UiT The Arctic University of Norway

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Energetics, energy availability and Relative Energy Deficiency in Sport (REDs) in female football players

Methodological perspectives and research implications

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Abbreviations

ACSM	American College of Sports Medicine
AEE	Activity energy expenditure
AME	Amenorrhea
AUC	Area under the curve
BIS	Bergen Insomnia Scale
BMD	Bone mass density
BMR	Basal metabolic rate
CFS	Chalder Fatigue Scale
CHO	Carbohydrate
CTX-1	Collagen type 1 C-telopeptide
DE	Disordered eating
DLW	Doubly labeled water
DXA	Dual-energy X-ray absorptiometry
EA	Energy availability
ED	Eating Disorder
EDE-Q 11	Eating disorder examination questionnaire 11
EE	Energy expenditure
EEE	Exercise energy expenditure
EI	Energy intake
EPOC	Excess post exercise oxygen consumption
EUM	Eumenorrhea
FFM	Fat free mass
FIFA	Fédération Internationale de Football Association
GHQ	General Health Questionnaire
HC	Hormonal contraceptives
IAEA	International Atomic Energy Agency
IGF-1	Insulin-like growth factor 1
IOC	International Olympic Committee
LDL	Low density lipoproteins
LEA	Low energy availability
LEAF-Q	Low energy availability in females questionnaire
NHANES	National Health and Nutrition Examination Survey
OSTRCQ	Oslo Sport Trauma Research Center Questionnaire
P1NP	Procollagen type 1 N propeptide
PAL	Physical activity level
PPV	positive predictive value
PRC	Precision recall curve
REDs	Relative Energy Deficiency in Sport
RMR	Resting metabolic rate
ROC	Receiver operating curve
T_3	Triiodothyronine
T_4	Thyroxine
TDEE	Total daily energy expenditure
TEF	Thermic effect of food
Triad	The female athlete triad
TSH	Thyroid stimulating hormone
VO_2	Oxygen consumption
REK	Regional Committee for Medical and Health Research Ethics

List of papers

Paper I

Dasa MS, Friborg O, Kristoffersen M, Pettersen G, Sundgot-Borgen J, Rosenvinge JH. Accuracy of tracking devices' ability to assess exercise energy expenditure in professional female soccer players: implications for quantifying energy availability. *Int J Environ Res Public Health*, 2022;19(8):4770.

Paper II

Dasa MS, Friborg O, Kristoffersen M, Pettersen G, Plasqui G, Sundgot-Borgen J, Rosenvinge, JH. Energy expenditure, dietary intake and energy availability in female professional football players. *BMJ Open Sport Exerc Med*, 2023, 1;9(1):e001553.

Paper III

Dasa MS, Friborg O, Kristoffersen M, Pettersen G, Sagen JV, Sundgot-Borgen J, Rosenvinge, JH. Evaluating the suitability of the Low Energy Availability in Females Questionnaire (LEAF-Q) for female football players. *Sports Med – Open*, 2023,13;9(1):54.

Paper IV

Dasa MS, Friborg O, Kristoffersen M, Pettersen G, Sagen JV, Torstveit MT, Sundgot-Borgen J, Rosenvinge, JH. Prevalence of indicators associated with Relative Energy Deficiency in Sport (REDs) among professional female football players. *In review.*

Summary

Background

For athletes, it is imperative that energy intake (EI) adequately addresses the total energy requirements inherent to their specific sport. Low energy availability (LEA), which is the failure to provide the body with adequate energy to sustain homeostasis, may potentially result in Relative Energy Deficiency in Sport (REDs). This is a syndrome that can have detrimental health consequences, including altered menstrual, bone and endocrine functions. There is limited understanding of the energy requirements, as well as the prevalence and health consequences of LEA and REDs among female football players. Further, several methodological limitations in measuring EA impede the validity of findings. Considering this, the objective of this thesis was to explore the concepts of energetics, energy availability (EA) and REDs among female football players, with additional focus on methodological approaches.

Methods

All four papers presented in this thesis are conducted on professional female football players from the Norwegian premier league (Toppserien) and first division. In *paper I* we assessed the measurement accuracy of commonly used tracking devices to estimate the energetic cost of exercise, a key factor in the calculation of EA. *Paper II* quantified the EI, energy expenditure, and EA. Dietary recalls, doubly labeled water and GPS measurements were used to provide a wide range of measures related to the energetics of female players. *Paper III* assessed the suitability of the commonly used Low Energy Availability in Females Questionnaire (LEAF-Q) by comparing the questionnaire scores against indicators associated with the female athlete triad (Triad) and LEA. In *Paper IV*, we assessed the prevalence of indicators associated with REDs and formulated a cumulative risk index, based on their strength of association with REDs, providing better risk information regarding the development of the syndrome.

Results

Paper I demonstrated that all examined tracking devices significantly underestimated the energetic cost of intermittent exercise, ranging between 10.7 – 20.6%. All devices revealed a systematic inverse relationship with increasing exercise intensity. Omitting excess post-exercise oxygen consumption from the calculation significantly improved the accuracy with underestimation between 3.1 – 7.2%. In *paper II*, the average total daily energy expenditure was 2918 ± 322 kcal (physical activity level of 2.0 ± 0.3). The average EI was 2274 ± 450 kcal. A significant difference was observed between EI on match vs rest days ($p < 0.05$), but not between match versus training or training vs rest days. Carbohydrate intake on both match and training days was below the recommended guidelines for large parts of the cohort. The prevalence of LEA was 36% and 23% on match and training days, respectively. *Paper III* examined the suitability of the LEAF-Q as an indicator of the Triad and LEA; however, the area under the curve (AUC) for the LEAF-Q showed poor performance (0.44 – 0.53) for all clinical markers, apart from detection of amenorrhea (AUC = 0.86). The questionnaire did not statistically differentiate players classified as *at risk* or *not at risk* based on broad indicators of LEA. In *paper IV,* the absence of any primary, secondary, or associated indicators ranged between 33-55%. However, 22% presented with clustered primary and secondary indicators, which was unrelated to player position. Amenorrhea was reported by 30% of the non-contraceptive users ($n = 27$). Amenorrheic players displayed a significantly greater number of cumulative indicators compared to eumenorrheic players ($p < 0.05$).

Conclusion

Current generation of tracking devices did not provide accurate estimates of the energetic cost of intermittent exercise, consequently undermining the validity of EA assessment using these devices. While the energetic requirements of female football players appear to be moderate, a substantial portion of the cohort did not meet the nutritional requirements, notably pertinent to carbohydrates. Furthermore, there was limited evidence of nutritional periodization. Despite a marked prevalence of LEA, the LEAF-Q was not able to reliably identify players at risk for developing of health detriments. Continued usage of the LEAF-Q to detecting LEA among female football players can not be recommended. Rather, methods to evaluate player health outcomes, such as identifying the presence of clinical indicators associated with REDs should be prioritized to provide better risk assessments for the individual athlete, as sole assessments EA seems to insufficiently reflect health risk among female football players.

Sammendrag (Norwegian summary)

Bakgrunn

For idrettsutøvere er det avgjørende at energiinntaket (EI) tilfredsstiller de totale energibehovene, som er spesifikke for deres idrett. Lav energitilgjengelighet (LEA), hvor kroppen ikke får nok energi til å opprettholde homeostase, kan potensielt resultere i relativ energibrist (REDs). Dette er et syndrom som kan ha skadelige helsekonsekvenser, inkludert forstyrrelser i menstruasjons-, ben- og endokrine funksjoner. Det er begrenset forståelse av energibehovene, samt forekomsten og helsekonsekvensene av LEA og REDs blant kvinnelige fotballspillere. Videre hindrer flere metodiske begrensninger i målingen av energitilgjengelighet (EA) validiteten av funnene. Med bakgrunn i dette, var målet med avhandlingen å utforske konseptene rundt energetikk, EA og REDs blant kvinnelige fotballspillere, med ytterligere søkelys på metodiske tilnærminger.

Metoder

Alle de fire artiklene presentert i denne avhandlingen er utført på profesjonelle kvinnelige fotballspillere fra den norske toppserien og førstedivisjon. I *artikkel I* undersøkte vi nøyaktigheten av alminnelig brukte sporingsenheter for å estimere energikostnaden ved trening og kamp, en nøkkelfaktor i beregningen av EA. *Artikkel II* kvantifiserte EI, energiforbruk og EA. Kostholdsregistreringer, dobbelt merket vann og GPS-målinger ble brukt for å gi et bredt spekter av mål relatert til kvinnelige spilleres energetikk, samt informasjon om forekomsten av LEA. *Artikkel III* vurderte validiteten til det ofte brukte spørreskjemaet Low Energy Availability in Females Questionnaire (LEAF-Q) ved å sammenligne spørreskjemaresultater med indikatorer assosiert med den kvinnelige utøvertriaden (Triaden) og LEA. I *artikkel IV* undersøkte vi forekomsten av indikatorer forbundet med REDs og formulerte en kumulativ risikoindeks. Dette gir en bedre risikovurdering angående potensiell utvikling av syndromet.

Resultater

Artikkel I viste at alle undersøkte sporingsenhetene betydelig underestimerte energikostnaden ved intermitterende aktivitet, som varierte mellom 10.7 – 20.6%. Alle enhetene viste et systematisk invertert forhold med økende treningsintensitet. Fratrukket etterforbrenning fra oksygengjeld etter fysisk aktivitet i beregningen, ble underestimeringen betydelig mindre (3.1-7.2%). I *artikkel II* var det gjennomsnittlige daglige energiforbruket 2918 ± 322 kcal (fysisk aktivitetsnivå (PAL) 2.0 ± 0.3). Gjennomsnittlig EI var 2274 ± 450 kcal. En signifikant forskjell ble observert mellom EI på kampdager versus hviledager ($p < 0,05$), men ikke mellom kampdager versus treningsdager eller treningsdager versus hviledager. Karbohydratinntaket både på kamp- og treningsdager var under de anbefalte retningslinjene for store deler av kohorten. Forekomsten av LEA var 36% og 23% på

henholdsvis kamp- og treningsdager. *Artikkel III* undersøkte validiteten av LEAF-Q som en indikator for Triaden og LEA; imidlertid viste området under kurven (AUC) for LEAF-Q dårlige verdier (0,44 - 0,53) for alle kliniske markører, bortsett fra deteksjon av menstruasjonsforstyrrelser (AUC = 0,86). Spørreskjemaet klarte ikke å skille spillere klassifisert som *i risiko* eller *ikke i risiko* basert på indikatorer assosiert med LEA. I *artikkel IV* var fraværet av alle primære, sekundære eller assosierte indikatorer mellom 33-55%. Imidlertid presenterte 22% av spillerne med kumulative primære og sekundære indikatorer, som var uavhengig av spillerposisjon. Menstruasjonsforstyrrelser ble rapportert av 30% av ikke-prevensjonsbrukere (n=27). Spillere med menstruasjonsforstyrrelser hadde et betydelig høyere antall kumulative indikatorer sammenlignet med spillere uten menstruasjonsforstyrrelser ($p < 0.05$)

Konklusjon

Nåværende generasjon av sporingssystemer gir ikke nøyaktige estimater av energikostnaden ved intermittent trening, noe som dermed undergraver validiteten av EA målinger ved bruk av disse systemene. Selv om energibehovene til kvinnelige fotballspillere ser ut til å være moderate, møtte en betydelig del av kohorten ikke de ernæringsmessige retningslinjene, særlig med hensyn til karbohydrater. Videre var det begrenset forekomst av ernæringsperiodisering i forhold til fysisk belastning. Til tross for en markant forekomst av LEA, var LEAF-Q ikke i stand til pålitelig å identifisere spillere i risiko for å utvikle helseproblemer knyttet til Triaden og LEA. Fortsatt bruk av LEAF-Q kan derfor ikke anbefales for kvinnelige fotballspillere. Snarere bør metoder som kan evaluere spillere sin helsestatus, som det å identifisere tilstedeværelsen av indikatorer assosiert med REDs prioriteres. Dette vil kunne gi bedre oversikt og risikovurdering for den enkelte, da måling av EA alene ikke ser ut til å gjenspeile potensiell helserisiko blant kvinnelige fotballspillere.

Introduction

Football is one of the most popular sports in the world, with over five billion football fans around the globe, according to the Fédération Internationale de Football Association (FIFA).[1] Presently, there are over 30 million female football players, and FIFA is aiming to double this figure by 2026.[1] As part of their 2020-23 global vision, FIFA is seeking to enhance the professionalization of women's football, leading to more investments, better media coverage and rising salaries.[1] Nevertheless, sex discrimination in terms of payment and player rights continue to persist within the world of football.[2,3] Despite a growing body of research on women's football,[4] there is still a sex bias in favor of research conducted on male participants, with findings often generalized to females.[5] A recent scoping review of the football literature underscored a remarkable disparity in the volume of articles published, with studies on men's football substantially outnumbering those focusing on woman's football.[6]

The last decades have seen a marked advancement in focus on athlete health, with several measures being put in place to protect athletes and understand the mechanisms ultimately compromising their ability to participate on the desired level.[7–9] Depending on the characteristics of a given sport, athletes are exposed to physiological and psychological stress, which may increase the risk of developing injuries or other health problems.[10] Professional female football players will generally cover 9-11 km during a match, with 22-28% performed as high intensity running or sprinting.[11,12] A typical seven day in-season training period usually consist of 1-2 matches, in addition to 4-6 training sessions.[13] Modern football is also becoming increasingly demanding, placing greater expectations on players and team support staff regarding strategies for improving performance, preventing injuries and reducing recovery time.[14]

Sufficient energy intake (EI) in athletes is necessary to support the energetic demands of training and competition, and further, promote restitution, recovery and adaptation to facilitate health and performance.[8,15,16] The physical and energetic requirements of male football players are well established through years of research.[17–20] However, in female football players, these requirements are less understood. At present, the nutritional guidelines for professional football players does not differ between sexes, as no current evidence supports sex specific nutrition.[21] However, "*absence of evidence does not equal evidence of absence",* and these guidelines are predominantly grounded in research conducted in males.[22] Therefore, more female specific research is needed, as the current guidelines does not necessarily account for potential sex-based differences, such as the effect of ovarian hormones on CHO metabolism.[21,22]

With the increasing physiological demands in women's football, emphasis on meeting the nutritional needs is becoming more crucial.[23] Energy availability (EA) is defined as the amount of energy available to sustain physiological functions after subtracting the energetic cost of exercise.[24] insufficient EA due to increased exercise energy expenditure (EEE), reduced EI, or a combination of the two, known as low energy availability (LEA), may have severe consequences, causing negative physiological alterations.[25–27] These alterations are described by the Female Athlete Triad (Triad), a concept that has its origins several decades ago.[26,28–30] The Triad describes the interrelationship between LEA, menstrual disturbances, and attenuated bone mass density (BMD). Notably, LEA may occur with or without disordered eating or eating disorders (DE/ED), and it is recognized as the underlying etiology. Previous studies have shown a causal relationship between LEA and symptoms of the Triad.[26,30] In 2014, a more comprehensive model (framework), building on the foundation of the Triad was developed by the International Olympic Committee (IOC) and their expert panel. This model is termed Relative Energy Deficiency in Sport (REDs), which also includes male athletes. [8,27] REDs is defined as "*impaired physiological function, including, but not limited to, metabolic rate, menstrual function, bone health, immunity, protein synthesis, cardiovascular health caused by relative energy deficiency"*. The REDs model offers a broader understanding of physiological alterations, with LEA still recognized as the causative factor.[8] Although proponents of the Triad have criticized the REDs model for not demonstrating a causal relationship between exposure and outcome variables,[31] the REDs model seems to have prevailed as it encompasses the Triad mechanisms while also recognizing broader elements associated with LEA. In contrast to most sports medicine research, females are overrepresented in research on LEA and REDs.[27,32] There is, however, several knowledge gaps in the literature, as most of the prior work is conducted on sports emphasizing high power to weight ratio and low fat mass (%), such as endurance and aesthetic sports. Less is therefore known about the manifestation of LEA and REDs in team-sports, such as football. It is also important to recognize that the specific methodologies employed may greatly influence the research outcome, highlighting the importance of deeper understanding of their implication.

Theoretical background and framework

Energetic requirements in women's football

Human, or animal energetics, may refer to the measurement and explanation of variations in energy expenditure (EE).[33] The energetic requirements of female football players will depend on several factors, including player position and tactical dispositions influencing the game.[34,35] Match performance analysis from the Women's Champions League have shown that similar to men, female players show temporary fatigue during and towards the end of games.[36] However, it was not until recently that the relationship between decline in sprint performance and alterations in muscle glycogen was shown in females. A recent study on female football players reported that post match glycogen was decreased by 80% and 69% in type I and II fibers, respectively.[37] Further, lactate levels peaked at 8.4 mmol/L, indicating elevated anaerobic energy production, which is known to be mainly glycolytic in nature.[37]

Most studies investigating physiological demands in women's football are based on GPS displacement data, focusing on performance parameters, such as high intensity running and sprinting.[34] Indeed, the majority of tracking devices additionally offer methods to estimate EEE, usually through the metabolic power concept.[38,39] Metabolic power calculates EEE based on accelerations and velocity data, which are based on various assumptions, including acceleration on flat terrain being equivalent to uphill running at constant speed [40]. While this may facilitate quantification of energy consumption, a number of studies on male participants have shown metabolic power to significantly underestimate EEE.[41–43] No studies have investigated how these devices perform, in terms of accuracy, using female participants, and the algorithms are based on data from males.[38] Disparities in physiological variables such as work economy, efficiency and body composition still remains undecided, and is not accounted for by metabolic power.[44] With this in mind, it is important to differentiate between studies investigating physiological demands through displacement data, where high sampling GPS is shown to be accurate,[45] and energetic demands quantified through EEE, as they serve to inform on different entities.

