measurement of total energy expenditure using the doubly labelled water method*

Measurement of total energy expenditure was quantified using DLW (Lifson, 1966; Speakman and Hambly, 2016) over 14-days. At 17.50 on the evening of day zero the player provided a single baseline urine sample into a labelled sample pot before sealing. Following collection of a baseline 35 ml urine sample to estimate background isotope enrichments, the player self-administered orally a weighed bolus dose of hydrogen (deuterium $^2H$) and oxygen ($^{18}O$) stable isotopes (Cortecnet, Voisins-Le-Bretonneux, France) in the form of water ($^2H_2{}^{18}O$) (witnessed by the lead researcher) at the time of 18.10. The player was dosed in accordance to their body mass with a bolus of DLW weighed to four decimal places. To ensure that the entire dose of DLW was consumed, additional water was added to the dosing vessel which was also consumed. The following morning (day one), the player was asked to provide a 35 ml sample of the second urine void of the day. Further second void urine samples were collected every second day between 8 – 10am, with the last collection on day 14. All samples were aliquoted and stored in 1.8 mL cryovials at -80°C until later analysis in compliance with the Human Tissue Act 2004. Body mass was recorded at the start and end of the protocol and exact times of all urine sample collections were also recorded. Analysis of the isotopic enrichment of urine was performed blind using a Liquid Isotope Water Analyser (Los Gatos Research, USA) at the University of Aberdeen, Scotland, UK (Hannon et al., 2021).

### *7.4.5 Energy availability*

Energy availability was calculated using the equations of Loucks et al. (2011).

### *7.4.6 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 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) (section 3.8). The coefficient of variation between the lead researcher and the second SENr nutritionists' food diary analysis were 3.78 ± 1.92%, 4.16 ± 2.21%, 3.83 ± 2.16% and 4.23 ± 1.83% for energy intake (kcal), carbohydrate (g), protein (g) and fat (g), respectively.

### *7.4.7 Training and match profiles*

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., 2021) (section 3.9).

### *7.4.8 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 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$^{o}$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 (section 3.6).

## 7.5 Results

| | **S1 Original Investigation** | | | | | **S2 Nutrition Intervention** | | | **S3 Additional Investigation** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aug-17 | Nov-17 | Feb-18 | Apr-19 injury | 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 | 1.6 | | | | | | |
| 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 25.** Summary of the main findings.

### *7.5.1 Body composition*

Fat free mass decreased by 2.6kg within three weeks of injury, returning to pre-injury levels within nine months (Figure 26).

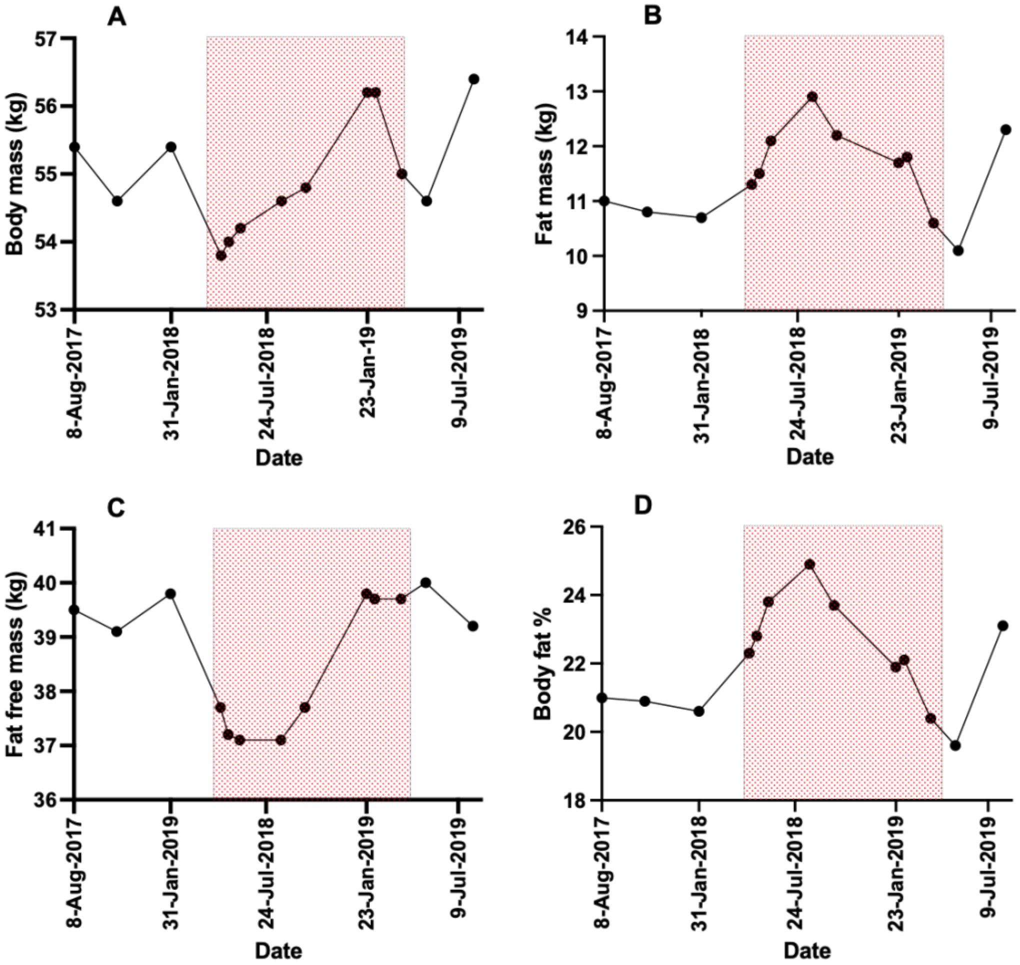


**Figure 26.** Changes in body mass(A), fat mass (B), fat free mass (C) and body fat percent (%) (D). The red zone indicates the time between the injury occurring to returning to full team training.

### *7.5.2 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 (Table 22).

**Table 22.** Resting metabolic rate indirect measurements, predictions, and ratios.

| RMR method | Aug 2017 | Nov 2017 | Feb 2018 | May 2018 | June 2018 | Feb 2019 |
|---|---|---|---|---|---|---|
| Indirect calorimetry (kcal·d$^{-1}$) | 1450 | 1474 | 1364 | 1375 | 1474 | 1450 |
| Watson BM (kcal·d$^{-1}$) | 1409 | 1422 | 1429 | 1415 | 1419 | 1455 |
| Cunningham (kcal·d$^{-1}$) | 1369 | 1360 | 1376 | 1329 | 1316 | 1373 |
| Ten Haaf (kcal·d$^{-1}$) | 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.

### *7.5.3 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 23 shows the average and range of relative energy availability per day using the Loucks et al. (2011) and Torstveit et al. (2018) equations (Table 23).

**Table 23.** Relative mean and per day energy availability.

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **Mean** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Loucks (kcal·kg·d$^{-1}$) | 49 | 53 | *53* | 28 | 40 | 40 | 39 | 63 | *65* | *68* | 41 | 58 | 40 | 43 | 45 |

Numbers *in italics* indicate a rest day

### *7.5.4 Daily estimated intake:*

Estimated EI increased by ~30% post intervention (February and May 2019), with the increase being made up from protein and fat intake (Table 24).

**Table 24.** 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$^{-1}$·d$^{-1}$) | 3.6 | 3.8 | 3.6 | 2.7 | 4.5 | 3.7 |
| Protein (g) | 91 | 73 | 96 | 82 | 116 | 143 |
| Protein (g·kg$^{-1}$·d$^{-1}$) | 1.6 | 1.3 | 1.7 | 1.5 | 2.1 | 2.6 |
| Fat (g) | 46 | 45 | 43 | 87 | 82 | 70 |
| Fat (g·kg$^{-1}$·d$^{-1}$) | 0.8 | 0.8 | 0.8 | 1.6 | 1.5 | 1.3 |

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

### *7.5.5 Training and match profiles:*

Table 25 provides an overview of combined absolute training and match load during data collection periods.