Most studies that have investigating the energetic requirements in female football players have relied on GPS and accelerometer data.[12,13,34,35] Only one study has used the gold standard method of doubly labeled water (DLW) to quantify total daily energy expenditure (TDEE).[46] Here, the average TDEE was ~ 2600 kcal with a physical activity level (PAL) of 1.8, classified as moderate. However, the study was conducted on a finite sample of international players at training camp, making generalization of the results difficult. There is conflicting evidence regarding the physiological demands of domestic versus international players, with recent evidence suggesting minor differences in physical attributes.[11] This stands in contrast to earlier studies and may be a result of advancements and increased professionalism within women's football, consequently reducing the physiological divide between high and lower tier teams.[47–49] Accordingly, there is a need for scaled up DLW studies to ascertain the energetic requirements for female footballers, across different tiers.

Nutritional requirements in women's football

Studies on elite female football players have reported EI ranging from ~ 1900 – 2400 kcal.[13,46,50,51] Morehen et al. reported that the mean value of EI among female internationals was 1923 kcal, which is ~ 700 kcal below their TDEE, measured with DLW.[46] Football, being a glycolytic sport, has around 60-70% of total energy production supplied by carbohydrates (CHO),[21] making it the principal macronutrient for performance.[17,52] Although current evidence suggests that the energetic requirements of women's football is moderate, there is significant strain on muscles through the high amount of eccentric loading, primarily due to repeated acceleration and deacceleration inherent to the sport.[46,53] Thus, the recovery period associated with match and training may exceed that of other sports with comparable energetic demands.[54] With the increasing demands in women's football, congested fixtures are becoming prevalent[55] Consequently, the focus on replenishing glycogen stores between games becomes increasingly important, a process which may take up to 72 h.[56] Apart from being the predominant energy source in football, studies have shown that CHO periodization may enhance skeletal muscle adaptation, as well as body composition (e.g. increase fat free mass (FFM)).[57] However, the CHO intake have been found to be well below the recommended guidelines in female professional football players.[46] With the knowledge that insufficient CHO intake can lead to adverse physiological changes even in the absence of LEA, it is important for female football players to adhere to current nutritional guidelines in order to optimize health and performance.[58,59] This includes augmenting EI in response to the physiological load, known as nutritional periodization.[21]

In addition to CHO, protein and dietary fat are important macronutrients essential to support skeletal muscle recovery following exercise and metabolic function, among other factors.[21,60,61] In contrast to CHO, these macronutrient requirements generally seem to be met among female footballers.[21,46]

Energy availability

Energy availability (EA) has been defined as the difference between EI and EEE, expressed relative to an individual's lean body mass, representing the daily amount of energy available to sustain all physiological functions outside of exercise.[24] The algebraic equation is the following:

$$Energy\ availability = \frac{(Daily\ energy\ intake - Daily\ exercise\ energy\ expenditure)}{FFM}$$

As such, LEA is the failure to provide the body with adequate energy to sustain this physiological homeostasis. The presence of LEA is generally defined as < 30 kcal/kg^{-1} FFM/day^{-1}.[25]This threshold is largely based on research measuring the effect of various levels of EA on hormonal status, the hypothalamic-pituitary-thyroid axis, hypothalamic-pituitary ovarian axis, and markers of bone metabolism in premenopausal sedentary women, below which a disruption of the hormonal milieu occurs.[25,62,63] Still, this threshold has been disputed as an absolute cut off value, and alternative approaches have been suggested,[31,64,65] to accommodate for the fact that there seems to be different physiological responses to LEA, and that individual thresholds may consist along a spectrum.[65,66] However, it is well documented that the presence of severe LEA may cause endocrinological and metabolic disruptions, that over time can have severe health outcomes.[25,63,67–71] Despite this, the understanding of the effects of LEA is still incomplete. For instance, controlled periods of LEA may yield performance enhancing outcomes, particularly in sports emphasizing high power to weight ratio.[72,73] As such, it is perceived by some, as necessary to achieve the desired athletic performance. Hence, it is important to acknowledge that athletes employ various weight manipulation strategies to enhance their capacity.[74] While a comprehensive examination of the direct influence of LEA on athletic performance is outside the scope of this thesis, it is essential to highlight that LEA is characterized by shades of gray rather than strict dichotomy. With that in mind, the subsequent definition of LEA implies a problematic level unless expressed otherwise, as it is conceived as severe in magnitude or prolonged in duration and includes negative health alterations.

One of the main challenges when measuring EA is the definition in itself, as it relies heavily on the measures of EI and EEE, which are both notoriously difficult to accurately assess.[75,76] In particular, measuring EEE proves challenging in intermittent sports, such as football, due to the high amount of anaerobic work above maximal oxygen consumption (VO_2).[11,37,77] For example, certain tracking devices have been shown to systematically underestimate EEE, compared to indirect calorimetry.[43,78] Further, the average underestimation of EI in athletes has been shown to be approximately 20%.[76] To overcome some of the challenges associated with measuring EA, some studies have shifted focus towards investigating the prevalence of symptoms related to REDs.[79–81]

In the effort to effectively screen athletes at risk for developing health issues from LEA, Melin et al. developed the Low Energy Availability in Females Questionnaire (LEAF-Q) in 2014.[82] The questionnaire, comprising 25 items, consists of the subcategories: ‘injuries’, ‘gastrointestinal function’, and ‘menstrual function’. The total and subscale scores have distinct cut-off values associated with an increased risk of Triad/LEA. Originally, the LEAF-Q was validated for endurance athletes and dancers, but has been widely applied in other sports, including football, despite distinctive differences in physiological characteristics and impact.[13,83,84] This widespread usage of the questionnaire can be problematic, as it raises the risk of invalid research findings.

Carbohydrate availability

Glucose can be stored in the muscle and liver as glycogen, which during exercise is converted back to glucose, and transported to active tissues though the bloodstream.[85] Thus, humans have a finite amount of CHO available for energy production, making exogenous CHO necessary to meet the energy requirements during prolonged or intense exercise.[86]

With the increasing amount of high-intensity running and sprinting in modern football, the need to fuel sufficiently to support the energetic needs is significant .[14,53] Recent years have seen a focus on the role of CHO availability and its importance in relation to both performance and athlete health.[58] Given the body's limited capacity to store CHO in the muscles and liver, strategically enhancing CHO intake pre, peri and post workout becomes essential during high-intensity exercise.[53,87,88] Recently, several studies have shown the importance of adequate CHO availability for bone turnover in response to exercise, and that low CHO availability may induce negative health outcomes independent of EA status.[59,89] The performance detriments following a low CHO diet on high-intensity exercise performance is well established.[87,90] However, the negative health consequences associated with insufficient CHO intake are less understood.[91] It was recently reported that professional English female football players exhibited fear of carbohydrate intake in relation to body composition, as well as body image issues.[92] Further, professional and international players have consistently displayed insufficient CHO intake in relation to physiological demands.[13,46] Hence, evidence indicating adverse physiological implications of reduced CHO availability, coupled with the recognized inadequacy of CHO intake in female footballers requires attention. At present, there is no set threshold defining low CHO availability. However, as recommendations for different scenarios defined for football exist, low CHO availability may be referred to as CHO intake below these recommendations.[21,23]

Relative Energy Deficiency in Sport (REDs)

Low energy availability is considered the underlying etiology of REDs.[8,30] As a result of LEA, suppression of energy demanding processes may occur to prioritize vital biological processes, necessary for survival.[93,94] These subsequent physiological alterations is outlined in the REDs model (**Figure *1***), along with potential performance detriments that may accompany the onset of REDs (**Figure 2**).[27] During periods of energy deficiency, signals to restore energy balance by stimulating EI or attenuating EE can also be augmented. These processes may result in adaptive thermogenesis, making measures such as weight change less reliable in treatment and assessment of athletes.[95] Since REDs is defined as a syndrome, it is characterized by clustering of symptoms, with no clearly defined diagnostic criteria. Greater clustering of symptoms may therefore be linked with increased risk of development of REDs, yet one or more indicators of REDs may be present without the syndrome manifesting.[96] Determining whether indicators are related to LEA or other factors, such as medical conditions, environmental influences, genetics, or natural variations can be challenging. It is therefore important to avoid over-interpretation of standalone symptoms when assessing athletes.

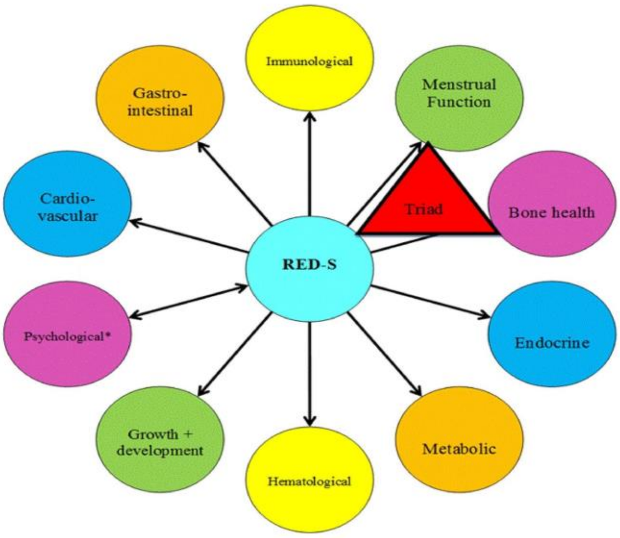


Figure 1. *Potential health consequences of REDs.*[27] *(With permission from the BMJ publishing group Ltd.)*

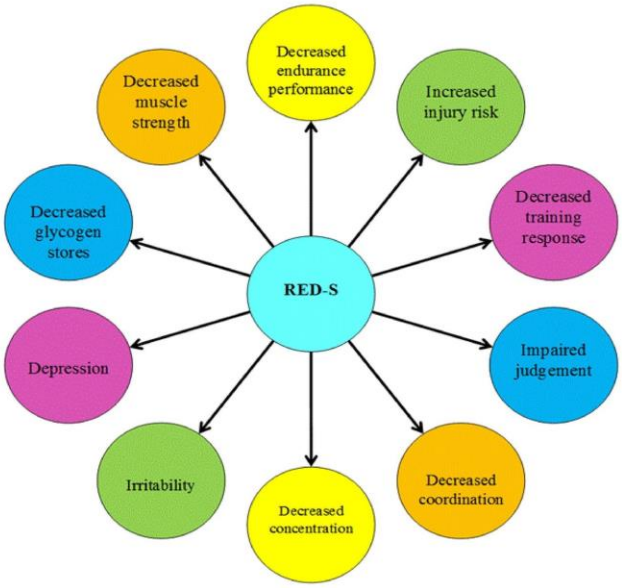


Figure 2. *Potential performance consequences of REDs.*[27] *(With permission from the BMJ publishing group Ltd.)*

The progression from LEA to REDs may look different depending on the individual. **Figure 3** introduces a model that outlines the possible progression towards development of REDs, emphasizing that the energetic demands of a sport can help determine the key source of potential health risk. The balance between EI and EE is fundamental, but the context of the sport may reveal the likely issue of the two. The model identifies sports that may typically be at risk for insufficient EI or excessive EE based on their metabolic profiles, which is illustrated by the magnitude of the bubble (EI/EE). For instance, triathletes or cyclists may exhibit extreme EE during intensive training or competition phases.[97] Despite increasing EI, they might struggle to accommodate the energetic needs, requiring reduced EE if persistent. Conversely, football players have been shown to exhibit conservative levels of EE, which theoretically, should be manageable to compensate, under normal circumstances. Thus, the sport's energetic profile suggests that inadequate EI may be the primary concern.

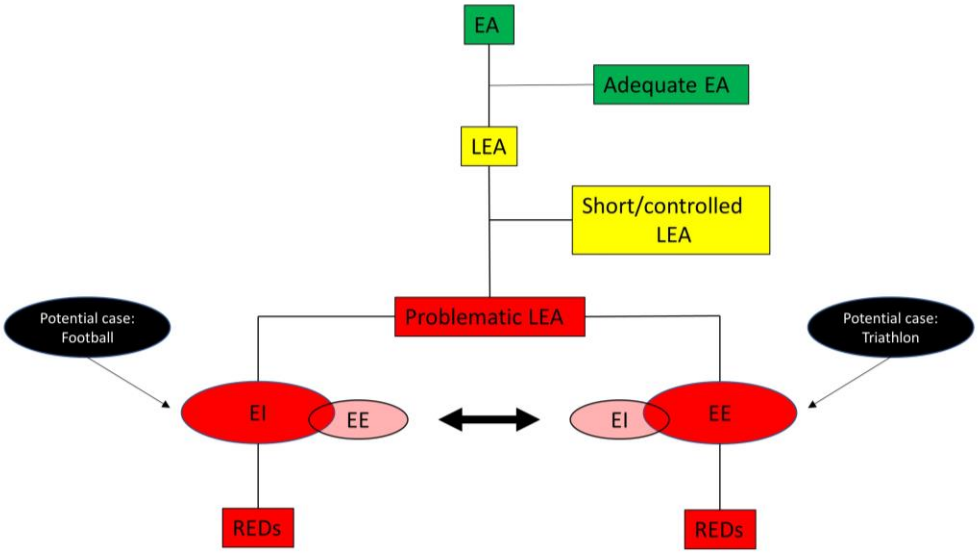


Figure 3. *Conceptual model depicting the potential progression towards development of REDs. Colors represents the associated risk for development of health detriments, in descending order. EA = energy availability; LEA = low energy availability; EI = energy intake; EE = energy expenditure; REDs = Relative energy deficiency in sport.*

Low energy availability (LEA) and REDs in female football

To summarize the available body of literature, a systematic search in the PubMed database was performed on the 05.04.2023. The two keywords 'football' and 'soccer' were searched with various combinations of the keywords: 'relative energy deficiency in sport', 'RED-S', 'REDs', 'low energy availability', 'LEA', 'energy availability', 'EA', 'female athlete triad', and 'triad'.

Inclusion criteria were English original publications available in full text, peer-reviewed journals, football/soccer specific, outcome including measures of EA, REDs, or the Triad and female players. Secondary data studies (e.g., reviews) and studies on males or mixed sport cohorts were not included. **Table 1** provides a summary of the included studies on LEA, REDs, the Triad, or a combination of them.

Table 1. *Summary of literature investigating LEA/REDs and/or associated indicators among female football players*.

Author	Level	N	Design	Aim	Summar
Braun et al. 2017 [50]	Young elite	56	Prospective cohort	Investigate EI, EE and nutritional status	Average EI: 2226 ± 195 kcal/d. LEA < 30 kcal/kg^{-1} FFM/day^{-1}: 53% (average EA: 30.0 ± 7.3) Low CHO intake (average: 5.4 ± 1.1 g/kg) Majority of players did not meet nutritional needs
Dobrowolski et al. 2019 [98]	Professional*	41	Cross-sectional	Evaluate EE and assessment of EI	Average EE: 2811 ± 493 kcal/d Average EI: 1476 ± 434 kcal/d. Energy deficiency: 98% (average kcal/kg BM/day: 24.3 ± 8.9) Low CHO intake (average: 3.2 ± 1.2 g/kg) Majority of players had insufficient macronutrient and EI
Leão et al. 2022 [99]	Elite	14	Repeated measurement	Evaluate training load and EI	Average EI: 1764 ± 495 kcal Average EA: 38.9 ± 13.2 Mismatch between self-reported EI and training load
Luszczki et al. 2021 [100]	Youth (13-18 years)	34	Cross-sectional	Assess prevalence of Triad and REDs	64.7% at risk for the Triad (LEAF-Q) Lower EI in “at risk” vs “not at risk” players Athletes with EI below the recommendations had increased risk of the Triad/REDs
Magee et al. 2020 [83]	Division 3, College	18	Prospective cohort	Prevalence of LEA and utility of the LEAF-Q	Average EI: 1806 ± 264 kcal LEA: 66.7 % (average EA: 23.0 ± 5.7) 56.3 % at risk of LEA (LEAF-Q) Overall high prevalence of LEA Low overall nutritional knowledge
Morehen et al. 2021 [46]	Professional	24**	Prospective cohort	Quantify EI, EE, and EA	Average TEE (DLW): 2693 ± 432 kcal Average EI: 1923 ± 367 kcal LEA: 88% (average EA: 18 ± 9) Low CHO intake (average: 3.3 ± 0.7 g/kg) High prevalence of LEA and little nutritional periodization
Moss et al. 2020 [13]	Professional	13	Cross-sectional	Measure EA and relationship with associated risk factors	Average EI: 2124 ± 444 kcal Average EEE: 418 ± 140 kcal/d LEA: 23 % (average EA: 35 ± 10) 23% at risk for LEA (LEAF-Q) with additional low RMR ratio Low CHO intake (average: 3.3 ± 0.6 g/day) Inconclusive relationship between EA and risk factors Most players displayed reduced EA and no nutritional periodization

Parker et al. 2022 [101]	Professional	1	Case study	Describe the challenges faced in developing LEA	Average TEE (DLW): 2062 kcal/d No LEA despite amenorrhea and anovulatory menstrual cycles Highlights that menstrual irregularities are not necessarily caused by LEA Important that players feel comfortable discussing menstrual status with practitioners to support performance and health
Prather et al. 2015 [102]	Elite	220	Cross-sectional	Determine prevalence of stress fractures, menstrual dysfunction, and DE	8 % history of low limb stress fracture 7.7 % at intermediate risk for eating disorders (EAT-26) EAT-26 score ≥ 10 had higher prevalence of menstrual dysfunction
Reed et al. 2013 [51]	Division 1, College	19	Repeated measurement	Examine change in EA across the season	LEA: 26% pre-season, 33% mid-season (35.2 ± 3.7), 12% post-season (44.5 ± 3.7) LEA was due to low EI during lunch and dinner EA inversely related to body dissatisfaction and drive for thinness Most players not at risk for LEA Higher prevalence of LEA during season
Reed et el. 2014 [103]	Division 1, College	19	Repeated measurement	Examine nutritional practices associated with LEA	CHO intake was lower in mid and post-season, compared to pre-season 100 % of LEA athletes did not meet the recommended CHO intake mid and post-season (average: 5 ± 1 g/kg) Identifying inadequate CHO intake may prevent LEA

**= Level of participants is unclear or doubtfully described. ** = Number of participants during specific segments of analyses differ. CHO = Carbohydrates; Energy availability (kcal/kg^{-1} FFM/day^{-1}) = EA; Energy intake = EI; Energy expenditure = EE; Total energy expenditure = TEE ; Low energy availability in females questionnaire = LEAF-Q; Resting metabolic rate = RMR; Low energy availability = LEA; EAT-26 = Eating attitudes test 26.*

The field of LEA and REDs research has predominantly focused on leanness and endurance sports, as the prevalence of REDs is considered to be higher in athletes competing in sports where body mass may be critical for optimal performance.[8,104,105] **Table 1** provides a summary of studies that have investigated REDs, EA, or the Triad, specifically focusing on female footballers. Few studies have focused on identifying concomitant REDs indicators. Instead, the prevalence of standalone symptoms like RMR, BMD or psychological variables has been investigated, with some exceptions, rather than the prevalence of clustered REDs indicators, which ultimately defines the syndrome. Based on the current literature, the prevalence of LEA among female football players is reported to range from approximately 20-80%, depending on the measurement methods applied.[13,46,50,51,84,103] Hence, despite estimates on the prevalence of LEA, the knowledge of how these transfers to manifestation of REDs indicators remains rudimentary.

The football season commonly consist of three periods: pre-season, competition, and transition/offseason, commonly eliciting different physiological loads.[106] Several studies have shown that the prevalence of LEA and associated risk factors described by the REDs model change throughout the season.[13,51,103] However, as the competition phase usually lasts 9-10 months, this is indeed the period of highest importance, encompassing the majority of the annual calendar. Moss et al. reported that 23% of players had in-season LEA, with several players also eliciting adverse health symptoms consistent with REDs.[13] Similar findings were also reported in collegiate football players, with LEA observed in 33% of the players, and being inversely related to drive for thinness and body dissatisfaction.[51]

A recent study by Morehen et al. reported considerable underfueling in relation to EE, especially in terms of CHO.[47] Although underreporting of EI is to be expected from athletes,[76] low CHO availability, indeed, makes football players vulnerable to develop LEA, possibly resulting in REDs.[46,76] Despite advancements in research regarding nutritional recommendations, there is still many unanswered question. While current research posits that no difference in dietary recommendations between male and female football players are needed,[21] the body of evidence is scarce.[46] Moreover, queries pertaining to substrate metabolism in female athletes remain undecided.[107] For example, females have been shown to rely more on fat metabolism than men at the same relative level of exercise intensity.[108] Thus, more research utilizing high quality methods across different levels of competition is opportune.

Investigations into LEA among female football players highlight the challenge in understanding its short and long-term health implications. Again, Morehen et al. reported that 88% of international professional female football players were categorized with LEA, using the threshold of < 30 kcal/kg^{-1} FFM/day^{-1}.[47] Similar findings have been demonstrated in other studies, but direct comparisons across studies are difficult as a result of different measurement techniques to quantify LEA.[13,103] Historically, football has not been considered a high-risk sport in terms LEA and REDs.[8] However, the recent findings regarding the prevalence of LEA among female players, suggest it might be higher than previously believed.

Despite several studies investigating the prevalence of LEA in female football players, there are few studies investigating the occurrence of indicators for REDs in this population. As outlined in the REDs model, there are several potential health consequences that may manifest themself with or without DE/ED.[27] These include attenuated BMD, menstrual dysfunction, hormonal dysregulation, metabolic alterations, and psychological disturbances. A recent study in male and female Kenyan endurance athletes showed that the prevalence of Triad-REDs symptoms was not higher in the LEA group, compared to the control group.[79] As such, there is a need to investigate the occurrence of valid indicators of REDs beyond the measure of LEA by itself, as this is what ultimately compromises athlete health.

Health consequences of LEA and REDs

The potential health consequences outlined in the REDs model (**Figure *1***) may alter a range of body systems including bone and menstrual function, as well as immunological, endocrinological, metabolic, hematological, psychological, cardiovascular, growth and development and gastro-intestinal functions.[8,27] While the REDs model highlights specific body systems as independent health consequences following LEA, these systems are interconnected, often eliciting subsequent reactions. The human body as an organism, adapts to internal and environmental stresses via complex biochemical processes, which are manifested through these systems.[109] Apart from growth and development', as well as gastrointestinal and hematological function, all these health consequences are explored in the present thesis.

Menstrual function

The normal menstrual cycle usually lasts between 21-35 days and individuals falling within this category is referred to as being eumenorrheic (EUM).[110] [111] The menstrual cycle consists of (i) the follicular, (ii) the ovulatory , and (iii) the luteal phase, respectively. The follicular and luteal phases can further be specified for extensive nuance.

In cases of severe or prolonged LEA (> 3 months), physiological adaptations may take place, attempting to preserve energy for the most important physiological processes, where reproductive function is not prioritized.[26,112,113] This occurs through the downregulation of metabolic pathways, which subsequently may result in alterations to the hypothalamic-pituitary-gonadal axis.[26,114,115] Consequently, energy will be shifted towards metabolic processes such as locomotion, energy conservation, thermoregulation and cell maintenance.[115] This may lead to functional hypothalamic amenorrhea (AME), also referred to as secondary AME or oligomenorrhea.[116] Given the underlying etiology of exercise induced menstrual disturbances, it is natural that individuals competing in sports eliciting high metabolic demands in terms of EE are more susceptible to LEA, compared to sports with lower levels of EE.[67,117] Nevertheless, as LEA can also be induced by decreased EI, often combined with ED or DE, the knowledge regarding prevalence and factors influencing this is important, including traditionally non-weight sensitive sports.[27,92,118]

Hormonal contraceptives

Hormonal contraceptives (HC) are exogenous hormones that primarily inhibit ovulation and alter endogenous production of sex hormones.[119] The various HC methods comprise oral contraceptive pills, intrauterine systems, implants, injections, and transdermal patch, and they may be mono, bi or triphasic, eliciting different hormonal milieus.[120] The useage of HC's has been reported to be higher in athletes compared to the general population, with a prevalence of ~ 50%.[121] However, a recent review of HC use in the English super league found that only 28% of the players were currently using a HC agent.[122]

The menstrual function is considered pivotal in the clinical assessment and diagnosis of long-term LEA.[123] In non-HC users, alteration of normal bleeding pattern is considered the principal indicator.[8,30,123] Usage of HC may alter or completely cease menses, potentially masking the presence of LEA.[124] Considering the high prevalence of HC usage among athletes, this constitutes a challenge for health care practitioners and researchers working with LEA in this population. It also emphasizes the need for continued development of "objective" indicators of LEA and REDs.

Endocrinological function

While numerous studies have reported hormonal alterations linked with LEA and REDs, the inherent challenges with accurately measuring LEA may influence the consistency of results across studies.[25] As such, caution should be exercised when interpreting hormonal results as direct indicators of REDs or LEA, since limited causal relationships have been firmly established.[67,114] However, certain hormonal markers have repeatedly occurred in response to LEA and could potentially serve as future indicators of REDs. These include but are not restricted to leptin, insulin-like growth hormone 1 (IGF-1), thyroid hormones and cortisol.[114]

Early detection of LEA is of importance since long-term exposure may elicit more severe symptoms like secondary amenorrhea and attenuated BMD. [67] Therefore, hormonal markers are seen as a potential mechanism of early detection, before these pronounced symptoms manifest themselves.[67,114] Laboratory studies, with brief exposure to LEA have provided marked changes in the hormonal milieu.[63,125,126] For example, in a laboratory study on female participants, a positive correlation in circulating leptin was found with decreasing LEA.[63] Other studies have found influences on triiodothyronine (T_3), glucose, and insulin.[67,114] The seminal EXCALIBUR studies by Loucks et al. have also consistently demonstrated that T_3 is attenuated in response to LEA, which is likely the hormone most closely linked with LEA.[62,63,127]

Bone health and metabolism

For athletes, low BMD may manifest itself as changes in bone microarchitecture and strength, and may increase the risk of injuries such as stress fractures and in worst case scenarios, osteoporosis.[70,128] Bone consists of three major cell types, i.e., osteocytes, osteoclasts, and osteoblasts, with the latter accounting for more than 90% of bone cells. These three cell types coordinate cell resorption and remodeling.[129] Indeed, bone remodeling is influenced by mechanical loading, and thus, athletes participating in weight-bearing sports with high levels of impact (e.g., football) seems to have lower risk of bone related injuries, compared to non-weightbearing sports.[70,130,131]

Identification of low BMD in athletes in normally done using age-matched Z-scores in premenopausal women. The term “below the expected range for age” has been defined as Z-score < - 2 by the International Society for Clinical Densitometry,[132] while the American College of Sports Medicine (ACSM), in their position stands, defines low BMD as < -1 for athletes.[27,30] Nevertheless, there has been a recent debate regarding the BMD Z-scores applied in sports medicine.[133] Athletes experiencing high amounts of mechanical loading are expected to have elevated BMD compared to controls. Consequently, utilizing the same Z-score of < -1 might mask potential consequences elicited by LEA.

Dual-energy X-ray absorptiometry (DXA) is normally applied to quantify BMD and calculate Z-scores.[8,26] Few studies investigating LEA have applied markers of bone remodeling and absorption to compliment DXA scores, which may further enhance the understanding of the athletes’ bone health. N-terminal propeptide of type 1 Procollagen (P1NP) and C-terminal telopeptide of type 1 collagen (CTX-1) have previously been identified as the most promising bone turnover markers when assessing loss in BMD,[134] and provide interesting options in relation to measurement of bone health in athletes. These markers, however, have not yet been much applied in the sports medicine literature.

Energy metabolism

As described by the REDs model, metabolic function may indeed be altered as a result of LEA.[8] In response to an energy deficit, the body may respond in a number of ways in order to minimize this shortfall and thus, attenuate excessive weight loss.[95] These mechanisms include hormonal alterations in thyroid hormones, which are important for the regulation of whole-body thermogenesis.[135] Other mechanisms involved in regulating metabolic processes include leptin, insulin and ghrelin, all serving as potential indicators of EA status.[95,136,137] RMR have consistently been applied as a surrogate marker for LEA in the literature, as it has the potential to adequately reflect the metabolic state of the body.[138] This is usually applied through $\text{RMR}_{\text{Ratio}}$, which is calculated by dividing an individuals measured RMR by a predictive value, based on an equation factoring in variables such as age, height, body mass and FFM.[138] In females, a value < 0.90 is usually considered indicative of LEA.[139] Another method is to estimate EA through RMR by dividing measured kcal/day^{-1} by FFM, in scenarios where direct measure of EA is not possible or deemed inaccurate.[75,80,140]

TDEE consists of several components, where the basal metabolic rate (BMR) constitutes the largest contribution, and is often proportional to body size.[141,142] Other contributions to TDEE are non-resting energy expenditure which comprise of EEE, non-exercise activity thermogenesis, and thermic effect of food (TEF).[95] Excessive TDEE, resulting in energy deficiency may also drive adaptive thermogenesis, a homeostatic process where energy is preserved in order to decrease EE and thus increase metabolic efficiency.[143] In an evolutionary perspective, this is an important mechanism as prodigious TDEE in times of food scarcity may have catastrophic outcomes.[144,145] In situations where athletes have been susceptible to long term or severe LEA, adaptive thermogenesis may also result in weight stability, despite inadequate EI in relation to EE, making weight monitoring a poor indicator.[25,145] Thurber et al. recently proposed an alimentary limit on sustained maximal human energy expenditure of 2.5 x basal metabolic rate (BMR), using DLW data from various demographics.[142] These data suggest that although TDEE may drive adaptive thermogenesis, this is likely a result of the inability of individuals to increase EI sufficiently. Given the available evidence on the energetic demands of football, it is unlikely that excessive EE is the primary driver of LEA and REDs, as most players display low to moderate PAL,[17,18,46] however, limited evidence exist in female football players.

Immunological function

There is evidence that the immune function may be altered by LEA, causing increased likelihood of illness and consequently time-loss from training and competition.[146,147] A cross-sectional survey of Olympic athletes found that athletes with LEA, as measured through the LEAF-Q, had increased risk of time-loss, compared to non LEA athletes.[146] Further, a study completed on healthy adults and elderly individuals reported that acute fasting decreased neutrophil function in both populations.[148] Although no causal inference to LEA is evident, the immune response caused through acute energy deficiency by decreasing neutrophil activity may well play a role in athletes with long-term or severe LEA. There is also evidence that AME athletes may elicit accelerated downregulation of mucosal immune function through decreased levels of salivary secretory immunoglobin, increasing their risk of upper respiratory infections.[147] While more research is needed to elucidate the mechanisms that compromises immune function in athletes with LEA, the observed time-loss of athletes in this group may serve as an interesting and important marker when screening for LEA/REDs.

Cardiovascular function

In athletes, there is some evidence supporting that LEA may affect cardiovascular health.[149] However, it is a well-established occurrence of cardiovascular abnormalities, including bradycardia, hypotension and dysregulation of vascular contractility among patients with anorexia nervosa.[150] The mechanisms behind increased cardiovascular risk in patients with anorexia nervosa is still unclear.[151] Nevertheless, LEA and anorexia nervosa share similar pathophysiological characteristics, as both conditions are driven by an energy deficiency, although LEA may be present with or without psychological problems.[152] Hence, research on anorexia nervosa patients may provide important information on the potential severe consequences of LEA. It have been reported that female athletes with AME may display decreased endothelial function, as well as increased levels of total cholesterol and low density lipoproteins (LDL), compared to EUM athletes.[153] This indicates that analogous to anorexic patients, cardiovascular function may be compromised in athletes with severe LEA. While interesting, the sensitivity of these markers across diverse sports and athlete profiles remains to be thoroughly established.

Psychological factors

Within the REDs model (**Figure *1***), a bidirectional arrow illustrates that psychological distress can either precede the onset of REDs or emerge as a consequence of it.[27] Severe LEA or amenorrhea is associated with pronounced psychological distress,[154,155] as underscored by multiple studies noting diminished mental well-being in athletes identified with REDs.[118,156,157] Although There is a link between REDs and poor mental well-being, the understanding of the psychological precursors leading to the development of REDs remains rudimentary.[27]

Evidence seems to suggest that the prevalence of ED is higher in elite athletes compared to non-athlete populations, as well as higher in females than males.[158–160] As many sports require body compositions with low fat mass (%), in order to perform at the top level, this may increase the risk of psychological distress among certain athletes (i.e., weight class sports).[161,162] In football, most research exploring psychological factors in relation to LEA or REDs have centered around DE/ED, generally reporting low prevalence numbers.[102,163,164] However, Sundgot-Borgen & Torstveit found a prevalence of 24% for ED among football players using clinical interviews.[165] This highlights the importance of methodological considerations and inherent variations within expansive groups like football players, when conducting research.

Decreased sleep quality have been linked to athletes with LEA and/or REDs, and may have negative consequences for general mental health and wellbeing.[27,156,166] These include depression and mood changes, which have been linked to REDs in previous studies, and are highlighted as a possible ramification in the IOC consensus statement.[27,167–169] As most depressive disorders are characterized by subjective sleep disturbance, the occurrence of these symptoms are likely to occur simultaneously, affecting both performance and health.[170] Overall, although multifaceted, the understanding of psychological factors and their manifested change in relation to LEA and REDs is important and need further attention.

Rationale for the thesis

Despite the surge in research focusing on EA and REDs, female football players remain underrepresented in the literature. The work that has been conducted in this population has primarily concentrated on estimating the prevalence of LEA using small sample sizes, leaving a knowledge gap regarding the health consequences of these estimates.[13,46,50,51,83,84,99,103] Of the studies also investigating health parameters in conjunction with EA, the sample size is restricted, or the provision of outcome measures is limited, resulting in numerous physiological systems outlined by the REDs model remaining uninvestigated.[13,46,51,103] Moreover, the methods employed to quantify EA have considerable limitations, which could introduce bias and inaccuracies distorting our understanding of LEA and its impact. Hence, it is opportune to examine methodological and energetic parameters related to LEA and REDs among female football players. In this context, understanding of the overall energetic requirements is crucial, as nutritional considerations deeply influence the abovementioned concepts.[21,46] The energetic understanding of female football players remains rudimentary, particularly lacking in-season data. This may potentially impact the formulation of nutritional guidelines and practical application, thereby influencing players' health and performance outcomes.

Aims

The principal objective of this thesis was to explore energetics, EA, and REDs among female football players, including a critical evaluation of research methodologies employed for this purpose. This is outlined across four distinct papers, with the following aims:

1. To examine the accuracy of commonly used tracking devices to assess EEE during intermittent exercise and evaluating the subsequent implications for quantifying EA *(paper I)*.