**Table 25.** An overview of combined absolute training and match load during a 7-day data collection period. The first three time-points in Table 25 were undertaken with the team pre-injury, with the last two time-points undertaken post-injury as individual sessions. These data [February 2019] were aligned with the DLW assessment.

| 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 |

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

### *7.5.6 Biomarkers:*

Iron markers were out of range (low) at the second testing time-point, otherwise all markers were within the normal clinical ranges (Table 26).

**Table 26.** Blood biomarker data over an 18-month study timescale.

| **Blood markers** | **Clinical range*** | **Aug-17** | **Nov-17** | **Feb-18** | **May-18** | **Jun-18** | **Feb-19** |
|---|---|---|---|---|---|---|---|
| Cortisol nmol·L$^{-1}$ | 140 - 500 | 436 | 476 | 261 | 284 | 211 | 382 |
| Luteinising hormone U·L$^{-1}$ | 3 - 80** | 2.2 | 7 | 15.2 | 19.5 | 11.5 | 5.4 |
| Follicle stimulating hormone U·L$^{-1}$ | 1 - 16** | 1.7 | 5.4 | 6.8 | 5.2 | 8 | 4.1 |
| 17 beta oestradiol pmol·L$^{-1}$ | 45 - 1200** | 464 | 62 | 244 | 366 | 268 | 361 |
| Total testosterone nmol·L$^{-1}$ | <1.9 | 1.1 | 1.3 | 1 | 1.6 | 1 | 1.1 |
| Sex hormone binding globulin nmol·L $^{-1}$ | 25 - 155 | 152 | 31 | 137 | 145 | 93 | 136 |
| Insulin growth factor 1 nmol·L$^{-1}$ | 11.2 - 54.5 | 29 | 40 | 29 | 35 | 30 | 29 |
| Insulin pmol·L$^{-1}$ | <174 | 48 | 65 | 41 | 42 | 36 | 53 |
| Thyroid stimulating hormone mU·L$^{-1}$ | 0.3 - 6.0 | 3.6 | 1.3 | 2.3 | 2.2 | 2.1 | 3.4 |
| Free thyroxine pmol·L$^{-1}$ | 10 - 22 | 15.5 | 15.5 | 15.3 | 16.4 | 19.6 | 15.2 |
| Free triiodothyronine pmol·L$^{-1}$ | 3.6 - 6.4 | 5.5 | 4.3 | 5.4 | 4.9 | 5 | 5.1 |
| Prolactin mU·L$^{-1}$ | <500 | 370 | 329 | 283 | 317 | 316 | 343 |
| Progesterone nmol·L$^{-1}$ | <1 - 70** | 15.5 | <1 | 7.3 | 13.7 | <1 | 18.5 |
| Ferritin ug·L$^{-1}$ | 13 - 150 | 36 | 20 | 39 | 40 | 88 | 35 |
| Vitamin B12 ng·L$^{-1}$ | 160 - 800 | 436 | 391 | 537 | 456 | 661 | 496 |
| Folate ug·L$^{-1}$ | 3.9 - 20 | 5.1 | 4.5 | 7.9 | 8.3 | 8.7 | 11.9 |
| Iron umol·L$^{-1}$ | 13 - 32 | 13.3 | 6.9 | 25.6 | 24.5 | 21.6 | 16.4 |
| Transferrin g·L$^{-1}$ | 2.2 - 3.6 | 2.69 | 3.42 | 2.61 | 2.45 | 2.65 | 2.63 |
| Total iron binding capacity umol·L$^{-1}$ | 41 - 77 | 61 | *78* | 59 | 56 | 60 | 60 |
| % Ferritin saturation | 20 - 55 | 22 | *9* | 43 | 44 | 36 | 27 |
| Zinc umol·L$^{-1}$ | 10.7 - 24.5 | 14.6 | 18.9 | 17.9 | 23.9 | 17.8 | 13 |
| Vit D nmol·L$^{-1}$ | 50 - 200 | 91 | 75 | 70 | 84 | 95 | 59 |
| Haemoglobin g·L$^{-1}$ | 118 - 148 | 140 | 133 | 139 | 146 | 142 | 145 |
| Haematocrit L·L$^{-1}$ | 0.36 - 0.44 | 0.4 | 0.4 | 0.398 | 0.417 | 0.407 | 0.424 |
| Magnesium nmol·L$^{-1}$ | 0.7 - 1.0 | 0.75 | 0.93 | 0.75 | 0.74 | 0.86 | 0.82 |

**Abbreviations:** Aug – August; Oct – October; Jan- January; Jun – June; Feb – February. *Values are indicative of internal laboratory references for females of various age ranges; **Values are whole ranges and depend on the stage on the menstrual cycle. Numbers *in italics* and underlined indicate out of range.

## 7.6 Discussion

### *7.6.1 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 FFM/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$^{-1}$; energy availability 34 kcal·kg·d$^{-1}$], placing her in the subclinical energy availability range (Loucks et al., 2011), despite reporting regularly menstruating (Figure 25) and her blood profile supporting this (Table 26). One possible reason for this is that this level of energy availability can be tolerated for short periods of times without any negative outcomes (Loucks et al., 2011). The other option is that DI was underreported, despite employing measures to reduce underreporting including dietary recalls and multiple daily reminders (Capling et al., 2017). Measuring DII 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 25; Z scores), which might be due to the loading nature of soccer (Baker, Chen, Larson, Bemben, and Bemben, 2020) and the fact that the player was not suffering from long-term LEA. In addition, at this time (S2 into S3), EI 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 (Dasa, Friborg, Kristoffersen, Pettersen, Sagen, et al., 2023; Rogers et al., 2021).

During S3 the player's RMR ratio (measured vs predicted RMR >0.90) and biomarkers (LH, FSH, oestradiol, prolactin, $FT_3$, $FT_4$ and IGF-1) were within normal ranges (Table 22, 26 and Figure 25) 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.

### *7.6.2 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 DI-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$^{o}$ 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. The work of Hecksteden and Meyer (2020) 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, making it difficult to establish a true baseline value, we did not feel confident interpretating the data this way.
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 FM and increasing FFM. 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.

## 7.7 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.

### 7.8 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 soccer 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 and is still playing topflight soccer with a regular menstrual cycle.

# CHAPTER 8

## Synthesis of findings

This chapter aims to summarise the findings of the thesis in relation to the original objectives outlined in Chapter 1. It begins with a summary of the key findings, followed by a general discussion focusing on how the data derived from this study has contributed to our understanding of the prevalence and factors affecting LEA in elite female soccer players. Finally, the practical implications, limitations, and recommendations for future research will be outlined.

## 8.1 Achievement of thesis aims

The primary aim of this thesis was to assess hormonal contraceptive use, menstrual function, energy expenditure, energy intake, energy availability, blood biomarkers and body composition among elite female soccer in WSL 1. The data derived from this thesis provides a greater understanding of the landscape regarding the prevalence and factors affecting low energy availability in WSL 1 players (Figure 27). The aim was achieved through a series of laboratory and field-based studies conducted in Chapters 4, 5, 6 and 7. An overview of each objective is provided below.