2. To quantify the overall energetic requirements of women's football including TDEE, EI and EA *(paper II).*

3. To assess the applicability of the LEAF-Q as a screening tool in female football players *(paper III).*

4. To determine the prevalence of indicators associated with REDs in female football players, with a secondary focus on variation across player positions and menstrual function (EUM vs AME) *(paper IV*).

Arrangement of papers

The arrangement of papers in this thesis follows a logical sequence, based on the methodological considerations and applicability of various measurement instruments. *Paper I* serves as a foundation for understanding the accuracy of measured EA in *paper II*, as well as the level of bias that could be expected from prior studies estimating EA. Considering that the LEAF-Q is widely used in studies across various athletic populations, it would seem fitting to apply this as an indicator associated with REDs. However, due to the difference between football and the sports for which the LEAF-Q were validated, we first found it necessary to evaluate the questionnaire's applicability (*paper III*). Results from *paper 1* guided the choice of tracking devices to be applied in *paper IV.*

Methods

Participants and ethics

The total study cohort consisted of 60 football players from three teams in Norway, specifically two teams in the Norwegian premier league (Toppserien) and one team from the Norwegian first division (it should be noted that the team competing in the first division was promoted to the premier league in the following season). All participants were classified as tier 3 (national level) or tier 4 (international) level.[171]

Paper 1 included 13 players from the study cohort all competing in the Norwegian premier division. *Paper II* included 51 players from both leagues, while *paper III* and *IV* included all 60 players in the cohort. Anthropometric measurements and demographic information for the whole cohort is presented in **Table 2**. Further information about the sub-groups is provided in the individual papers (*I-IV*).

Table 2. *Age and anthropometric characteristics of participants included in the studies.*

Age	22.5 ± 3.7
Body mass (kg)	64.1 ± 6.3
Height (cm)	168.9 ± 6.0
Fat mass (%)	24.7 ± 4.2
Fat free mass (kg)	49.3 ± 4.7
Body Mass Index (kg/m^2)	22.4 ± 1.7

Ethics

Written consent was obtained from all participants included in the studies. The entire research protocol was approved by the Norwegian Center for Research Data (807592) and published in order to provide full transparency.[172] The Regional Committee for Medical and Health Research Ethics (REK), Region North, deemed the full research protocol outside the scope of the Health Research Act. However, because blood analyses were conducted at Haukeland University Hospital, Bergen, Norway under the jurisdiction of REK, Region West, we collected their approval (2016/787) for the project related to the collection, storage, and analyses of blood and urine samples relevant for *papers 2-4.*

Ideally, all participants should have been non-HC users, as the usage of HC's may potentially attenuate or mask symptoms associated with LEA or REDs.[124] Consistent with previous findings, more than 50% of our participants used HC, mainly for contraceptive purposes.[122] Despite the inconvenience of HC use for the intended overall aim of the papers, it was deemed unethical to promote the discontinuation of HC usage.

Study design

The data collection can be divided into four parts. In April 2021, two teams completed the procedure that serves as the foundation for *paper I.* Between October 2021 and May 2022, three teams completed the data collection which constitutes *Papers II-IV*. For each team, the data collection period lasted 14 days, with some additional days following the main period. For each study, a brief outline is described below. A detailed explanation of study designs is provided in the individual papers.

Paper I- was completed as a cross-sectional observation study to determine the accuracy of commonly used tracking devices, one which would also be used to assess EEE in *paper II* of the thesis. Participants completed a single visit protocol measuring VO_2 during exercise (criterion measure), while simultaneously wearing various tracking devices, measuring estimated EEE. The participants completed a course on artificial grass, designed to reflect the requirements in women's football [36,47]. The course length was 549.5 meters (**Figure 4**) and was completed five times, with one minute break between each round. Excess post exercise oxygen consumption (EPOC) was measured to account for EEE derived work above VO_2 max. The tracking devices were compared against the criterion measure to determine their accuracy in reflecting EEE.

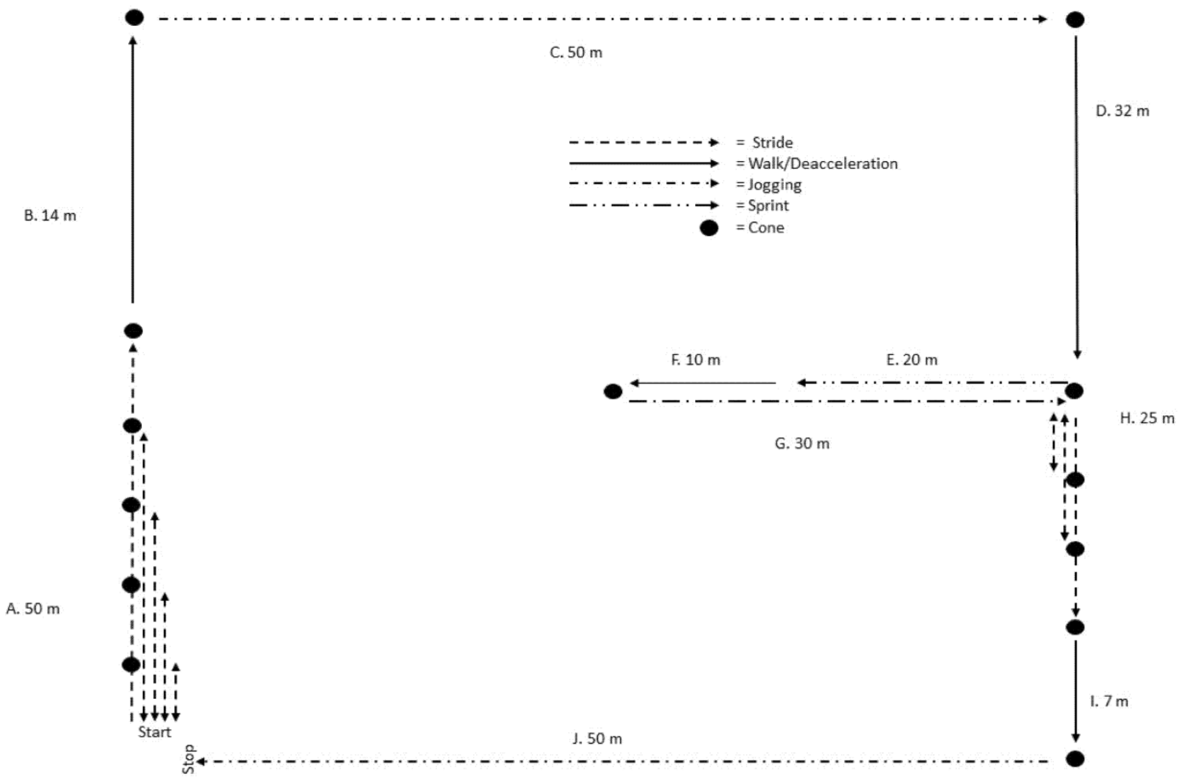


Figure 4. *Illustration of the intermittent exercise protocol, indicating the type of movement, length, and order of segment (numbered A-J).*

Picture 1. *Athlete during pilot-testing of the protocol in paper I.*

Paper II- was designed as a prospective observational study aiming to quantify EE, EI, and EA. All participants ingested stable isotopes through DLW to determine TDEE, as well as quantifying dietary intake on match, training, and rest days. During the data collection period, players were instructed to continue with their normal habitual pattern to reflect the physiological response seen during a typical in-season week.

Paper III- was a cross-sectional study aiming to assess the applicability of the LEAF-Q for female football players. The published LEAF-Q cut-off values were used to group participants as *at risk* or *not at risk* for development of the Triad and LEA. These groups were then compared against physiological measures of the Triad, as well as a broader panel of indicators related to LEA and REDs.

Paper IV- was conducted as a cross-sectional investigation including all three teams. A wide range of indicators associated with REDs were used to estimate the point prevalence. These indicators were categorized as primary, secondary, or associated, based on the strength of evidence associated with REDs.

Procedures

Assessment of Exercise Energy Expenditure (Papers I & II)

In *paper I,* the participants were equipped with three wearable tracking devices (**Table 3**) estimating EEE using metabolic power.[38]

Table 3. *Tracking devices specifications*

Manufacturer and model	Specifications	Mounting position
Statsports, Apex, Newry, Northern Ireland, UK (GPS[1])	18Hz GPS, 952Hz tri-axial accelerometer, gyroscope & magnetometer	Thoracic spine between scapulae
Catapult Innovations, Vector, Melbourne, Australia (GPS[2)]	10 Hz GPS, 1KHz tri-axial accelerometer, gyroscope & magnetometer	Thoracic spine between scapulae
Playermaker™, Playermaker, Tel Aviv, Israel (IMU)	1KHz inertial sensor, 3 x tri-axial accelerometer and gyroscope	Fixed to boots

All GPS devices were placed outside in recording mode at least 20 minutes prior to testing to ensure optimal satellite connection. For testing in *paper I,* the GPS devices were securely positioned within a bespoke vest, maintaining a separation of 2-3 cm between devices, whereas the inertial sensor was fixed to the participants' footwear utilizing the included strap, all in accordance with the manufacturer guidelines. Simultaneously, participants were equipped with a portable breath-by-breath analyzer (VO_2 Master Health Sensors INC, Vernon, BC, Canada) measuring VO_2 consumption. Post-hoc comparisons were made between estimates of EEE derived from metabolic power and VO_2 data to determine the level of agreement both with and without the inclusion of EPOC. *Paper I* provides extended details on the methods and calculations of EEE. In *paper II,* EEE was derived from StatSports Apex and calculated automatically via the manufacturer software (StatSports Sonra). All training sessions and matches were subjected to post-hoc editing to ensure synchronization and inclusion of data exclusively representing the physiological work completed during exercise.

Quantification of training and match load (Paper II)

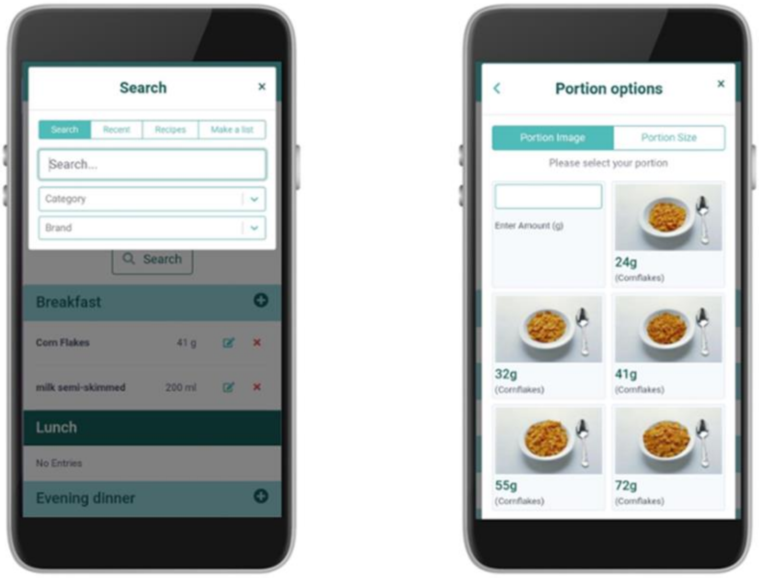
Both training sessions and match load were monitored using GPS displacement data (Statsport, Apex, Newry, Northern Ireland, UK). We applied predetermined variables to quantify total distance covered and meters in speed zone 1-5 (see *paper II* for specific speed zones) to determine physiological load. The coaching staff were familiar with the usage of GPS and applied with the guidelines from the manufacturer to attain optimal results. The goalkeepers were not included in the training and match load analysis.

Doubly Labeled Water (paper II)

The DLW method for measuring TDEE in free-living conditions was administered in *paper II*.[173,174] The protocol applied in this study was the *Maastricht protocol* and is described in detail elsewhere.[175] Briefly, individual doses with DLW were calculated from total body water, based on Body Mass Index. The players collected a baseline urine sample at home, following training, in the evening before going to bed (day 0). After collecting the baseline sample, the participants consumed a weighted amount of $2^{H}2^{O}$ and $H^{2}18^{O}$, providing a body water enrichment of approximately 155 p.p.m. for H^{2} and 235 p.p.m. for 18^{O}. Following this, urine samples were collected on day 1, 7 and 14, from the second voiding in the morning. On the same days, a second urine sample was collected in the afternoon or evening. Participants were instructed in how to collect urine samples and conducted this procedure at home using standardized urine cups. Urine samples were then stored in the refrigerator at the participants' home for maximum 24 h. A member of the research team then collected the urine sample within the given time frame. Urine samples were then immediately taken to the lab, aliquoted to a 2 mL airtight glass vial and stored in -20^{o} fridge until analysis. Urine samples were analyzed with an isotope ratio mass spectrometer (Thermo Scientific Delta V Advantage; Thermo Fischer Scientific, Bremen, Germany). Carbon dioxide production was calculated from the difference between the elimination rates of H^2 and 18^O using the equation as recommended by the International Atomic Energy Agency (IAEA) DLW database consortium.[173] TDEE was calculated from carbon dioxide production, assuming a respiratory quotient of 0.85. Readers are referred to *paper II* for extended details on the methods and application of the DLW method.

Energy intake (paper II)

EI was measured on training, match, and rest days, respectively, using the 24-hour diet recall method. The diet recalls were conducted using a single blinded nutritional analysis software, developed for research purposes, and with access to the Norwegian nutritional register (Myfood24, Leeds, UK). Specifically, at 7 a.m., participants received an automated e-mail and text message, containing a link to the diet recall. Following the initial communication, subsequent reminders were disseminated until the task was finalized. Prior to the data collection, participants had been instructed to complete the diet recall as soon as possible following receipt of the link and received training in completion of the task. To assess the average daily EI, weighted means from training, match and rest days was calculated to ensure that EI accurately reflected the differing proportions of days within the period under consideration.



Picture 2. *Picture illustrating food search and portion size determination modules on mobile phone from the Myfood24 database. Picture printed with permission from Dietary Assessment Ltd ©.*

Energy availability (Papers II-IV)

For *paper II*, EA on match and training days were calculated using the estimated EEE derived by the GPS system (Statsports, Newry, Norther Ireland) assessed during *paper I.* EA was calculated using the formula EA = (EI-EEE)/FFM.[24] Further, the estimation of average EA during the entire 14-day DLW measurement period was calculated. The TEF was assumed to be 10 % across all individuals,[176] allowing for the estimation of activity energy expenditure (AEE) through the formula AEE = TDEE – (RMR + TEF) and subsequently EA = EI – (AEE/FFM). All participants from the entire cohort did not complete the protocol in *paper II* as they did not fulfill the inclusion criteria (e.g., not free of injury). As such, paper *III and IV* quantified EA using RMR < 30 kcal/kg^{-1} FFM/day^{-1}.

Resting metabolic rate (Papers I-IV)

In *Paper 1*, RMR was measured using the VO_2 Master prior to completion of the testing protocol. This was done as the mean VO_2 (L/min) was subtracted from the total EEE measured to strictly quantify EE derived from activity above rest. Participants wore the device for 10 minutes laying down in a supine position in a dimmed room at the training facility where the testing took place. For *Paper III* and *IV,* RMR was measured with a canopy system using the Vyntus CPX (Carefusion, Höchberg, Germany, Sentrysuit v. 2.21.4). The measurements were completed with participants arriving the testing facility between 06 – 09 a.m. in an overnight fasted state, by motorized transportation. Participants were placed in a quiet and dimmed room, laying in the supine position for 5 minutes, before a ventilated canopy hoodie was positioned. VO_2 and carbon dioxide production were then measured for 25 minutes, where the average value for the last 20 minutes was used to determine RMR.

Body composition, bone mineral density, and anthropometrics (Papers I-IV)

In *paper II, III* and *IV,* body composition and BMD was measured using DXA (Prodigy, Encore, SP 4.1, Version 18, GE Medical systems, Madison, Wisconsin, USA) according to best practice guidelines,[177] using the NHANES database. The DXA was calibrated with a standard phantom supplied by the manufacturer each day before starting the measurements. All participants were instructed to arrive the measurement in an overnight fasted state, and preferably, avoid strenuous exercise the day prior to testing. Further, they were encouraged to void and defecate after arriving the testing facility before starting the measurement. The measurement was completed with participants wearing minimal clothing (e.g., t-shirt and tights/shorts) and positioned in the supine position, ensuring the body was properly aligned with the central longitudinal axis of the scan table. Both arms were positioned alongside the body, in neutral position to minimize overlapping of anatomical structures. All measurements were completed using the standard mode and auto-analysis provided by the software and manually adjusted if necessary. First, a total body scan to assess the body composition was conducted, followed by an anteroposterior scan of the lumbar spine (L1-4) and hip (femoral neck). The same certified technician completed all DXA measurements to avoid inter-rater variability. Low BMD was defined as Z-score < -1.0.[30] In *paper III*, the usage of Z-score < 0 was also utilized to explore the applicability of the LEAF-Q questionnaire. To measure body composition in *paper I,* multi-frequency bioelectrical impendence (IN-body 720, Biospace, Tokyo, Japan) was applied, following the manufacturer guidelines.[178] In brief, participants were instructed to stand barefoot on the device, holding on to the handles with arms in approximately 40 ° for the duration of the measurement.