**Objective 1: Assessing hormonal contraceptive and non-hormonal contraceptive use and associated symptomatology among Women's Super League 1 players (Chapter 4).**

Using a previously validated questionnaire by Martin et al. (2018), the first audit of HC and non-HC use was carried out among WSL 1 players. It was found that HC use in WSL 1 players (28%) closely matched that of the general population (30%) although was lower than other elite athletes (49.5%). Most HC users reported benefits from its usage. However, it is crucial to note that the study does not endorse HC as there is a lack of comprehensive understanding regarding potential adverse health effects or impacts on performance resulting from long-term HC use. In terms of self-reported negative symptoms during their menstrual cycle, almost three-quarters of the players reported negative symptoms during their menstrual cycle, with 4% having to abstain from training due to such symptoms.

**Objective 2: Assessment of RMR, exercise energy expenditure, energy intake, menstrual function, blood biomarkers, and body composition, among elite female soccer players participating in a WSL 1 team during pre-season.**

During a 3-day assessment period none of the squad members exhibited LEA. Measured energy availability, hormonal profiles ($T_3$) and responses to menstrual cycle questionnaires, suggest that LEA or menstrual dysfunction were not present during the initial weeks of pre-season. However, players did not adjust energy or carbohydrate intake based on the intensity of training or match programme, with almost half (47%) the squad consuming $<3g \cdot kg^{-1} \cdot d^{-1}$ of carbohydrates on match day, compared to the recommended $7g \cdot kg^{-1} \cdot d^{-1}$ (Collins et al., 2021; McLay et al., 2007). Most of the squad were within normal clinical ranges for micronutrient, hormone, and iron markers, suggesting that players dietary intakes were providing sufficient nutrients. For markers that were out of range in individual players, it is likely that these deviated due to the increased training load and physiological stress endured during pre-season training.

### Objective 3: Longitudinal assessment of RMR, exercise energy expenditure, energy intake, menstrual function, blood biomarkers and body composition, among elite female soccer players throughout an entire season in a WSL 1 team

Over a 10-month season, players were measured at four distinctive time points over the season. Although the LEAF-Q indicated that nearly one-third of the squad were at risk of LEA, only one player presented with symptoms (menstrual dysfunction) and markers of suffering from LEA (energy availability of 34 kcal·kg FFM$^{-1}$·day$^{-1}$, decrease in FM, BF%, BM and RMR ratio). Players significantly increased their EI and carbohydrate intake on training and match days compared to days off. However, the mean carbohydrate consumption on match day was only 4.5 g·kg$^{-1}$·day$^{-1}$ falling short of the recommended 7 g·kg$^{-1}$·day$^{-1}$ (Collins et al., 2021; Maughan and Shirreffs, 2007; Williams and Rollo, 2015). This highlights that further work is still required to fuel female soccer players for match performance. Body composition notably changed over the season with a significant decrease in FM and increase in FFM, while bone health remained within normal clinical ranges throughout the season. Vitamin D concentrations significantly declined during the season, with only one player falling below the clinical deficiency concentration (50 nmol·L$^{-1}$). However, with >75 nmol·L$^{-1}$ seen as sufficient, 70% of the squad had vitamin D concentrations below this in February. Hence, a more individualised supplement and education protocol is necessary to counter the decline in vitamin D and its potential impacts on bone health, muscle function, and protein synthesis during winter months. Similarly, though remaining within clinical norms, ferritin concentrations dropped in 50% of players at various points in the season, classifying them as iron deficient according to athletic classifications (Peeling et al., 2007). Over half of the players saw their ferritin concentrations return to normal without intervention, while two players remained iron deficient at the season's end. These individual cases of iron and vitamin D deficiency highlight the importance of longitudinal data in understanding player health, with continuous monitoring allowing early identification of issues before symptoms manifest (Sim et al., 2019).

**Objective 4: Provide a detailed two-year examination of a professional internationally capped female soccer player suffering from secondary amenorrhea and anovulatory menstrual cycles.**

In Study 3 (Chapter 6), none of the players were classified with LEA within the team, however, further investigations were initiated when one of the players suffered a serious injury, underwent surgery, and subsequently reported eight months without menstruation. Initially, there were indications suggesting the possibility of LEA, with the player scoring 13 on the LEAF-Q. However, thorough investigations involving RMR, EE (DLW), EI, and biomarker analyses concluded that LEA was unlikely the cause of the player's condition. Unfortunately, due to the onset of the COVID-19 pandemic and the player relocating abroad, an official diagnosis could not be obtained. Subsequent follow-ups revealed that the player's menstrual function returned to normal, and she later became pregnant. As a result, it was concluded that her menstrual dysfunction was unlikely attributable to LEA but rather to other psychological and stress-related factors. This serves as an important learning point for practitioners, highlighting the need to consider various factors beyond just LEA when addressing menstrual dysfunction in elite soccer players.

## 8.2 General discussion of findings

### *8.2.1 Hormonal contraceptive and non-hormonal contraceptive use*

Despite nearly half of female athletes using HCs (Martin et al., 2018), Study 1 (Chapter 4) revealed a lower prevalence among WSL 1 soccer players, with only 28% using HCs, aligning more closely with the general population (30%) (Cea-Soriano et al., 2014). This trend persisted across longitudinal measurements in Studies 2 and 3 (Chapters 5 and 6), with 28% of players using HCs. This lower prevalence may be attributed to the relative infancy of professional soccer in England, as previous research suggests higher HC usage among athletes at an ‘elite’ level (43%) compared to sub-elite athletes (22.5%) (Martin et al., 2018). Notably, Schaumberg et al. (2018) demonstrated that female athletes often use HCs to manipulate their menstrual cycle. Study 1 data suggests that there could be a reluctance to endure anticipated negative symptoms, such as weight gain (33%), which may deter some players. Despite concerns, only 38% of HC users reported experiencing negative symptoms, with mood swings and continued menstruation being the most common issues (Table 10). In contrast, 86% reported positive symptoms, such as absence of menstruation, regular periods, and reduced pain (Table 10). This disparity suggests potential benefits of HC usage for some players, which may prompt increased adoption as the sport professionalises.

Non-HC athletes experience menstrual cycle abnormalities ranging from 6% to 79%, depending on factors such as sport type and competition level (Warren and Perlroth, 2001). While previous studies have explored menstrual function in female soccer players (Moss et al., 2020; Prather et al., 2016; Reed et al., 2013; Sundgot-Borgen and Torstveit, 2007), these have focused on individual teams rather than across entire leagues. In Study 1 (Chapter 4) menstrual cycle symptoms were prevalent across the league (6 out of 10 teams), with 72% reporting at least one negative symptom, primarily cramps (70%) during menstruation. Fourteen players (26%) reported menstrual dysfunction, though only one had been clinically diagnosed. These findings highlight the varied responses to HC and non-HC usage.

While this thesis does not advocate for or against HC usage in elite female soccer players, it presents data showing that nearly three-quarters of non-HC users experienced negative side effects, while 86% of HC users reported positive symptoms. Although some studies suggest slightly inferior exercise performance among HC users compared to naturally menstruating females (Elliott-Sale et al., 2020), recent data from British track and field athletes echoes our findings, with 76.8% reporting negative impacts of the menstrual cycle on performance (Jones et al., 2024). We believe it is crucial to acknowledge these findings and emphasise the need for further research and investigation into effectively managing these symptoms. Given the lack of research on the long-term health and performance consequences of HC use, our data underscores the need for comprehensive support and education regarding menstrual health, along with individualised, multi-disciplinary strategies to help mitigate the negative impact of menstrual dysfunction and optimise athlete health and performance.

### *8.2.2 Energy availability and menstrual function*

In Studies 2, 3 and 4 (Chapters 5, 6 and 7), the LEAF-Q identified several players as potentially at risk of suffering from LEA. However, upon evaluating energy availability using the criterion of $< 30$ kcal·kg·d$^{-1}$ along with associated markers (RMR ratio and biomarkers) and symptoms (menstrual dysfunction), it became apparent that the LEAF-Q significantly overestimated the number of players at risk. Recent findings by Dasa, Friborg, Kristoffersen, Pettersen, Sagen, et al. (2023) support our observations, reporting that a third of female soccer players were classified as at risk of LEA without showing signs or symptoms associated. The discrepancy may stem from the fact that the LEAF-Q was primarily validated in endurance athletes and dancers (Melin et al., 2014). These athletes are more prone to overuse injuries (Lopes et al., 2012; van Gent et al., 2007), compared to soccer players, who participate in a high-contact sport with potential for both acute and overuse injuries (Ekstrand et al., 2011). Consequently, the LEAF-Q may produce skewed scores in this context, leading to overestimations of LEA risk through normal soccer related injuries being potentially picked up as LEA derived stress-related injuries. Given the tendency for overestimation of LEA by the LEAF-Q in our study, future research should develop a more specific screening tool for LEA in female soccer players. This new tool should be designed to accommodate the distinctive demands and injury susceptibilities specific to soccer, distinguishing it from non-contact sports.

In Study 2 (Chapter 5), markers associated with LEA (RMR ratio and biomarkers) and symptoms (menstrual dysfunction) suggested that the female soccer players in the squad were not suffering from LEA during the pre-season. However, a snapshot assessment may only provide information relevant at the measured timepoint. Therefore, in Study 3 (Chapter 6), we longitudinally measured the same markers at four time points across a full playing-season. Only two players (11%) over the season suffered menstrual dysfunction, a lower rate than the self-reported dysfunction league-wide in Study 1 (Chapter 4) (26%). Upon further investigation, one player did not exhibit altered markers of LEA and reported a return to her normal menstrual cycle at the next time point. The second player reported menstrual dysfunction at the end of the season measurement (May 2018). The player’s energy availability was 34 kcal·kg FFM$^{-1}$·day$^{-1}$ with reductions in fat mass (17%), body fat percentage (12%), and body mass (4%) observed throughout the season. Resting metabolic rate ratio remained above the threshold but decreased from 1.05 to 0.95 over the course of the season. Unfortunately, this player declined to have blood tests, so no biomarkers were available, however, her hip and lumbar Z scores remained consistent above the threshold for bone disorders throughout the season. Finally, in Study 4 (Chapter 7), despite suffering from amenorrhea and anovulatory menstrual cycles, the player did not have LEA, with an energy availability of 45 kcal·kg FFM$^{-1}$·day$^{-1}$ RMR ratio > 0.90 and hormonal markers within range. It is likely that an alternative cause, such as psychological stress, contributed to her menstrual dysfunction (Mountjoy et al., 2014).

The assessment of energy availability in Studies 2, 3, and 4 (Chapters 5, 6, and 7) revealed that none of the players in the squad were below the threshold of LEA (<30 Kcal･kg $FFM^{-1}$). This finding contrasts significantly with previous studies on elite female soccer players. Moss et al. (2020) (23%), Reed et al. (2013) (24%) and more recently Dasa, Friborg, Kristoffersen, Pettersen, Plasqui, et al. (2023) (29.5%) and Morehen et al. (2022) (88%) have reported LEA as prevalent in domestic and international elite female soccer players. However, there were very few symptoms or other markers indicating energy availability as an issue in these studies. This raises questions about the discrepancies between this study and others. It is widely acknowledged that energy availability is extremely difficult to measure in an applied setting (Burke et al., 2018) due to a high level of burden (e.g., time and effort) on both the player and investigator. There have previously been no set protocols to measure the elements that make up energy availability, with all methods subject to errors associated with accurately measuring EI, EEE, and FFM. This is evident in the previous studies of elite female soccer players, which have used a variety of measures for both EI (food diary vs weighed food diary vs researcher present weighed inventory vs dietary recalls vs RFPM) and EEE (DLW vs GPS vs METs). This lack of consistency makes comparisons between studies extremely difficult. However, more recently, an update to the RED-S model (Margo et al., 2023) has acknowledged these limitations and has attempted to address them. The focus has shifted from using a threshold as the main indicator of LEA to utilising clearly defined methods and markers to assess every element associated with RED-S. Furthermore, LEA is viewed as being on a sliding continuum with moderating factors that can either protect against or add additional risk to LEA and its expression of disturbances to health, well-being, and performance. Data from this study aligns with the latest updates from Mountjoy et al. (2023), indicating that menstrual disturbances are multifactorial and often extend beyond LEA.

### *8.2.3 Body composition*

Studies 2 and 3 (Chapters 5 and 6) provided insight into body composition fluctuations within a WSL 1 team over the course of a season. A significant reduction in FM and increases in FFM were observed during the season. The most notable decrease in FM occurred between pre-season and the end of pre-season, likely attributed to players gaining FM during the off-season when EE is reduced, a phenomenon previously reported in both female (Minett et al., 2017) and male (Hoshikawa et al., 2005; McEwan et al., 2020; Reilly and Peiser, 2006) elite soccer players. While previous studies in elite female soccer players have reported either decreases

(Minett et al., 2017) or maintenance (Reed et al., 2013) of FFM over a season our study found a significant increase from pre-season to mid-season (February 2018). One reason for this disparity could be the duration of the season. The current study spanned a 10-month season, whereas the other studies lasted only 3-4 months. A longer season allows for prolonged periods for body composition changes to occur, alongside variations in training methodologies. Minett et al. (2017) reported a reduction in strength training frequency from 3 days a week in pre-season to once a week during the season, which may have influenced their results. Given these observations, our data from Studies 2 and 3 (Chapters 5 and 6) underscores the significance of pre-season in inducing substantial body composition changes.

Mean BM (59.8 ± 5.8), FM (12.4 ± 2.5 kg) and FFM (42.3 ± 2.6 kg) in Studies 2 and 3 (Chapters 5 and 6) using the gold standard DXA contribute valuable insights to the existing literature on elite female soccer players. Fat mass aligns closely with previous studies on US collegiate athletes (13.3 kg) (Reed et al., 2013), WSL 1 players (12.9kg) (Emmonds et al., 2019) and (11.5kg) (Moss et al., 2020) and more recently international players (11.8kg) (Morehen et al., 2022). However, discrepancies exist in FFM, with our study (42.3 ± 2.6 kg) reporting lower values compared to other studies (Reed et al., 2013) (44.8kg), (Emmonds et al., 2019) (46.3kg), (Minett et al., 2017) (48kg) and (Moss et al., 2020) (49.5kg). Despite similar player stature across studies, differences in BM primarily stem from variations in FFM. Discrepancies may also arise due to differences in DXA machines used, including beam path, software algorithms, and scanning frequencies (Bazzocchi, Ponti, Albisinni, Battista, and Guglielmi, 2016). Therefore, it is important to acknowledge that when comparing to other studies, although the gold standard DXA is being used, it still has its limitations (Kasper et al., 2021).

During Study 4 (Chapter 7), body composition changes were tracked over two years, including during a long-term injury. Figure 25 illustrates how post-injury and surgery, the player lost 2.6 kg of FFM, while simultaneously gaining 2 kg of FM, consistent with similar injuries in elite male soccer players (Milsom et al., 2014). Maintaining muscle mass and function is a primary aim during long-term injury rehabilitation, as muscle losses can prolong rehabilitation and affect performance outcomes (Buckthorpe, La Rosa, and Villa, 2019). Applying strategies similar to those used during rehabilitation from ACL injuries in elite male soccer players, such as maintaining EI at levels similar to when the player was active and loading non-injured muscle groups, have been shown to reduce FFM losses (0.6kg vs 5.8kg) (Anderson et al., 2019;

Milsom et al., 2014). This novel insight into body composition changes during long-term injury in elite female soccer players highlights the need for tailored nutritional support to minimise muscle loss and aid recovery.