Blood markers (Paper III and IV)

Venous blood samples were analyzed in *paper III and IV* for both plasma and serum. The samples were completed with participants arriving the hospital outpatient clinic (Haukeland, laboratory for blood analysis) in a fasted state between 7-10 a.m. All analytes were assayed at the Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway. The laboratory is accredited in compliance with ISO 15189:2012. Glucose, Total-cholesterol, and LDL were analyzed using Cobas 8000 c702, whereas thyroid-stimulating hormone (TSH), T_3, free thyroxine (T_4), and ferritin was assayed using Cobas 8000 e801. CTX-1 and P1NP were assayed using Cobas c602. Insulin and IGF-1 were analyzed using Immulite 2000 XPi, whereas leptin was assayed using an enzyme-linked immunosorbent assay kit (Mediagnost Cat#E07, RRID: AB_2813737) (not accredited analysis). Serum cortisol was analyzed using an in-house-developed high-performance liquid chromatography-tandem mass spectrometry.[179] For *paper III* and IV, reference ranges were adopted from Haukeland laboratory for blood analysis and can be found in the specific papers as well as the laboratory's overview.[180]

Self-reported outcomes (Paper III and IV)

During the data collection, a panel of questionnaires were administered for all participants. These were administered after participants completed RMR and DXA measurements, while receiving breakfast. In *Paper III,* the participants completed the LEAF-Q.[82] Participants were classified as *at risk* (total score ≥ 8) or *not at risk* (total score < 8) for the Triad and persistent LEA, according to the original publication.[82] Additionally, cut off values for the subcategories; injury (≥ 2), gastrointestinal function (≥ 2) and menstrual function (≥ 4), all associated with increased risk for development of Triad and LEA dysfunction in the original publication was applied. In *paper IV,* participants underwent assessments for various conditions, including the Eating Disorder Examination Questionnaire 11 (EDE-Q-11),[181] the General Health Questionnaire (GHQ),[182] the Bergen Insomnia Scale (BIS),[183] the Chalder Fatigue Scale (CFS),[184] as well as an adapted version of the Oslo Sport Trauma Research Center Questionnaire (OSTRCQ).[185] These questionnaires were administered to evaluate behaviors associated with DE/ED, symptoms of distress related to anxiety and depression, problems with initiating and maintaining sleep, physical and mental fatigue, as well as time loss from training and competition. Additionally, a non-validated custom-made questionnaire were completed by the participants, examining the eating habits in relation to training and matches, as well as specific questions about a history of stress fractures. All questionnaires were completed with a portable tablet (Ipad pro, Apple Inc, California, USA) using a digitally encrypted platform (Nettskjema, University of Oslo, Oslo, Norway).

Quantification of REDs indicators (Paper IV)

In *paper IV* the prevalence of indicators associated with REDs was assessed. To do this, indicators were categorized as primary, secondary, or associated, based on the strength of evidence associated with REDs and LEA from previous literature. Primary indicators included: secondary amenorrhea (based on self-reported menstrual status from the LEAF-Q),[63] low levels of free $T_{3,}$[114] elevated score on the EDE-Q-11,[181] BMD Z-score at the hip or lumbar spine (L1-4) < -1,[30] and history of stress fracture.[186] The secondary indicators included: low levels of IGF-1,[114] blood glucose,[187] TSH, [188] elevated LDL,[153] and major time loss from both training and match participation caused by illness/sickness measured by the OSTRCQ.[185] The associated indicators included: low RMR (defined as < 30kcal/kg^{-1} FFM/day^{-1} [139,189]), ferritin,[190] leptin,[114] free T_4,[114] P1NP,[134] ; elevated total cholesterol,[191] CTX-1,[134] and cortisol.[192] These indicators where then scored dichotomously (positive = 1, negative = 0) to provide a cumulative score index, indicating individual risk of REDs.

Statistical analyses

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 26 (IBM, Armonk, NY, USA) (*paper I & III),* the open software Jeffrey's Amazing Statistics Program (JASP) 0.16.4 (*paper II & III),* and the open software R project for statistical computing 4.3.1 (*paper IV).*

Paper I

In *paper I,* we applied a two-way mixed intraclass correlation coefficient (ICC) model to determine the level of agreement between tracking devices and the criterion measure, reporting both absolute and relative agreement.[193] Paired sample t-tests were used to examine mean differences between the measurement devices, indicating the level of bias. Effect sizes were reported using Hedge's g correcting for small sample sizes.[194,195] Lastly, a linear regression model was used to examine the possible presence, level, and direction of systematic bias.

Paper II

Weighted means were calculated to determine the average EI. Specifically, EI on training, match and recovery days were multiplied by the respective number of days represented within data collection period, providing weighted total EI for each category. The sum of these were then divided by the total number of days, yielding the overall average EI. To evaluate if nutritional periodization was applied in accordance to changes in physiological load, the difference between TDEE and EI, training, and match load, as well as the difference between energy and macronutrient intake on match, training and rest days were analyzed using Student t-tests, with post-hoc corrections to account for familywise error. Previous findings indicate that positional differences elicit disparate physiological needs.[34,196] The mean EI, as well as positional differences for TDEE and EI were examined using one-way analysis of variance (ANOVA) or a generalized linear model. Lastly, we applied a linear regression model to examine if player position, indicating the physiological load elicited during match and training, or other physiological variables known to influence EE would have the greatest impact on TDEE.

Paper III

Welch's test with post-hoc corrections were applied to compare the participants classified as *at risk* and *not at* risk by the LEAF-Q. Receiver operating curves (ROC) analyses to estimate the area under the curve (AUC) were conducted to examine the accuracy of the LEAF-Q in determining the presence of clinically defined markers of the Triad and persistent LEA. Youden's index were also calculated to indicate the most optimal cut-off scores for this cohort, and to propose alternative cut-offs, where applicable.[82,197]

In the case of significant AUC values, precision recall curves (PRC) were calculated. PRC's can be informative for binary classification problems (*at risk/not at risk* in this dataset) if the dataset is imbalanced, usually manifested by negative cases outweighing the positive.[198] As ROC curves do not account for the positive predictive value (PPV), they may present an overly optimistic view faced with imbalanced datasets, since the specificity will not be affected by the skew.[199] The PRC is based on the PPV and thus, may give us additional information about the prediction of future classifications as they evaluate the portion of true positives among positive predictors.[198]

Paper IV

Here, we calculated the point prevalence of indicators associated with REDs using the formula $\frac{number\ of\ cases}{population\ size\ for\ assessment}$ = prevalence, for each indicator. The cohort was analyzed as one, before being stratified into sub-groups based on player positions and menstrual status and analyzed using either the Welch's test, t-test, ANOVA, chi square test, or Pearson's correlation

Results

The following section summarizes the overreaching findings. Extended details are provided in *papers I-IV.*

Paper I: Accuracy of microtechnology to assess exercise energy expenditure in professional female football players: Implication for quantifying energy availability.

All tracking devices significantly underestimated the EEE compared to the criterion measure (EEE_{VO2}). By adjusting the EEE measurement through subtraction of EPOC (resting periods) the remaining degree of underestimation was no longer statistically significant. Unstandardized residuals from the regression analyses, using mean centered values showed that the underestimation was 0.42, 0.85 and 0.28 kj per unit increase in in VO_2 consumption, estimated by GPS^1, GPS^2 and IMU (devices), respectively. Extracting EPOC from the calculation produced lower unstandardized residuals indicating an underreporting of 0.23, 0.66 and 0.11 kj per unit increase for the GPS^1, GPS^2 and IMU values, respectively. The regression analyses indicated an inverse relationship between all tracking devices and the criterion measure as total EEE increased (**Figure 5**).

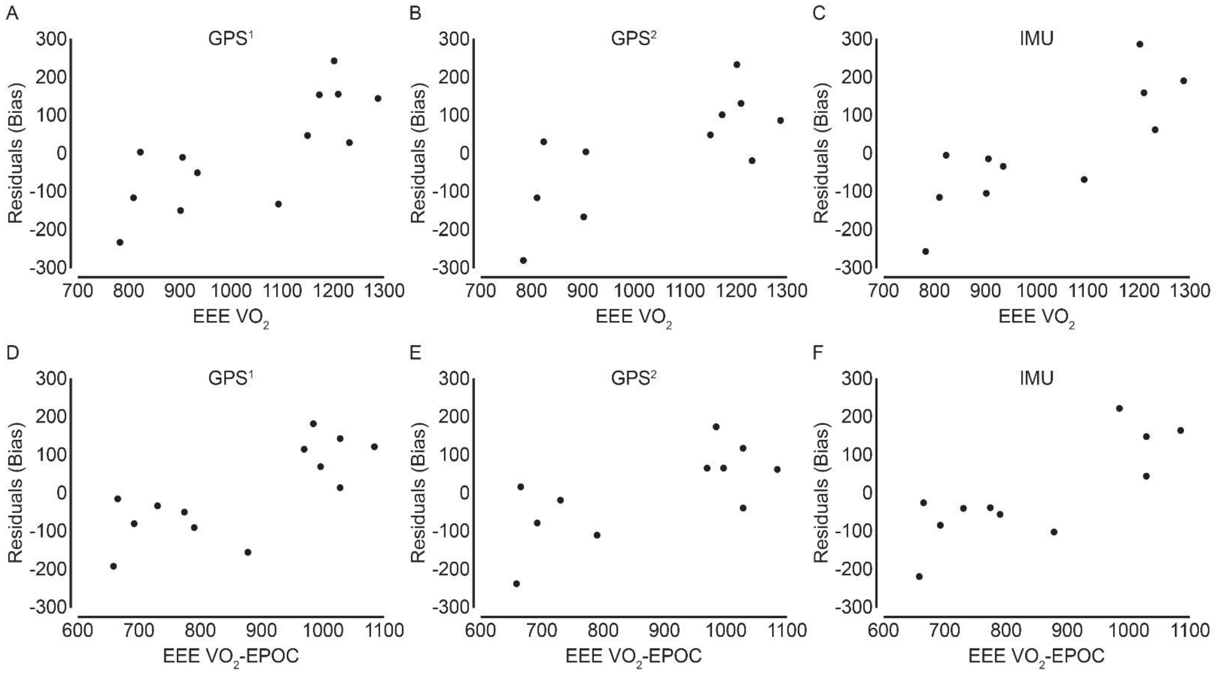


Figure 5. *Residual plot indicating the difference between the predicted EEE and the measured EEE_{VO2} in the upper panel and EEE_{VO2}-EPOC in the bottom panel (values displayed in kj). Negative values indicate overestimation and positive values indicate underestimation. Exercise energy expenditure = EEE; Kilojoules = kj; Oxygen consumption = VO_2;* Excess post-exercise energy consumption = EPOC.

Overall, the percentage error for EEE was 10.7%, 20.6% and 14.5%, for GPS^1, GPS^2 and IMU, respectively. When removing EPOC measurements from the result by subtracting rest periods, only measuring moving time, the percentage error was 7.2%, 3.1% and 3.7% for GPS^1, GPS^2 and IMU, respectively.

Paper II: Energy expenditure, dietary intake, and energy availability in professional female football players

We found a statistically significant difference in intensity across all speed zones (1-5) on match vs training days ($p < 0.001$) and for total distance ($p < 0.001$). The average TDEE was moderate (2918 ± 322 kcal) based on the corresponding measured PAL, which was 2.0 ± 0.3 (see Paper II for specific PAL values). Furthermore, we found a significant linear relationship between TDEE and weight, FFM, height and RMR. During the measurement period, no significant change in body mass occurred, suggesting an underreporting of ~ 22%, on average. There was a significant difference in EI on match days (2468 ± 843 kcal) compared to rest days (2195 ± 834 kcal), but no difference between training (2247 ± 485) vs match, or training vs rest days, respectively. For CHO, there was a significant difference for match vs training days and match vs rest days, but not for training vs rest days. Protein and fat intake was similar across match, training, and rest days, with no statistical difference. In terms of player positions, no statistical difference was found for energy and macronutrient intake.

Energy availability derived from EEE showed that the average EA was nearly akin on match and training days (36.7 ± 17.7 vs 37.9 ± 11.7 kcal) and the prevalence of LEA was 36 and 23%, respectively. In terms of average EA, the prevalence was high (74%), with a mean value of 21.6 ± 10.7 kcal.

Paper III: Evaluating the suitability of the Low Energy Availability in Females Questionnaire (LEAF-Q) for female football players.

The mean overall LEAF-Q score was 7.0 ± 3.0 (≥ 8 indicates high risk of LEA/Triad), whereas the mean values for the subcategories were 3.0 ± 2.3 (injury, ≥ 2), 2.0 ± 1.7 (gastrointestinal symptoms, ≥ 2) and 2.0 ± 2.4 (menstrual symptoms, ≥ 4), respectively. Moreover, the LEAF-Q identified 32% of participants as *at risk* for the Triad. For the subcategories, 68%, 55%, and 15%, respectively, scored above the LEAF-Q cut off value, indicating problems related to the Triad and LEA. There was no statistical difference between the groups categorized as *at risk* or *not at risk* for any physiological or biochemical measures.

The AUC analysis revealed poor performance across all clinical markers associated with LEA, except for the detection of amenorrhea. This was also the case for the PRC. The Youden’s index showed that an increase in cut-off score to ≥10 for the overall LEAF-Q would slightly optimize the overall diagnostic performance of the questionnaire.

For the LEAF-Q subcategories, the AUC index was excellent for detection of amenorrhea, and fair for detection of compromised BMD. The AUC index showed that the subcategories were inadequate in revealing any other markers associated with LEA. The PRC for amenorrhea was excellent, while the questionnaire failed to correctly identify any other symptoms associated with LEA. According to the Youden's index, an increase in cut-off score to ≥ 5 for the injury subcategory would only slightly optimize the diagnostic performance.

Paper IV: Prevalence of clinical markers associated with Relative Energy Deficiency in Sport (REDs) among professional female football players.

Of the entire cohort, 55% presented with no primary indicators. Moreover, 33 % presented with a single primary indicator, 9% with two primary indicators, and 3, % with three primary indicators. In terms of secondary indicators, 33% presented with no indicators, 42% with one indicator, and 25% with two indicators. For the associated indicators, 33% presented with none, 50% with one, 12% with two and 5 % with three, respectively.

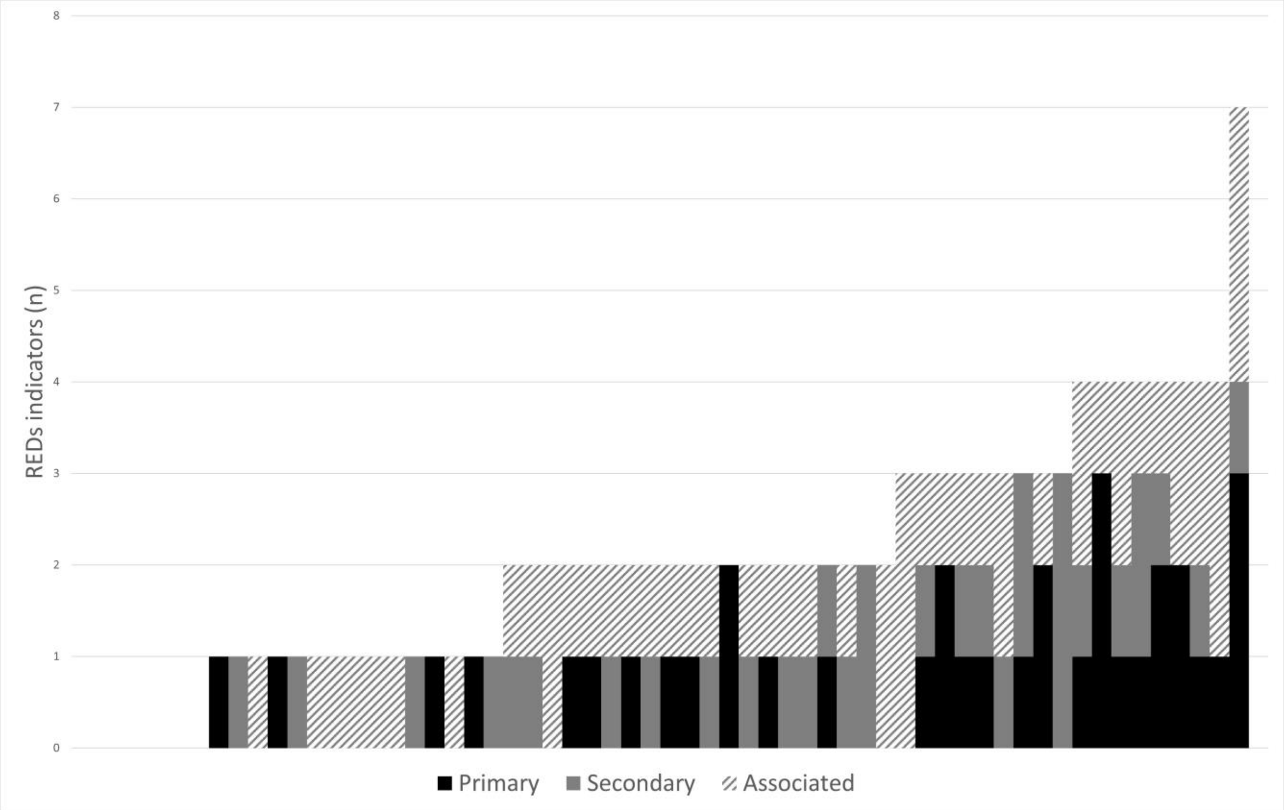


Figure 6. *Distribution of primary, secondary, and associated REDs indicators in ascending order from left to right.*

We found a significant difference in anthropometric measurements between goalkeepers and all other player positions (p = 0.035), but the players' position was unrelated to the prevalence of primary, secondary, or associated indicators of REDs. For the whole cohort we found a significant positive correlation between the EDE-Q and BIS (r = 0.33, p = 0.01), GHQ (r = 0.43, p < 0.001), and CFS (r = 0.40 p = 0.002), respectively.