### *8.2.4 Dietary intake and nutritional biomarkers*

Energy intake throughout the season was notably higher on training days (2306 ± 371 $kcal \cdot day^{-1}$) and match days (2188 ± 351 $kcal \cdot day^{-1}$) compared to days off (1960 ± 330 $kcal \cdot day^{-1}$). These dietary intakes are consistent with previous reports in collegiate and international female soccer players (1865 ± 530 - 2794 ± 233 $kcal \cdot day^{-1}$) (Clark et al., 2003; Gravina et al., 2012; Reed et al., 2014; Reed et al., 2013), as well as WSL 1 players (2124 ± 444 $kcal \cdot day^{-1}$) (Moss et al., 2020). However, what sets this study apart is that players seemed to adjust their intake based on training and match demands, possibly indicating intentional periodisation or the impact of nutrition education.

Similarly, carbohydrate intake followed the same pattern, with players consuming significantly more on training (4.1 ± 1.1 $g \cdot kg^{-1} \cdot day^{-1}$) and match days (4.5 ± 1.1 $g \cdot kg^{-1} \cdot day^{-1}$) compared to days off (3.3 ± 0.9 $g \cdot kg^{-1} \cdot day^{-1}$). These dietary intakes align with previous reports in international players (3.2 – 4.7 $g \cdot kg^{-1} \cdot day^{-1}$) (Martin et al., 2006; Morehen et al., 2022; Mullinix et al., 2003) and domestic players (3.3 – 4.1 $g \cdot kg^{-1} \cdot day^{-1}$) (Dasa, Friborg, Kristoffersen, Pettersen, Plasqui, et al., 2023; Moss et al., 2020). However, as noted in previous studies (Dasa, Friborg, Kristoffersen, Pettersen, Plasqui, et al., 2023; Moss et al., 2020), players tended to under consume carbohydrates on match days (4.5 ± 1.1 $g \cdot kg^{-1} \cdot day^{-1}$) compared to the recommended 7 $g \cdot kg \cdot day^{-1}$, with 19% consuming less than 3 $g \cdot kg \cdot day^{-1}$. This trend is consistent with recent findings with McHaffie et al. (2022) suggesting a "carbohydrate fear" among players due to concerns about weight gain. However, this study's data indicates that increasing carbohydrate intake around match days can be achieved without adverse effects on BM and FM. Given that players consumed more than sufficient amounts of protein (2.0 ± $0.2 g \cdot kg^{-1} \cdot d^{-1}$) and fat (1.3 ± $0.2 g \cdot kg^{-1} \cdot d^{-1}$), adjustments can be made to reduce protein and fat intake around match days to accommodate the additional calories from carbohydrates. What is clear from these data and supported by McHaffie et al. (2022) is that there is a continued need for targeted nutrition education to improve players' understanding of the critical role of carbohydrates for fueling match day performance.

Iron is an essential micronutrient for exercise and athletic performance, playing key roles in energy production and oxygen transport (Sim et al., 2019). Despite the importance of iron, iron deficiency is prevalent globally and among female athletes, affecting approximately 15-35% of this population (Sim et al., 2019). Previous studies in female soccer players have reported reduced iron concentrations in 29-56% of players (Braun et al., 2018; Landahl et al., 2005; Tan et al., 2012). However, comparing these studies is challenging due to differences in measured biomarkers and definitions. In our studies, we classified players' iron status using the stages of iron deficiency proposed by Peeling et al. (2007) and found that although players' ferritin concentrations remained within clinical norms throughout the season, 50% of the players were classified with stage 1 iron deficiency at least once during the season. While stage 1 depleted iron stores typically have minimal impact on performance (Burden et al., 2015; Rubeor et al., 2018), early detection could prevent progression to more severe stages (Peeling et al., 2007). However, our data demonstrated that without intervention, three of the five players with stage 1 iron deficiency experienced an increase in ferritin concentrations ($>35\ \mu g/L^{-1}$) by the subsequent time point, while the other two players' ferritin concentrations remained low until the end of the season, leaving them classified with stage 1 iron deficiency going into the off-season. It would have been insightful to assess the two players iron profiles upon returning to pre-season to evaluate the impact of the off-season. Potential reasons for the players iron deficiency were hypothesised to include acute iron loss (e.g., sweating, blood loss from menses or gastrointestinal bleeding), insufficient iron intake, or hormonal fluctuations impacting iron absorption (DellaValle, 2013; Telford et al., 2003). It is likely that a combination of factors influenced players' iron concentrations. These individual cases highlight iron deficiency as a concern among elite female soccer players in the WSL, emphasising the need for monitoring by teams to mitigate risks such as fatigue, cognitive impairment, and immune deficiencies (McClung et al., 2014). Additionally, it underscores the importance of longitudinal data in understanding players' health, with future assessments involving comparisons to both clinical norms and historical data, enabling tailored education and supplement protocols.

Vitamin D has been shown to impact muscle regeneration (Owens et al., 2015), immune function (Jung et al., 2018) and bone health (Lappe et al., 2008) in athletes. Since vitamin D is primarily synthesized in the skin through sun exposure, comparisons to female soccer players in other countries may not be meaningful. Thus, the most relevant comparison is with elite male soccer players in the English Premier League (Morton et al., 2012). Research on elite male soccer players in the English Premier League showed a significant decrease in vitamin D concentrations from August to December, with 65% of players being insufficient (<50 $nmol·L^{-1}$). In Studies 2 and 3 (Chapter 5 and 6) although players also experienced a significant decrease during a similar period, only one player (10%) fell below the same threshold. However, when using a threshold of >75 $nmol·L^{-1}$ as sufficient (Owens et al., 2018), in February, 70% of the squad were below this concentration. Vitamin D supplementation was provided to the squad during the winter months, with a recommended intake of 2000 IU per day, though adherence was not measured. Consequently, it is challenging to determine whether this supplement was insufficient or if adherence in the squad was poor. Given the vital role of vitamin D in bone health, muscle function, and protein synthesis (Lee et al., 2017), data from Studies 2 and 3 (Chapters 5 and 6) underscore the need for more individualised supplementation and education to mitigate the potential negative effects of decreased vitamin D during the winter among WSL 1 players.

### *8.2.5 Resting metabolic rate*

Resting metabolic rate typically constitutes the largest portion of TEE, ranging from 60-75% (Carpenter et al., 1995; Speakman and Selman, 2003). Accurate measurement or prediction of RMR is crucial for optimising body composition and fuelling strategies around training and matches. Previous studies have reported varying RMR values in female soccer players, with Fogelholm et al. (1995) finding a mean RMR of 1383$kcal·day^{-1}$ in Finnish female soccer players, though they were not full-time professionals. Moss et al. (2020) has reported 1510 ± 186$kcal·d^{-1}$ in WSL 1 players at the end of a season which aligns closely with the findings in Study 2 and 3 (Chapter 5 and 6) where RMR was measured to be 1543 ± 73$kcal·d^{-1}$.

In situations where direct measurement of RMR is not feasible (e.g. applied setting), prediction equations are often utilised. In Studies 2 and 3 (Chapters 5 and 6), we employed the Ten Haaf equation (Ten Haaf and Weijs, 2014) alongside direct measurement of RMR. The Ten Haaf equation has previously been reported to be within 10% accuracy of measured RMR in 86% of professional female rugby players (O'Neill et al., 2022). Although it could be argued that there are differences in lean mass between soccer and rugby players, these data provided a framework to explore the efficacy of the Ten Haaf method in female soccer players. In Study 3, we reported that there was a 5% difference between mean measured and mean predicted RMR, with 81% of players predicted RMR falling within 10% of the measured values. While an ideal scenario would involve the development of a prediction equation specific to female soccer players, our data suggests that the Ten Haaf equation accurately estimates RMR in this population and provides evidence to support the use of this equation in applied practice where direct measurements are not always possible.

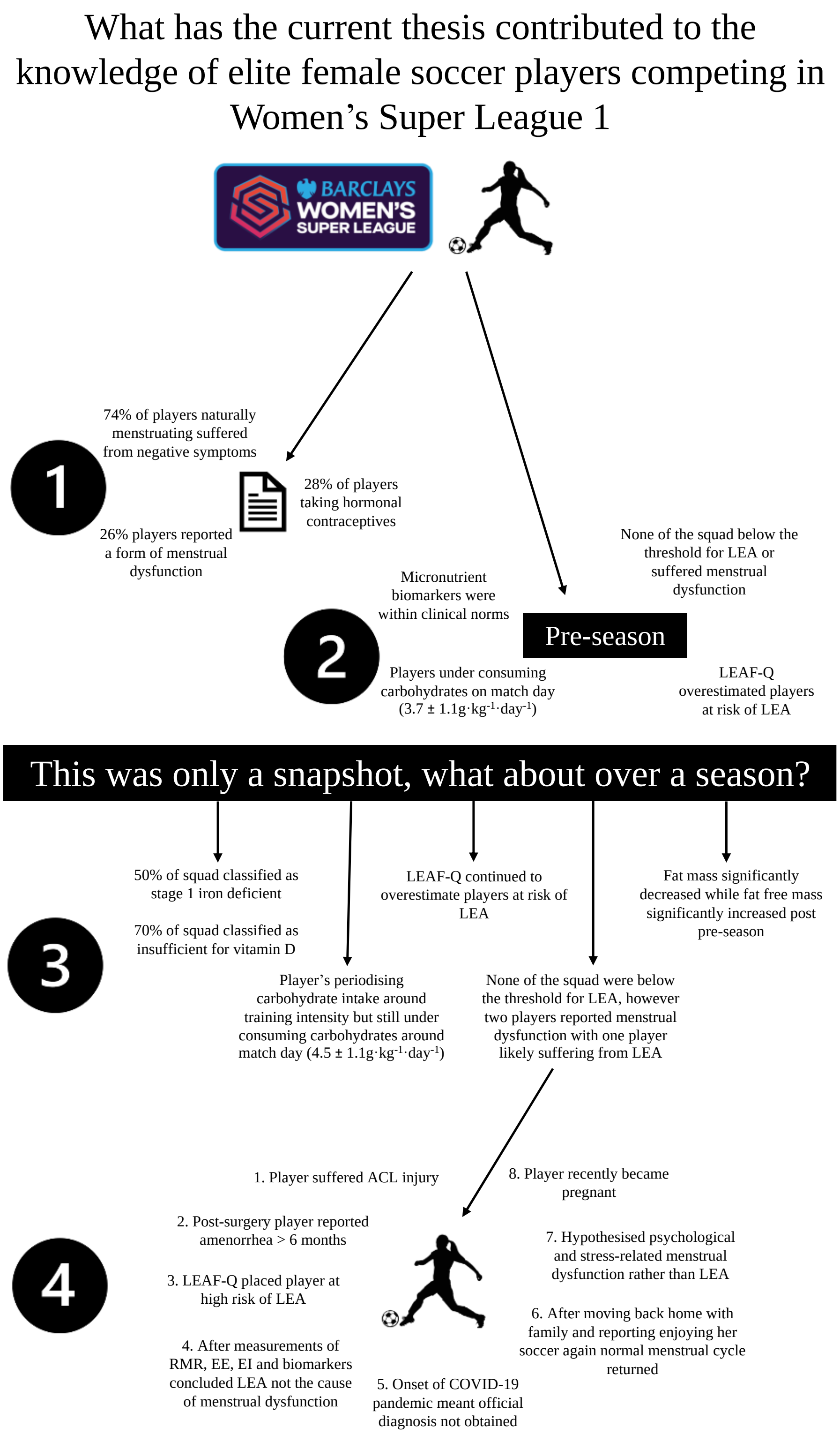


**Figure 27.** Overview of the main findings from the thesis. 1 = Study 1 (Chapter 4), 2 = Study 2 (Chapter 5), 3 = Study 3 (Chapter 6), 4 = Study 4 (Chapter 7). LEA = low energy availability, LEAF-Q = low energy availability in female's questionnaire, ACL = anterior cruciate ligament, RMR = resting metabolic rate, EE = energy intake, EE = energy expenditure.

## 8.3 Limitations

While the studies conducted in this thesis have contributed novel data to our understanding of elite female soccer players, they are not without limitations, some of which are applicable across multiple studies. Firstly, the data were collected from female first-team players competing in the WSL 1 league. Therefore, generalising these findings to young academy female players may not be appropriate due to potential differences in training intensity, nutritional needs, and physiological development. Moreover, the data collected in Studies 2, 3, and 4 (Chapters 4, 5, and 6) were obtained from players belonging to a single WSL 1 team, which may not be representative of other teams in the same league or professional female teams worldwide. Variations in coaching philosophies, training regimens, nutritional strategies, and organisational structures among different clubs could influence the outcomes observed in each study. Additionally, each study within this thesis had specific limitations that should be acknowledged.

### *Study 1 (Chapter 4)*

While 74% of non-HC users reported symptoms associated with their menstrual cycle, it is important to note that the questionnaire did not inquire whether these symptoms could have been influenced by factors other than their menstrual cycle, such as underlying medical conditions like endometriosis. Furthermore, the questionnaire did not assess the severity of these symptoms, the frequency of missed training sessions due to them, or whether players trained despite feeling unable to do so.

### *Study 2 (Chapter 5)*

While collecting measurements of DI, energy availability, body composition, and nutritional biomarkers during a 3-day period in pre-season provides valuable data, it is important to recognise the limitations of this approach. A single measurement during pre-season may not accurately reflect changes over the entire season, especially considering the variation in training loads and match frequency between pre-season and in-season periods. Additionally, DI, a component of energy availability, is notoriously challenging to measure accurately (Burke et al., 2018), although we utilised the RFPM, which has been validated previously within athletic populations (Costello et al., 2017). However, implementing this method for monitoring an entire squad simultaneously poses logistical challenges for researchers. Reflecting on Study 2, we found it difficult to follow up with players throughout the day, suggesting a need for a team-based approach involving one player and two research staff during

RFPM collection to enhance data quality in future studies. Unfortunately, due to a technical error RMR data was not collected in Study 2, this was a factor beyond the control of the research team with the option to conduct measurements using an alternative device not possible.

*Studies 2 and 3 (Chapters 5 and 6)*

To assess bone health during Study 2 and 3 DXA was undertaken, however, to gain a more comprehensive bone health profile, additional measurements such as bone biomarkers and HR-pQCT could have been utilised. Bone biomarkers have been shown to provide a more sensitive measure of bone health beyond DXA (Hagman et al., 2021; Kuo and Chen, 2017), with potential markers such as NTX, β-CTX and P1NP shown to be useful markers of bone turnover (Elliott-Sale et al., 2018). The use of HR-pQCT could have been employed to measure densitometry of the cortical and trabecular regions, along with the geometric properties of bone, providing a more nuanced indication of bone health (Ammann and Rizzoli, 2003; Minett et al., 2017). The integration of DXA, HR-pQCT and bone biomarkers would have provided a comprehensive bone health profile.

Vitamin D concentrations have been observed to decrease over a season in elite male soccer players (Morton et al., 2012). Similarly, in Studies 2 and 3 (Chapters 5 and 6), we observed a decline in vitamin D concentrations. However, due to the lack of measurement of supplement adherence, it is challenging to determine the reasons for this decrease in elite female soccer players. Thus, while our findings suggest a decrease in vitamin D concentrations during the winter months, future studies should incorporate individual supplement protocols and monitor adherence to evaluate the effectiveness of interventions.