The AME group displayed significantly greater number of cumulative (p = 0.002) REDs indicators, compared to the EUM group. We found no difference between the groups for any individual REDs indicators, yet the AME group consistently displayed unfavorable outcomes, compared to the EUM group (Figure 7-11). Additionally, the AME group reported higher frequency of previous stress fractures (AME 38% vs EUM 16%) as opposed to time-loss caused by sickness/illness, which was greater in the EUM group (AME 0% vs EUM 26%).

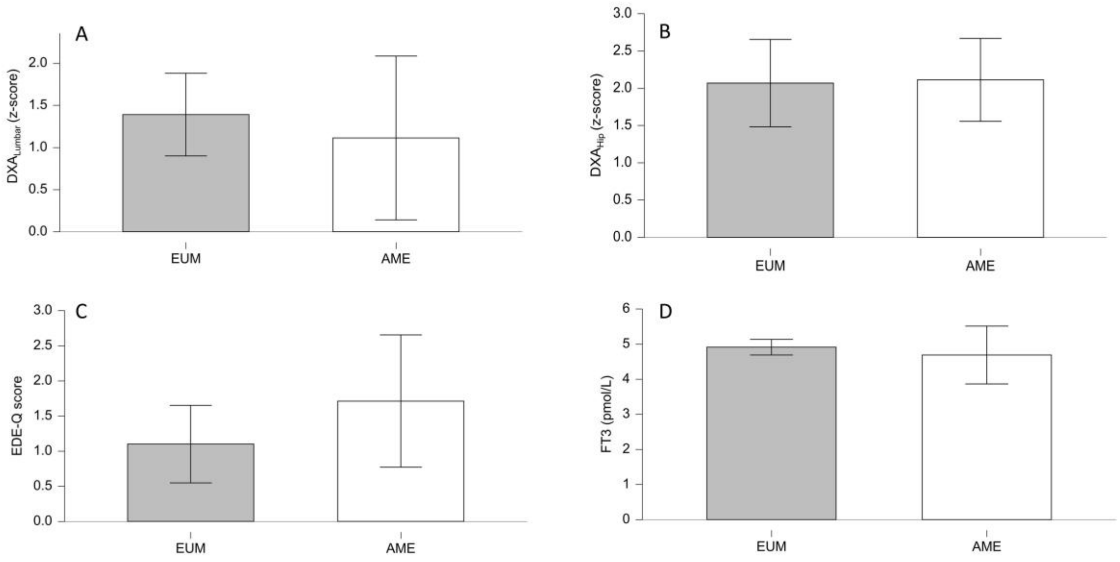


Figure 7. *Comparison of primary indicators between the AME and EUM group*

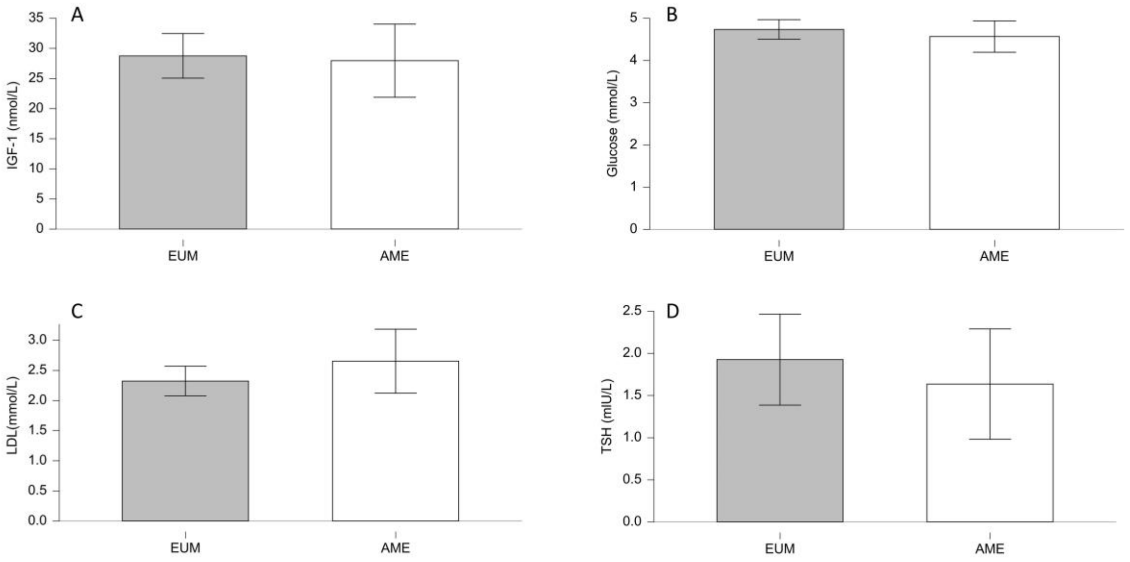


Figure 8. *Comparison of secondary indicators between the AME and EUM group*

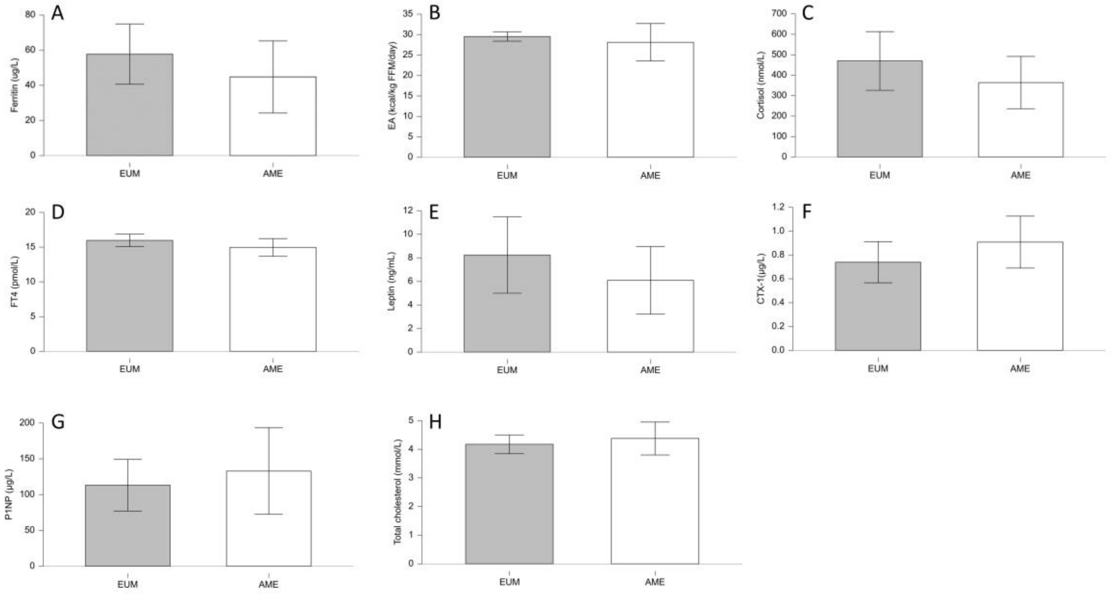


Figure 9. *Comparison of associated indicators between the AME and EUM group*

Discussion

Main findings

The aim of this thesis has been to investigate various dimensions of professional female football players' health through the concepts of energetics, EA, and REDs. Significant emphasis was also placed on evaluating the methods used and how this might influence the outcome measures.

The main findings can be summarized as follows:

- Tracking devices measuring EEE, which are required for quantification of EA, generally underestimated the caloric consumption during intermittent activity.

- The players exhibited moderate levels of EE independent of player position.

- CHO intake was generally well below the recommended levels and there was little to no nutritional periodization based on physiological demands exhibited.

- The estimated prevalence of LEA was low to moderate, depending on the calculation. The primary facilitator of LEA appeared to be insufficient EI rather than excessive EE, given the energetic requirements in football.

- The LEAF-Q is not applicable for female football players, apart from determining menstrual function. This is mainly due to the characteristics of football, compared to the sports for which the questionnaire was originally developed.

- Female football players may be susceptible to development of REDs based on the prevalence of indicators associated with the syndrome.

Measurement of energy availability in football

The last decades have seen a considerable burst in research focusing on athlete health and subsequently EA.[8,26,27,30,82] Knowledge about the prevalence, mechanisms and consequences of LEA is necessary, since adequate EA is vital for both health and performance.[27] Unfortunately, such knowledge is hampered by the fact that the measurement of EA is challenging in real word settings, as it relies on inaccurate methods.[67,75] *Paper I* investigated the accuracy of the latest available tracking devices (microtechnology), commonly used among teams, as this has direct consequences for studies investigating EA, including *paper II*. The overall accuracy of microtechnological devices was indeed poor, which may give rise to substantial discrepancies relative to the actual EEE of players.[78] Since the traditional calculation of EA relies heavily on EEE, this may partly explain diverse findings in the literature when assessing EEE in intermittent sports. Considering the inverse relationship between estimated EEE and VO_2 reported in *paper I,* this discrepancy will also magnify with an increase in total energetic cost. Consequently, games and hard training sessions will likely exhibit the largest error margins.

EI is another required component of the EA equation. Similar to EEE, measurement of EI is challenging, mainly due to subjective reporting and biases, such as recall and social desirability, with propensity towards underreporting.[76,176,200–202] One common method when assessing validity of dietary estimates is the usage of DLW, as this may serve as a reference, provided that the weight of the subjects is known.[202] Given the first law of thermodynamics, "*Energy can neither be created or destroyed, only alter in form",* the change in body weight is a direct consequence of the magnitude of disparity between EI and EE.[203] Although mechanisms like adaptive thermogenesis may cause weight stability, despite actual energy deficiency, this concept generally applies on a group level.[25,95] Thus, by introducing DLW in *paper II,* we were able, within the limitations highlighted, to quantify the amount of underreporting, which was ~ 22%. This again, highlights the limitations of the EA concept, relying on methods which are inherently imprecise.[75] The underreporting (*paper II)* was similar to what has been found in previous studies on athletes from various sports, reinforcing the validity of our results.[76]

The findings in *paper I* and *II* raise questions about the applicability of measuring EA in female football players, due to the intermittent and partly anaerobic nature of the sport, which imposes methodological difficulties for quantification of EA.[34,204]. Therefore, the time to actively pursue surrogate markers of LEA may be pertinent.[67,75,205] Recently, discussions around the universally applied thresholds defining LEA have gained traction, commonly denoted as < 30 kcal/kg^{-1} FFM/kg^{-1}.[25,67,125] The methodological challenges outlined in *paper I and II,* as well as prior audits of field-based measurements correspond well with subtle physiological differences observed in athletes, when stratified based on homogenous EA thresholds.[125,205,206] Findings from controlled laboratory studies, from which the EA thresholds are derived, may not be generalizable to elite athletes, individuals of varying sexes and adolescent populations.[67] Recent data also suggest that daily EA patterns exhibit significant day-to-day fluctuations, in dispute with the laboratory models.[66] It is therefore likely that individual responses to EA exist on a spectrum, and that uniform measurement of EA may be misleading.[93]

Energy requirements for female football players

Paper II aimed to quantify EE, EI, and EA. Our findings revealed moderate levels of TDEE, with no difference between players at a national or international level. The mean value was 2913 ± 322 kcal, corresponding to a PAL of 2.0 ± 0.3. These values are substantially lower compared to typical endurance sports, such as cross country skiing, and cycling,[97,207] yet similar to the only comparable DLW study, conducted on international level female football players.[46] In that study, Morehen et al.[46] argued that international level players possibly elicit greater EE, compared to national level players, consequently requiring higher EI. This stands in contrast to our results, indicating similar EE levels. Our results are also supported by findings from the National Women's football League (USA), showing that physiological characteristics and match performance are similar between the international and national level.[11] Hence, for international level selection, it may be the case that individual attributes such as technical and tactical skills are more important than metabolic fitness. Despite the energetic demands being moderate, the characteristics of the game inflict substantial strain on muscles, primarily through eccentric loading derived from repeated accelerations and deaccelerations.[53,208] In addition, the playing style of different teams may influence the demands imposed on the players.[35] Hence, the distribution of macronutrient intake becomes increasingly more important to obtain adequate recovery from match and training. This kind of knowledge should therefore be emphasized by team-support staff in relation to pre, peri and post-match nutrition.

In *paper II*, the mean weighted EI was 2274 ± 450 kcal. Using DLW to estimate TDEE allows for a valid reference in relation to EI.[202] Since no significant changes in body weight during the study period occurred, an average underestimation of ~ 22% was likely present. This is similar to what has been reported in previous studies on athletes.[76] Hence, most of the players likely had adequate total EI, albeit in the lower end. However, little to no nutritional periodization was observed between match, training, and rest days. As the physiological demands are considerably higher on match vs training, and training vs rest days, also apparent in *paper II*, there is a need for increased EI during periods of strenuous load, and especially congested fixtures.[21,209,210] As previously mentioned, the high eccentric component of football implies that extensive emphasis should be put on adequate CHO intake. The most recent UEFA expert group statement in elite football states that 6-8 g/kg of CHO is recommended on match day and 3-6 g/kg on training days.[21] In *paper II*, only 24 % met these recommendations on match day, while over 50% had a CHO intake of < 4 g/kg on training days. When correcting for the underreporting, assuming equal distribution of macronutrient intake, the average CHO intake was still only 5.4 g/kg on match day, with over 30% of players presenting with an intake of < 4 g/kg. It therefore seems that female players generally consume inadequate amounts of CHO. Conversely, both protein and fat were well within the recommended guidelines.[21] Although difficult to define, recent findings suggest that low CHO availability may be more detrimental to athlete health and performance than LEA.[58,59] Therefore, emphasis should be put on increasing CHO intake to support athlete health in general. Professional players on average will play 1-2 games, in addition to 4-6 training sessions per week.[13] Given this schedule, it is unlikely that sufficient glycogen re-synthesis will occur with the current nutritional regime. Recently, qualitative data suggest that reduced adherence to nutritional guidelines, among professional female football players, may be due to misconceptions on the impact of CHO on body composition and weight gain.[92] While these perspectives have not been studied in the present thesis, it stands out as a possible explanation of our findings. Accordingly, prioritizing efforts to enhance nutritional literacy among female football players on all seems important.

Low energy availability

In *Paper II,* direct measurement of EA was applied through measurement of EEE derived from a tracking device tested in *paper I* and EI. Further, in *paper III* and *IV,* we applied surrogate markers of EA through $\text{RMR}_{\text{Ratio}}$ and/or RMR kcal/kg^{-1} FFM/day^{-1}. The directly measured prevalence of LEA was 23% on training days, 36% on match days, while the average prevalence of LEA derived from AEE using the formula (AEE = TDEE – [RMR + TEF]) was 76%. However, when applying the correction factor based on the DLW reference by increasing EI by 22%, the prevalence decreased to 7% on training days and 23% on match days (**Figure *10***), while the AEE derived prevalence for the 14- day period was 48%. Previous studies on female football players report an estimated prevalence of LEA ranging from approximately 20-80%.[13,46,50,51,83,84,103] Our findings, however, indicate that these estimates are inflated. In general, it is more feasible to measure EEE in sports which in larger part consists of steady state efforts, with the possibility to employ tools such as power meters, offering valid translation from mechanical power to energetic cost (i.e., Kilojoules).[75,204,211] Hence, studies on endurance sports (i.e., cycling) likely represents more valid measures of direct EA, compared to team sports.