Menstrual function was assessed using a questionnaire at the four designated time points throughout the season. However, to gain a more comprehensive understanding of players' menstrual cycle health and to establish individual baseline patterns, continuous tracking throughout the season would have been beneficial. Continuous monitoring would offer a deeper insight into each player's cyclical changes over time and enable early detection of any menstrual dysfunction. With advancements in technology, various smartphone applications are available that could facilitate this process, reducing the burden on both players and practitioners.

To gain a deeper understanding of eating attitudes and their underlying motivations, utilising an eating attitudes questionnaire, like the Eating Disorder Inventory 2 (Garner & Olmsted, 1984) or the Eating Disorder Examination – Questionnaire (Fairburn & Beglin, 1994) would have been beneficial. These assessment tools could have offered supplementary perspectives on the factors influencing food choices and helped identify potential instances of disordered eating or eating disorders within the cohort.

Alongside the markers taken in Studies 2 and 3 (Chapters 5 and 6), incorporating performance-based testing markers could have offered valuable contextual information to these studies. As the primary objective of the performance team is to enhance players' performance, integrating performance insights alongside nutrition and health markers would have provided comprehensive insights. Although gym and field performance testing were conducted by the sports science team, logistical challenges, and the infrequency of testing (twice in a season) rendered it unfeasible to include these data in the thesis.

*Study 4 (Chapter 7)*

As LEA was ruled out as the primary cause of the player's menstrual dysfunction, the research team had planned further medical investigations to be carried out (*e.g.*, polycystic ovary syndrome, hyperprolactinemia, primary ovarian insufficiency). However, the global COVID-19 pandemic and the player's transfer to another club hindered the execution of these tests. Despite maintaining communication with the player, by the time COVID-19 regulations permitted testing again, the player had relocated to Australia, making it impossible to conduct the necessary medical assessments or establish a definitive diagnosis.

## 8.4 Recommendations for future research

Building upon the findings from this thesis, further research is required to advance our knowledge and understanding of how we can better support elite female soccer players from a sports science and nutrition perspective. Some of the questions that remain unanswered may be addressed via the following research recommendations:

1. Replicating studies 1, 2 and 3 in a cohort of female academy players given they are susceptible to the same potential issues regarding LEA and micronutrient deficiency whilst acknowledging the anthropometric, physiological, and metabolic differences

between adults and youth players that means data should not be extrapolated (Desbrow et al., 2014).

2. While the Ten Haaf equation successfully predicted 81% of players RMR within 10% of the measured value, this still leaves a fifth of the squad with significant error. Thus, the development and validation of a novel prediction equation using elite female soccer players would likely provide a more accurate estimation of RMR.

3. Development and validation of a custom screening tool for measuring risk of LEA in female soccer players. This new tool should be designed to accommodate the distinctive demands and injury susceptibilities specific to soccer (Dasa, Friborg, Kristoffersen, Pettersen, Sagen, et al., 2023).

4. Measuring the effectiveness of a behaviour change model such as the COM-B framework and behaviour change wheel (Atkins and Michie, 2015) to aid with the nutritional intervention of increasing carbohydrates around match days in elite female soccer players.

5. Season-long study assessing the vitamin D status in elite female soccer player, with individual supplement protocols that are monitored for adherence throughout the season.

### 8.5 Summary

In summary, the research undertaken in this thesis provides novel data on the prevalence and factors affecting LEA among elite female soccer in WSL 1 (Figure 27). It was observed that fewer elite female soccer players utilise HCs compared to elite athletes from other sports, and menstrual dysfunction is prevalent across the league. Despite evidence of periodisation in energy and carbohydrate intake, players consistently under-consume carbohydrates on match days. The LEAF-Q classified a third of the squad at risk of LEA, however, when measured none of the squad were under the LEA threshold, which challenges previous estimations of LEA in elite female soccer players. The occurrence of menstrual dysfunction should prompt a holistic consideration of potential causes beyond LEA. Changes in FM and FFM post pre-season highlight the possibility to alter body composition in elite female soccer players. Moreover, players are at increased risk of vitamin D insufficiency during winter months, and half of the squad experienced stage 1 iron deficiency at some point during the season. Overall, LEA did not appear to be prevalent in the WSL 1 squad assessed, however, these findings emphasise the importance of adopting a comprehensive, multidisciplinary approach to monitor and support each female soccer player individually, aiming to optimise both health and performance.

# CHAPTER 9

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# CHAPTER 10

## Appendices

### 10.1 The low energy availability in females questionnaire (LEAF-Q)

### The LEAF-Q - A Questionnaire for Female Athletes

### 1. Injuries - Please mark the response that most accurately describes your situation

**A:** Have you had absences from your training, or participation in competitions during the last year due to injuries?

☐ No, not at all
☐ Yes, once or twice
☐ Yes, three or four times
☐ Yes, five times or more

**A1:** If yes, for how many days absence from training or participation in competition due to injuries have you had in the last year?

☐ 1-7 days
☐ 8-14 days
☐ 15-21 days
☐ 22 days or more

**A2:** If yes, what kind of injuries have you had in the last year?

### 2. Gastrointestinal function

**A**: Do you feel gaseous or bloated in the abdomen, even when you do not have your period?

☐ Rarely or never
☐ Yes, several times a day
☐ Yes, several times a week
☐ Yes, once or twice a week or more seldom

**B**: Do you get cramps or stomach ache, which cannot be related to your menstruation?

☐ Rarely or never
☐ Yes, several times a day
☐ Yes, several times a week
☐ Yes, once or twice a week or more seldom

**C**: How often do you have bowel movements on average?

☐ Several times a day
☐ Once a day
☐ Every second day
☐ Twice a week
☐ Once a week or more rarely

**D**: How would you describe your normal stool?

☐ Normal (soft)
☐ Diarrhoea-like (watery)
☐ Hard and dry

### 3. Menstrual function and use of contraceptives

3.1 Contraceptives - Mark the response that most accurately describes your situation

**A:** Do you use oral contraceptives?

☐ Yes | ☐ No

**A1:** If yes, why do you use oral contraceptives?

☐ Contraception ☐ Reduction of menstruation pains ☐ Reduction of bleeding
☐ To regulate the menstrual cycle in relation to performances etc.
☐ Otherwise menstruation stops ☐ Other

**A2:** If no, have you used oral contraceptives previously?

☐ Yes | ☐ No

**A2:1** If yes, when and for how long?

**B:** Do you use any other kind of hormonal contraceptives? (e.g. hormonal implant or coil)

☐ Yes | ☐ No

**B1:** If yes, what kind?

☐ Hormonal patches ☐ Hormonal ring ☐ Hormonal coil
☐ Hormonal implant ☐ Other

3.2 Menstrual function Mark the response that most accurately describes your situation

**A**: How old were when you had your first period?

☐ 11 years or younger ☐ 12-14 years ☐ 15 years or older
☐ I don’t remember ☐ I have never menstruated *

***If you have answered “I have never menstruated” there are no further questions to answer – proceed to page 17.***

**B:** Did your first menstruation come naturally (by itself)?

☐ Yes ☐ No ☐ I don’t remember

**C:** Do you have normal menstruation?

☐ Yes ☐ No **(go to question C6)** ☐ I don’t remember **(go to question C6)**

**C1:** If yes, when was your last period?

☐ 0-4 weeks ago ☐ 1-2 months ago
☐ 3-4 months ago ☐ 5 months ago or more

**C2:** If yes, are your periods regular? (Every 28th to 34th day)

☐ Yes, most of the time ☐ No, mostly not

**C3:** If yes, for how many days do you normally bleed?

☐ 1-2 days ☐ 3-4 days ☐ 5-6 days
☐ 7-8 days ☐ 9 days or more

**C4:** If yes, have you ever had problems with heavy menstrual bleeding?

☐ Yes | ☐ No

**C5:** If yes, how many periods have you had during the last year?

☐ 12 or more ☐ 9-11 ☐ 6-8
☐ 3-5 ☐ 0-2

**C6:** If no or "I don't remember", when did you have your last period?

☐ 2-3 months ago ☐ 4-5 months ago
☐ 6 months ago or more ☐ I'm pregnant and therefore do not menstruate

**D:** Have your periods ever stopped for 3 consecutive months or longer (besides pregnancy)?
☐ No, never ☐ Yes, it has happened before ☐ Yes, that's the situation now

**E:** Do you experience any changes with your menstruation when you increase your exercise intensity, frequency or duration?

☐ Yes | ☐ No

**E1:** If yes, in what way(s)? (Check one or more options)

☐ I bleed less ☐ I bleed fewer days ☐ My menstruations stops
☐ I bleed more ☐ I bleed more days

## 10.2 Adapted version of Martin et al. (2018) questionnaire

Please complete this questionnaire as accurately and as thoroughly as possible. All data collected from this questionnaire are **confidential** and **anonymous**. You have the right to withdraw your data at any time. If you wish to withdraw your data we will require a way of identifying your responses, therefore, please provide a word (unique identifier) that can be used identify your data (e.g., pets name).

Unique identifier (optional): ____________________________________________________________

**General information**

Date of Birth: ________________________________________________________________________

Height (approximate in meters **or** feet and inches): __________________________________________

Weight (approximate in kilograms **or** stones and pounds): ___________________________________

What age (approximately) were you when you had your first period? [*e.g. 14 years old*]

______________________________________________________________________________________

**Competition and training**

1. How long have you competed at this level (either for **CLUB** or any other professional team)?

[*e.g., 2.5 years*] _____________________________________________________________________

2. How many training sessions do you undertake in an average week? __________________________

3. How long are these training sessions on average (minutes)? _________________________________

Do you <u>currently</u> use any type of ***HORMONAL CONTRACEPTIVE**? YES / NO

***HORMONAL CONTRACEPTIVES** include:

- Oral contraceptives
- Implant
- Injection
- Intrauterine device/coil (hormone releasing – **NOT** copper-based)
- Vaginal ring
- Contraceptive (transdermal) patch

If **NO**, please proceed to SECTION **A**

If **YES**, please proceed to SECTION **B**

*Please do **NOT** answer both sections; only answer the section that represents your <u>current</u> use/status*

**Section A: Menstrual cycle characteristics**

**(Remember do NOT answer this section, if you intend to answer section B)**

**1.** Do you use a non-hormonal intrauterine device (copper-based coil)? YES / NO

**2.** Approximately, what is the length of your menstrual cycle?

This is **NOT** how long you bleed for. The length of your menstrual cycle is the time, in days, from the first day of bleeding, to the start of the next bleed [*e.g., 28 days*].

________________________________________________________________________

**3.** Is the length of your menstrual cycle variable? YES / NO

If YES, please state the range of your cycle: [*e.g., 27-33 days*]

________________________________________________________________________

**4.** Are you amenorrheic [you have missed more than 3 consecutive periods]? YES / NO

- If so, has this been diagnosed/confirmed by a doctor? YES / NO

**5.** Are you oligomenorrheic [the time between your periods is more than 35 days long]? YES / NO

- If so, has this been diagnosed/confirmed by a doctor? YES / NO

**6.** Do you get pain or any other symptoms during your menstrual cycle? YES / NO

- If YES, please state the symptoms: [*e.g., headaches, stomach cramps*]

________________________________________________________________________

________________________________________________________________________

- If YES, please state when during your cycle you suffer these symptoms: [*e.g., first 2 days of bleeding*]

________________________________________________________________________

**7.** Do you avoid exercise/training during any point of your menstrual cycle? YES / NO

- If YES, please state when during your menstrual cycle: [*e.g., first day of bleeding*]

________________________________________________________________________

- If YES, please state the reasons why you avoid exercise/training: [*e.g., too tired*]

________________________________________________________________________

**8.** Do you take any medication? YES / NO

If YES, please state what medication you use: ________________________________________

If YES, please state how often you use this medication: __________________________________

Please proceed to SECTION **C**

*Please do **NOT** complete SECTION B*

**Section B: Current hormonal contraceptive use**

**(Remember do NOT answer this section, if you have answered section A)**

**1.** Which type of hormonal contraception do you **currently** use (please circle your answer):

oral contraceptive implant injection intrauterine device/coil vaginal ring

contraceptive (transdermal) patch

**2.** What is the ***BRAND** name of this contraception? ________________________________________

There are different ***BRANDS** for each type of contraception; the brand is usually the name on the packet of pills (e.g., Microgynon 30® or Cilest®), the name of the intrauterine device (e.g., Mirena® coil), the name of the contraceptive implant (e.g., Norplant®), the name of the injection (e.g., Depo Provera®), the name of the vaginal ring (e.g., NuvaRing®) or the name of the contraceptive patch (e.g., Ortho Evra®).

**3.** How long (years/months) have you used this method of contraception? ______________________

**4.** Why did you choose this type of contraceptive? [*e.g., my friend recommended it*]

________________________________________________________________________________

**5.** Have you discussed your hormonal contraceptive use with your coach/team doctor? YES / NO

**6.** When deciding whether or not to use this method of hormonal contraception, did you <u>consider</u> possible side effects? YES / NO

If YES, what side effects were these: [*e.g., might give me spots*]

________________________________________________________________________________

**7.** Have you <u>received</u> any negative side effects from use of this method of contraception? YES / NO

If YES, which side effects: [*e.g., put on weight*]

________________________________________________________________________________

**8.** Were there any positive effects of hormonal contraceptive use (other than preventing pregnancy), that you <u>considered</u> when opting for hormonal contraceptive use? YES / NO

If YES, what were these: [*e.g., might help with acne*]

________________________________________________________________________________

**9.** Have you <u>received</u> any positive effects from use of this method of contraception? YES / NO

If YES, which positive effects: [*e.g., knew when my period was coming*]

________________________________________________________________________________

**10.** Do you take any other form of medication? YES / NO

If YES, please state what medication you use: ____________________________________________

If YES, please state how often you use this medication: ______________________________________

Please proceed to SECTION **C**

**Section C: Previous use of hormonal contraceptives**

**(Remember this section CAN be completed by everyone)**

If you have used (other) forms of ***HORMONAL CONTRACEPTIVES** in the past, please complete the table below.

Remember types of ***HORMONAL CONTRACEPTIVES** include:

- Oral contraceptives
- Implant
- Injection
- Intrauterine device/coil (hormone releasing – NOT copper-based)
- Vaginal ring
- Contraceptive (transdermal) patch

Remember there are different ***BRANDS** for each type of contraception; the brand is usually the name on the packet of pills (e.g., Microgynon 30® or Cilest®), the name of the intrauterine device (e.g., Mirena® coil), the name of the contraceptive implant (e.g., Norplant®), the name of the injection (e.g., Depo Provera®), the name of the vaginal ring (e.g., NuvaRing®) or the name of the contraceptive patch (e.g., Ortho Evra®).

| **Type** | **Brand** | **Duration of use** | **Reason you stopped using it** |
|---|---|---|---|
| *For example:*<br>Oral Contraceptive | *For example:*<br>Microgynon 30 | *For example:*<br>2 years | *For example:*<br>Persistent headaches |
| | | | |
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THANK YOU for completing this questionnaire.

**Please cite Martin et al. as the reference for this questionnaire.**