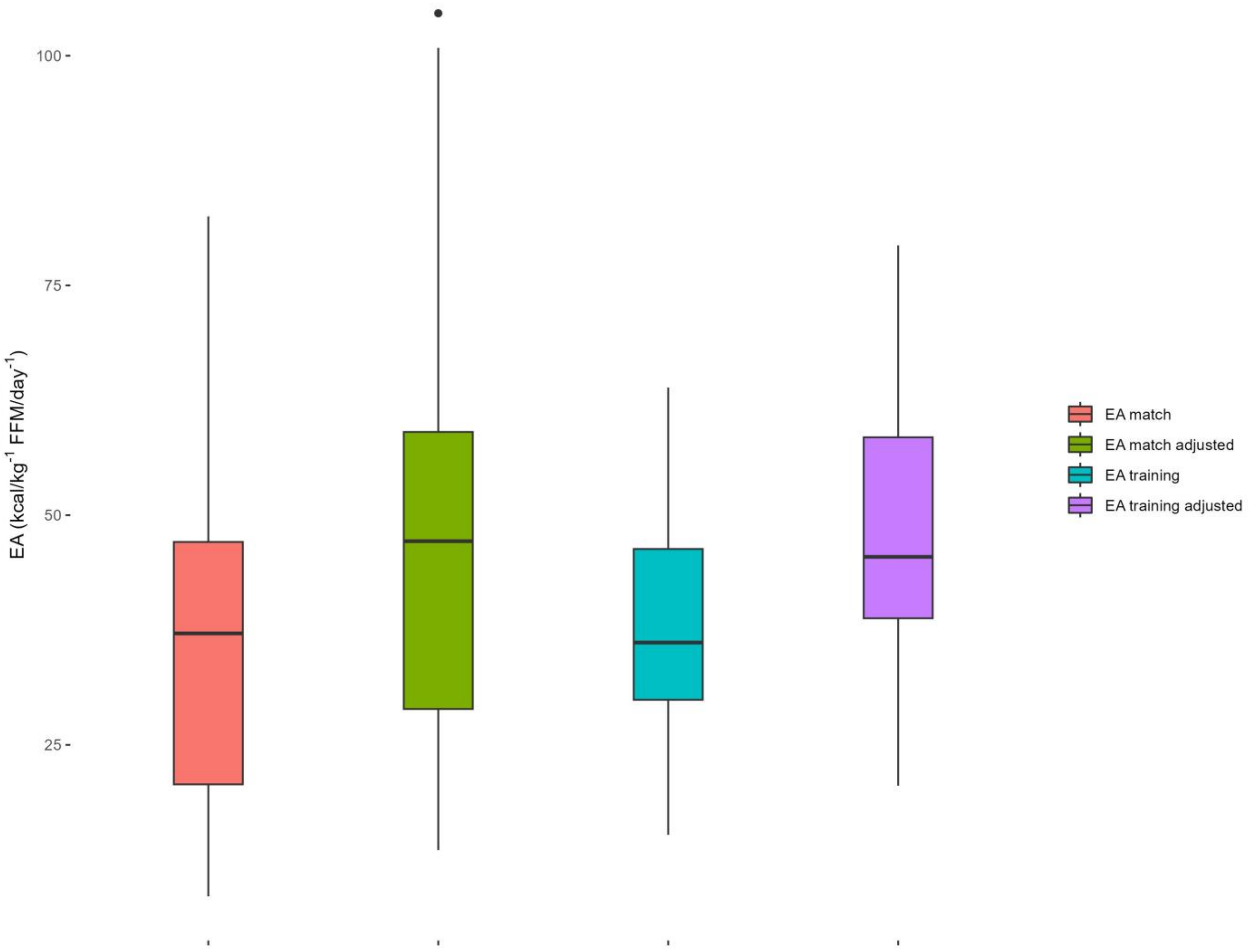


Figure 10. *Box plot showing the EA distribution as measured on match and training day vs the adjusted distribution assuming 22% underreporting of EI. EA = energy availability; EI = energy intake.*

The prevalence of LEA using surrogate markers (36% and 42% for EA< 30 kcal/kg^{-1} FFM/day^{-1} and RMR$_{\text{Ratio}}$ <.90, respectively) was substantially greater than that reported within an Australian elite and pre-elite mixed sport cohort (11% and 3%, respectively).[80] Interestingly, these authors reported that 37% of the cohort exhibited two to three symptoms consistent with the REDs. This is compatible to our findings, where 22 % of the cohort manifested both primary and secondary indicators associated with REDs. Moss et al.[13] reported no relationship between LEA and associated risk factors; however, there was a discernible relationship between attenuated RMR and LEA, measured directly, suggesting prolonged energy deficiency may have been present.[13] The interindividual variance evident across various studies, underscores a substantial discrepancy between LEA and its associated risk factors, delineated in the REDs model.[27]

In total, the prevalence of LEA in female football players seems to depend on the type of estimate utilized. With respect to practitioners measuring EA in the field, it is notoriously difficult and provides high degrees of uncertainty.[67] Since unfavorable physiological alterations likely occur at varying levels of EA, it may be advisable to focus on measuring indicators associated with REDs instead, enabling individual risk assessment in terms of development of negative health outcomes.

Screening for LEA

The LEAF-Q has consistently been used among populations for which it was not intended, including female football players.[13,83,100] This is unfortunate, as football is a high impact sport with risk of both overuse and acute injuries.[212] The LEAF-Q does not account for significant variations in sport-specific features, leading to skewed results. Interestingly, a previous study centered on young female football players (13-18 years) concluded that the LEAF-Q functions effectively as a screening instrument for identifying the Triad/REDs.[211] However, the study did not assess the utility or validity of the LEAF-Q. Instead, the authors compared groups that were determined based on risk assessment, and few physiological differences were actually present. A recent investigation concluded that the LEAF-Q was suitable to “rule out” LEA-related conditions in athletes who scored below the originally published cut-off value. However, it failed to identify athletes at risk for the Triad or LEA with its associated symptoms, using a mixed sport cohort.[140] Thus, the LEAF-Q performed better in a diverse cohort of athletes, compared to the observed findings in *paper III.* This is likely, in large part, explained by the fact that the mixed cohort primarily consisted of non-contact sports. The findings from *paper III* indicate that the overall score is subjected to bias through artificially inflated scores on the injury subcategory of the questionnaire. This confirms that the LEAF-Q does not account for the injury mechanisms seen in football, likely unrelated to LEA. Changing the cut-off values for the overall and injury subcategory to ≥ 10 and ≥ 5, respectively, increased the predictive power of the questionnaire, but without remedying the limitations as noted above; hence rendering its use in the context of female football players inadvisable.

REDs

Menstrual function

Changes to the menstrual cycle following LEA known as AME or oligomenorrhea (depending on the manifestation of the changes) are considered the principal indicator of REDs.[8] One of the challenges in applying menstrual cycle to assess REDs is the widespread use of HC agents.[120] In *papers III* and *IV,* 55% of the participants reported using HC agents, limiting the applicability of this marker. Of the non-HC users, eight players presented with AME, translating to a prevalence of 30%. While there was a statistically significant difference between the AME and EUM group regarding cumulative indicators, no individual REDs indicator displayed statistically significant differences between the groups. Despite this, the AME group consistently presented unfavorable outcomes across the REDs indicators. One possible explanation for this is that female athletes may elicit different hormonal profiles.[213] Our findings demonstrate that despite the presence of AME, other markers of REDs do not invariably ensue, which highlights the difficulty of correctly diagnosing athletes, particularly since the identification of REDs largely relies on clinical judgement.[96] Hence, although athletes may be categorized as AME according to pre-defined criteria (i.e., loss of menstruation), it may not evoke additional physiological alterations. This scenario would be somewhat akin to what is observed with EA, where the physiological alterations observed are not necessarily proportional with the magnitude of LEA.

Endocrinological function

Alterations in the hormonal milieu resulting from LEA have been extensively documented in controlled laboratory studies.[63,68,114,127] T_3, which is the hormone most closely linked to LEA,[63,127] had a prevalence of 10%, in *paper IV*. This is a thyroid hormone that affects several physiological processes in the body, including metabolism and thermoregulation, central in the REDs model [27,214]. Loucks et al. showed that suppression of T_3 occurred at an EA threshold between 19 and 25 kcal/kg^{-1} lean body mass/day^{-1}, within 4 days.[62] Since our data was cross-sectional, it is unknown if the attenuation observed in free T_3 reflects current or preceding energy status or is related to other factors, potentially compromising T_3. Additionally, compromised levels of several other blood markers were prevalent in *paper IV,* including cortisol, leptin, and IGF-1. The main hypothesis in terms of endocrine function, related to REDs, is that alterations in these hormones occur to conserve energy for vital physiological processes.[27] Hence, there is a need to investigate the collective status of several blood markers to assess the overall risk. Multiple individuals had more than one compromised blood marker in *paper IV,* which likely increase their risk for development of the syndrome. Despite this, arriving at definitive conclusions is elusive given the limited understanding of the causal relationship between several of the individual blood markers and REDs. Notwithstanding, the relationship between T_3 and REDs/LEA is strong, highlighting the fact that several of the participants possibly were experiencing symptoms of LEA.

Bone health and metabolism

Prior research have established links between LEA and BMD, which forms an integral part of the Triad, as well as the REDs model.[26] Several studies have demonstrated that LEA may trigger unfavorable alterations in both bone resorption and formation processes.[70,71,215,216] The high-impact nature of football may elicit ameliorated BMD levels compared to non-weightbearing sports.[71] In *paper IV,* the prevalence of Z-score < - 1 was 0 and 2% measured at the hip and lumbar spine, respectively. This is lower than expected in this cohort, considering the prevalence of LEA measured either directly or with surrogate markers (*papers II-IV*). Considering the osteogenic nature of football, there is an ongoing debate about the suitability of the < - 1 Z-score threshold, and whether this should be increased (i.e., < 0).[133] When we applied a < 0 Z-score threshold the prevalence of low BMD was not significantly increased, reinforcing the original findings.

Recently, evidence have emerged around the effect of low CHO availability in athletes, demonstrating that short-term CHO restriction may impair bone formation at rest and during exercise to a greater extent than LEA.[59] This was not evident in our data, as negative alterations in BMD were virtually non-existence, even though the CHO intake was inadequate in relation to EE, for large parts of the cohort. These findings were paired with low prevalence of compromised levels of CTX-1 and P1NP, which are markers of bone remodeling and resorption, respectively.[134] Together, these findings show that female football players are not at risk for development of low BMD. Based on the osteogenic nature of football, it is likely that the sensitivity of BMD as a marker of REDs is low in this population. This underscores the importance of emphasizing other markers associated with REDs when assessing female football players. Should BMD be considered a sensitive indicator among football players, comprehensive studies are required to create specific databases that establish bespoke thresholds for high-impact sports.[133]

Energy metabolism

Metabolic alterations, primarily manifested through attenuated thyroid function are considered a cardinal symptom of REDs.[8] Thyroid hormones are known for controlling human metabolism and decreased RMR is therefore closely linked with attenuated levels of thyroid hormones.[214] In *paper IV,* RMR was applied as an associated indicator of REDs, with a prevalence of 42%. Currently, RMR may be considered an ambiguous indicator, needing more research.[138,189] It is, however, interesting to note the linear correlation between RMR and TSH levels reported in *paper IV,* as TSH is strongly connected to metabolic regulation in the human body.[214] This could imply that RMR serves as an indicator of metabolic attenuation, which is reflective of energy deficiency.[139] Despite ambiguous evidence, the application of RMR seems warranted when used in longitudinal, repeated measures studies, or in conjunction with other indicators of REDs, as in *paper IV*.[138,139]

The conceptual model of REDs, is to some extent, inherently reliant on what is termed the “additive model” of EE, which is the conventional approach to considering human energetics.[145] This model hypothesizes that increasing physical activity directly adds to the total EE of humans, and thus, must be accompanied by increased EI to avoid LEA in athletes. This hypothesis has been challenged by a "constrained model" of EE (**Figure 11**) which proposes that increased physical activity, over time, directly decreases other metabolic processes, without necessarily being in LEA.[144,145,217]

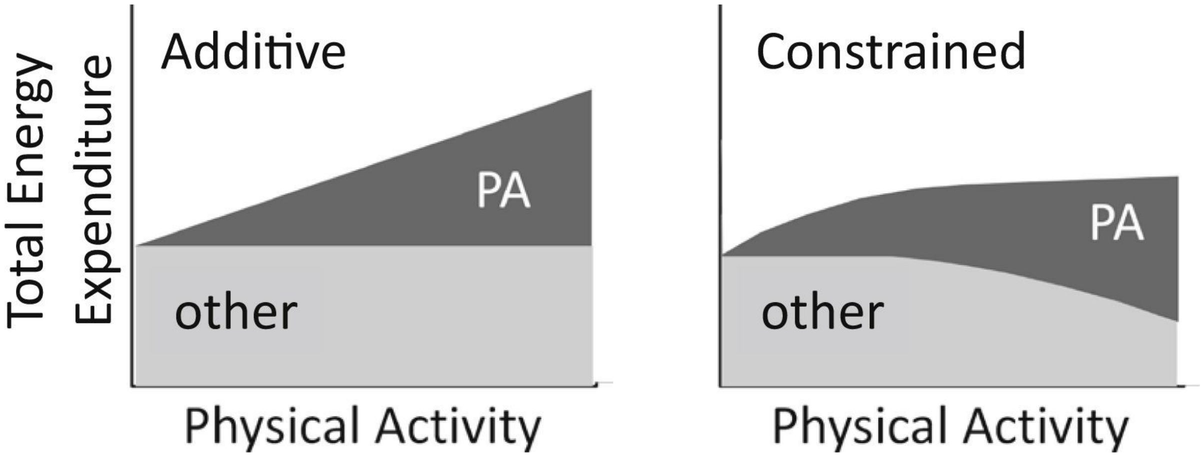


Figure 11. *Visualization of the additive and constrained total energy expenditure models. In the additive model, EE is a linear function of physical activity. In the constrained model, the body adapts to increases in physical activity by decreasing available energy for basal physiological functions (e.g., BMR). The figure is re-printed from Pontzer el al.,[144] with permission from Elsevier publishing.*

If the constrained model of EE is accurate, it is possible that the reduced RMR observed is a result of increased physical activity, leading to attenuation of other metabolic processes such as basal metabolic rate (BMR) or improved metabolic efficiency, reflected through RMR measurements. In *paper II,* where we quantified the TDEE of female football players, using the gold standard method of DLW, the mean PAL was 2.0 ± 0.3 (range 1.6 – 2.4). It has been suggested that an energetic sealing exist around ~ 2,5 x BMR for prolonged durations, making this the maximal sustained metabolic scope in humans.[142] None of the participants in *paper II* exceeded the 2.5 x BMR ceiling. However, the observational period was only two weeks and without any knowledge of PAL pre -or succeeding the study. Currently, evidence supporting the constrained energy model has substantial limitations including statistical assumptions.[217] At present, the physiological response to increased EE is likely explained by a combination of the two models.[217]

Immunological function

In *paper IV*, immunological function was measured through time loss from training or match, defined as major and caused by sickness/illness as defined by the OSTRCQ.[185] Overall, there was a 10% prevalence of time loss in the cohort. Surprisingly, for the sub-group analysis, there was 0% prevalence in the AME group, compared to a 26% prevalence in the EUM group. According to the

REDs conceptual framework, immunological function may be altered by LEA.[27] A study by Shimizu et al. reported that AME athletes displayed downregulation of mucosal immune function, compared to EUM-athletes.[147] This finding stands in contrast to those from *paper IV*. The fact that we did not specifically measure immunological markers may account for the disparity in prevalence among the AME and EUM groups, as self-reported time loss is vulnerable to recall bias. Although pivotal in the REDs model, there is little evidence to provide causal inference between LEA and altered immunological function. Rather, most of this research is based on questionnaires and observational studies.[146,218] Altered immune function subsequent to LEA exposure is conceptually coherent. Since the immune system is energy demanding, downregulation in response to LEA is conceivable as the body must prioritize acute processes for optimal functioning.[93] However, at present, scant definitive evidence substantiates this connection. Consequently, it becomes crucial to furnish our understanding, establishing direct links between LEA exposure and downregulated immune function, if present.

Cardiovascular function

Unfavorable lipid profiles have been reported in athletes suffering from long term or severe LEA, as well as in anorexic subjects.[153,191] The overall prevalence of LDL in *paper IV* was 22%. However, no relationship between increased LDL-levels and other risk factors of REDs were present. As such, it seems that altered LDL levels were normally distributed throughout the cohort. Several of the participants displayed higher body fat (%) levels, compared with previous studies investigating the relationship between blood lipids and LEA.[153] Elevated blood lipid profiles are primarily associated with increased body fat (%) in the general population.[219] Thus, the prevalence reported in *paper IV* is not necessarily related to LEA. This is important to consider when evaluating lipid markers in athletic populations that do not incorporate athletes strictly from sports associated with low fat (%). Overall, it is questionable if lipoproteins are sensitive markers for football players, as the body composition, on average, is expected to be higher than endurance and weigh class athletes, among whom associations with LEA have been found.[46,205,220]

Psychological factors

It is well established that REDs may be present either with or without DE/ED.[8,221] These conditions are reported to be higher in women participating in leanness sports.[159,160] However, the prevalence in football have previously been reported to be as high as 24%.[165] A recent study investigating the prevalence of DE among male and female football players found that 13% of female football players were suffering from DE, using the Eating Attitudes Test - 26 questionnaire.[163] In *paper IV,* the EDE-Q -11 was used to determine the prevalence of DE showing that 10 % of players were suffering from problematic behavior related to nutrition and body image. This is significantly lower than what was reported by Sundgot-Borgen & Torstveit, using clinical interviews for ED.[165] It is worth noting that questionnaire-based tools generally yield higher estimates compared to clinical interviews.[222] Further, the diversity in findings may be due to sample differences, researcher perspectives, and purposes, which are factors that should be considered when analyzing and interpreting results. Qualitative evidence have suggested that female football players exhibit unhealthy behaviors regarding EI and body image.[92] Although apparent in *paper IV*, it is possible that qualitative interviews, thoroughly exploring the psychological status of players, as well as external factors, influencing the mental wellbeing is necessary to uncover these beliefs in detail. Further, cultural differences between countries and teams may also play an important role in explaining the polarization related to well-being in seemingly homogenous populations.[92,220] Interestingly, our results found positive correlations between increased score on the EDE-Q, associated with DE/ED and several measures: the BIS, measuring sleep problems; the GHQ, measuring mental distress; and the CSF, measuring physical and mental fatigue. Although the overall prevalence of psychological issues was modest, this finding highlights the importance of thoroughly assessing mental well-being when screening for REDs, as it may offer an additional layer of precision in pinpointing those at an elevated risk.

Summary of REDs indicators

The measurement of REDs indicators may provide a more nuanced understanding of the risk to which an athlete is exposed, transcending many of the limitations associated with measuring EA. In *paper IV*, the prevalence of cumulative primary, secondary, and associated indicators of REDs ranged from 0-7, while 22% presented with both primary and secondary indicators. Further, 30% of non-HC-users classified as AME, which was surprising as team sports are generally regarded as a low-risk arena for development of REDs.[96] Identifying REDs is challenging and cannot be established based on findings in the scientific literature.[8] Rather, health professionals must evaluate the individual presentation of symptoms and their expertise to accurately diagnose athletes. Nonetheless, the potential for evolvement of the syndrome is represented on a spectrum, ranging from the absence of risk to an escalated probability. This evolvement is tied to the cumulative presence of indicators and their respective classification. Understanding of the prevalence and manifestation of these indicators among female football players is important for the strategic allocation of resources and implementation of targeted interventions. Our findings indicate that the risk for development of REDs is largely individual, and not linked to female footballers as a group.

Methodological considerations

Quantification of EA (Paper I-IV)

Energy availability is susceptible to inaccuracies due to its reliance on the quantification of EI and EEE.[75] In *paper I,* we investigated the comparing different tracking devices against a criterion measure. The device being used in *paper II* displayed a 10.7% error rate, compared to the criterion measure.[78] Despite the apparent inverse relationship with increasing energetic demands, the pattern of estimated EEE did not provide sufficient basis to develop a correction factor for refining the GPS algorithm applied in *paper II.* Further, although the StatSports (GPS device used in *paper II*) algorithm is based on the metabolic power concept by Osgnach et al. it is not open source. Thus, the exact method used to estimate EEE is unknown.[38] Consequently, the EEE estimations involved in the calculation of EA in *paper II* is likely underestimated, based on the results in *paper I.* This limitation should be noted when interpreting the findings related to directly measured EA throughout this thesis. Alas, to date there is no precise method to measure EEE during football or intermittent sports, but the best tracking device (*paper I*) should minimize the error rate. Several previous studies have utilized microtechnology to estimate EEE during football.[13,51,83,103] Moss et al. utilized an older system from the same manufacturer as applied in *paper II*, shown to be less accurate in terms of error rate for speed.[13,223,224] Other studies have applied systems known to be inaccurate at evaluating positions, and distances at different speeds.[225] Hence, this constraint is not unique to our investigations, but rather, a common challenge across studies examining intermittent and anaerobic sports.

Energy intake is known to be difficult to assess, as it normally places a significant burden on the individual, making it prone to error. In their study, Morehen et al.[46] applied a stringent food surveillance system in which dieticians monitored the EI of all major meals, and all snacks were recorded using the validated remote food photography method.[46] Despite this, there was a 30% discrepancy between EI and TDEE, with no significant change in bodyweight. These results are akin to the findings presented in *paper II.* Conversely, we chose to use 24-hour diet recalls recording the dietary intake of the players to increase compliance and participant burden. This method may be criticized for lack of accuracy by some, however, it has been shown to provide equally good or better results compare to methods such as dietary records and weighting of food.[76,201] This is exemplified by comparing our results to the ones by Morehen et al., which in addition are comparable cohorts.[46] While we did not assess EI on all individual days, the calculation of weighted means enables a valid estimation of EI across a different day, each characterized by disparity in physiological load. Hence, it is unlikely that employing an alternative method would have altered the general outcome.

In *paper III and IV*, it was not possible to complete measurement of direct EA for all participants. We therefore opted to use surrogate markers through RMR. This method is associated with certain limitations, including ambiguities regarding the impact of variables such as equipment, age, body mass, body composition, training volume, nutrition status and acute exercise status.[80,226,227] Nevertheless, RMR have also been shown to accurately reflect energy deficiency status in females and considered an interesting surrogate method when measuring EA in conjunction with other indicators.[139,189] It has been suggested that athletes presenting with less severe cases of energy deficiency may not present with attenuated RMR, despite being in LEA.[189] Since the sensitivity of these measurements can be decreased for individuals with minor energy deficiencies, the risk of false negatives are present. Nevertheless, as duration and magnitude of LEA are important predictors for developing negative health outcomes, the possibility of athletes with minor energy deficiencies being overlooked in the analysis is of less importance.[25,66,67]

Average EA derived from AEE using DLW have not been applied much in the literature.[46] Although it may provide insight into the EA of players over longer periods, it is obviously prone to error. However, it does provide a measurement of the EA based on all activities such as gym work, walking/commuting, stair walking etc. For certain individuals, activities of daily living may also possess EE qualifying as a training session. Nevertheless, this method is also prone to inaccuracies in reported EI.[75] Given the high prevalence of LEA found in *paper II*, as well as other studies utilizing this approach,[46] it is likely that the AEE overestimates the entity it is replacing (EEE), causing exaggerated estimates of LEA. This approach may therefore be better suited for non-athletic populations.

The measurement of direct EA is problematic for the above stated reasons, thereby rendering the concept inherently flawed, outside of laboratory settings. Determination of LEA thresholds are likely highly individual, thus questioning the application of universal thresholds.[65,125] Finally, the utilization of a surrogate marker in the form of RMR may be employed. However, neither this measure is unproblematic, and evidence is ambiguous. Collectively, these constraints enhance the appeal of direct measurement of REDs indicators as an alternative approach, as the outcome variables described by the REDs model is what ultimately compromises athlete health, with LEA as the causal factor.

Measurement of REDs indicators

The evaluation of bodily systems, as delineated by the REDs model, presents interesting possibilities, circumventing many of the shortcomings associated with measurement of EA. However, this approach is not without limitations either, most notably the fact that many of the proposed indicators do not exhibit robust causal relationship with the outcomes outlined by the model.[31] Furthermore, many of the cut-off values commonly applied in the literature lacks robust evidence and require further examination. Biomarkers may also be sensitive to external factors, causing misinterpretation of results.[228]

Paper II investigated the TDEE of elite level female football players, utilizing the DLW method.[175] Defined as the factor by which total EE exceeds BMR,[229] PAL serves as a proxy which enables quantification and comparison of physical activity regardless of sex, anthropometrics, and body composition. The Cunningham equation is the most used predictive model for estimating RMR in the literature relating to female athlete health.[13,80,230,231] Nevertheless, our results in *paper II*, suggests that the Harris-Benedict equation, another popular method for estimating RMR, is more accurate for this population.[232] In *paper II*, the difference between measured RMR and the Harris-benedict estimation was 0.02. Conversely the difference between measured RMR and the Cunningham estimation was 0.11. When using PAL to compare different populations, the magnitude in difference seen between the measured level and the Cunningham equation may have implications for determinants of EI, among other factors. As such, based on our results in *paper II*, to go forward, it may be advised to apply the Harris-Benedict equation when using RMR as a surrogate marker in female football players.

Study design

Fitting the aims of this thesis, we have used cross sectional (*paper I, III and IV)*, prospective observational (*paper II)* and survey based (*paper III and IV)* designs. Hence, It is important to acknowledge that causal inference cannot be drawn,[233] and the design cannot provide information about the longitudinal development of the symptoms and EA status. Further, while the cross-sectional studies within the thesis offer a snapshot of the players' status at the time of testing, it may also inadvertently emphasize their current condition, without reflecting potential fluctuations over time for certain measures.

Generalizability

Following the classification system by McKay et al.,[171] the participants were classified as tier 3 or tier 4, corresponding to highly trained/national and elite/international, respectively. According to this classification, tier 5 (world class) will only encompass top players within the top teams in the most competitive leagues. Our results show that energetic requirements are likely similar between tier 3, 4 and 5 athletes, in women's football. In terms of the LEAF-Q (*paper III*), our findings are likely generalizable to football players on all performance tiers. This assertion is grounded in the inherent characteristics of the sport, which inflicts risk for both acute and impact injuries, regardless of competition level. Although our cohort included participants from Europe, Asia, and the Caribbean, it primarily represents a homogenous group of white athletes, operating in a Norwegian sociocultural and sport context, which may limit the generalizability of our findings. This is also reflective of most research conducted on LEA and REDs, in general.[27] Considering the universal nature of football, as well as biological processes and mechanisms, the psychological factors studied may warrant particular consideration. In general, to robustly generalize findings in health science research, replication of studies across diverse populations may be needed.[234]

Perspectives

Prevention of LEA and REDs in practice

For female football players, the risk of REDs seems to be primarily individual in nature. As an initial and non-invasive approach, universal strategies should be prioritized. Coaches and team support staff should remain vigilant of indicators through established tools and metrics such as internal/external load, menstrual function, and sleep, parameters that are now routinely monitored in most clubs. Further, the insufficient CHO intake reported in *paper II,* as well as in previous studies among female footballers,[46,220] requires practical consideration, especially since many players seem to lack requisite nutritional knowledge.[92] One way to accommodate this is by focusing on nutritional periodization. At present, evidence-based, and practical frameworks are available for teams to adopt. If properly implemented, these frameworks can enable players to meet the nutritional requirements, and serve as preventive measures against the development of LEA, and subsequently REDs.[23] **Table 4** provides a practical model for CHO periodization throughout a typical one-match week, serving as an example to ensure that nutritional needs are met.

Table 4. *Example of practical model to "fuel for the work required" through CHO periodization in a tentative one game week for starters. The framework is adapted from Anderson et al.,* [23] *using data based on our findings with permission from Taylor & Francis ® (Journal of Sports Science).*

	Load	Breakfast	During training	Lunch	Snacks	Dinner
Monday (MD +2)	Light	Medium CHO 0.5-1 g/kg^{-1}	No CHO	High CHO 1.5 g/kg^{-1}	Medium CHO 0.5-1 g/kg^{-1}	Medium CHO 1 g/kg^{-1}
Tuesday (MD-4)	Medium	Low CHO 0.5 g/kg^{-1}	No CHO	Medium CHO 0.5-1 g/kg^{-1}	Medium CHO 0.5-1 g/kg^{-1}	Medium CHO 1 g/kg^{-1}
Wednesday (MD-3)	Hard	Medium CHO 1 g/kg^{-1}	No CHO	High CHO 1.5-2 g/kg^{-1}	Medium CHO 0.5-1 g/kg^{-1}	Medium CHO 0.5-1 g/kg^{-1}
Thursday (MD -2)	Medium	Low CHO 0.5 g/kg^{-1}	No CHO	High CHO 1.5-2 g/kg^{-1}	Medium CHO 0.5-1 g/kg^{-1}	Medium CHO 0.5-1 g/kg^{-1}
Friday (MD-1)	Light	High CHO 2 g/kg^{-1}	Medium CHO 40 g/hr^{-1}	High CHO 2 g/kg^{-1}	High CHO 1.5 g/kg^{-1}	High CHO 1.5-2 g/kg^{-1}
		Breakfast	**Pre-match**	**During game**	**Post-match**	
Saturday (MD)	Very hard	High CHO 1.5-2 g/kg^{-1}	High CHO 1.5-2 g/kg^{-1}	High CHO 60 g/hr^{-1}	High CHO 1 g/kg/hr^{-1} for 4 hours	
		Breakfast	**During training**	**Lunch**	**Snacks**	**Dinner**
Sunday (MD+1)	No training	High CHO 2 g/kg^{-1}	No training	High CHO 1.5-2 g/kg^{1}	High CHO 1.5 g/kg^{-1}	High CHO 1.5 g/kg^{-1}

Carbohydrates = CHO; MD = Match Day.

Depending on their financial capacity, clubs should prioritize nutritional guidance, such as appointing a dietician. This would ensure the successful implementation of specific nutritional strategies and increase nutritional literacy, important for both performance and health. In certain settings, adopting regular screening procedures comprising measures like blood markers, RMR, BMD and psychological status assessment could aid early identification of athletes who might be at risk for REDs. In combination with resources like the REDs clinical assessment tool, this may provide practitioners with a more standardized approach to manage athlete health.[96] However, caution should be exercised when utilizing these procedures, considering their validity may not be universally applicable. For instance, the commonly applied BMD Z-score threshold may not be as relevant for female football players, as discussed in *paper IV*.

Future research

Opinions regarding the risk LEA and REDs possesses to female football players remains equivocal. Low energy availability is prevalent among female football players, with varying numbers reported, depending on factors like time of season, level of competition, and age.[13,46,50,51,83,84,103] Recognizing the limitations in measuring EA, alternative approaches like the energy balance method have been suggested.[235] This method emphasizes the use of more objective measures to quantify EI, thereby reducing substantial constraints from the EA measurement process, including participant burden. At present, this method mainly possesses theoretical relevance within the context of athletic populations. If validated, it could lead to advancements in enhancing the accuracy and reliability of EA measurements.

Similar to LEA, using universal cut-off values for different REDs indicators is also a complex issue. As most reference values and cut-offs are derived from the general population, it may not be directly transferrable to athletic cohorts.[62,188,236] This is further complicated by interindividual variation that can exist within these ranges. Adopting longitudinal repeated measure designs may mitigate such variations and improve our understanding of these issues, including exposure/outcome variables.[237] Incorporating analytical approaches, such as Bayesian statistics, could further refine these analyses by utilizing population-based prior distributions to establish athlete-specific reference ranges, depending on the heterogeneity of the indicator and distribution under study.[238,239] While it was feasible to analyze the correlation between different measures of EA (i.e., direct vs surrogate) and associated risk factors within our data set, we chose not to. This decision was influenced by the fact that RMR likely reflects the metabolic state of preceding behavior, including possible adaptations to energy deficiency, as opposed to direct EA. Therefore, to extract meaningful insights from such analyses, it would be advisable in future research to follow the proposed study designs, including simultaneous measurements of both methods.

As delineated in *paper III,* the LEAF-Q proved unsuitable for female football players due to the distinctive differences of football compared to the sports for which the questionnaire was developed.[187] Thus, future research should also aim to develop an easily administrated screening questionnaire validated for football to be used on a regular basis for monitoring purposes.

Considering the energetic demands of football, it seems probable that challenges associated with LEA and REDs are primarily attributable to insufficient EI, possibly driven by psychological factors.[92] It is therefore coherent to focus on these elements, to effectively address underlying issues of REDs and LEA. In light of the extensive body of research addressing the prevalence of LEA, further studies on the specific prevalence may be of diminished priority, as practical impact is of higher importance. Hence, increasing our understanding of “objective” indicators, such as blood markers that reflect the physiological state of an individual, emerge as another interesting direction for future research on these complex matters.

Conclusion

- Current generation of tracking devices fails to provide accurate estimates of EEE, consequently undermining the assessment of EA in intermittent sports, such as football.

- Female football players display moderate levels of EE and generally consume inadequate amounts of CHO, with little sign of nutritional periodization, suggesting a need for better nutritional education to support optimal health and performance.

- The estimated prevalence of LEA in football is substantial, however, due to measurement errors associated with the applied methodologies, the reported numbers are likely inflated.

- The LEAF-Q is not suitable as a screening tool for female football players and is therefore not advisable for application in this population.

- Some female football players may be at risk for developing REDs, emphasizing the importance of team support staff to be aware of pervasive REDs-indicators.

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Paper I

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Article

Accuracy of Tracking Devices' Ability to Assess Exercise Energy Expenditure in Professional Female Soccer Players: Implications for Quantifying Energy Availability

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Abstract: The purpose of the study was to assess the accuracy of commonly used GPS/accelerometer-based tracking devices in the estimation of exercise energy expenditure (EEE) during high-intensity intermittent exercise. A total of 13 female soccer players competing at the highest level in Norway (age 20.5 ± 4.3 years; height 168.4 ± 5.1 cm; weight 64.1 ± 5.3 kg; fat free mass 49.7 ± 4.2 kg) completed a single visit test protocol on an artificial grass surface. The test course consisted of walking, jogging, high-speed running, and sprinting, mimicking the physical requirements in soccer. Three commonly used tracking devices were compared against indirect calorimetry as the criterion measure to determine their accuracy in estimating the total energy expenditure. The anaerobic energy consumption (i.e., excess post-exercise oxygen consumption, EPOC) and resting time were examined as adjustment factors possibly improving accuracy. All three devices significantly underestimated the total energy consumption, as compared to the criterion measure ($p = 0.022$, $p = 0.002$, $p = 0.017$; absolute ICC = 0.39, 0.24 and 0.30, respectively), and showed a systematic pattern with increasing underestimation for higher energy consumption. Excluding EPOC from EEE reduced the bias substantially (all p's becoming non-significant; absolute ICC = 0.49, 0.54 and 0.49, respectively); however, bias was still present for all tracking devices. All GPS trackers were biased by showing a general tendency to underestimate the exercise energy consumption during high intensity intermittent exercising, which in addition showed a systematic pattern by over- or underestimation during lower or higher exercising intensity. Adjusting for EPOC reduced the bias and provided a more acceptable accuracy. For a more correct EEE estimation further calibration of these devices by the manufacturers is strongly advised by possibly addressing biases caused by EPOC.

Keywords: female athlete; exercise expenditure; energy availability; team sport; exercise metabolism; technology

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1. Introduction

To support basic physiological functions and aid adaptations to training, an athlete's energy intake (EI) should be matched against the energetic needs a given sport activity require. An athlete's energy availability (EA) is quantified as the residual energy after subtracting the exercise energy expenditure (EEE) from the EI, divided by fat-free mass (FFM) [1]. In soccer, nutritional intake may impact a player's body composition, resulting in performance alterations. As such, sport nutrition experts can assist players in manipulating EI to meet the desired goals [2]. Nevertheless, athletes should be warned against the accidental or deliberate mismatch of EI and energy expenditure (EE), resulting in EA below 125 kilojoules (kJ) (30 kcal) per kg FFM. Such a low energy availability (LEA) may cause disturbances to hormonal, metabolic, and immune functions [3–5]. Although monitoring of body mass can provide insight into an athlete' s energy balance, long term LEA may result in "metabolic adaptation" causing weight stability, despite inadequate energy balance [6]. Thus, body mass is not sufficient to detect LEA in athletes. Despite this, limited knowledge exists regarding the occurrence and implications of LEA in female soccer players.

In soccer and intermittent field sports, tracking devices (GPS or accelerometer-based) is the most commonly used microtechnology to quantify physical activity [7], and to estimate EA [8–10]. Such devices may provide valid estimates of EEE within sports characterized by steady-state exercise such as running and cycling [11]. However, their accuracy is questionable within sports characterized by intermittent exercises, notably soccer, with high amounts of directional changes, accelerations, and deaccelerations [12]. In part, this is due to the anaerobic energy production, resulting in lactate accumulation and oxidation, which is not accounted for in aerobic energy production and therefore difficult to measure [13]. Further, different manufacturers may operate with disparate algorithms when processing data, potentially resulting in dissimilar output of EEE [14,15].

Some tracking devices, which are specifically developed for intermittent sports such as soccer, build on the metabolic power concept [16,17], yet several studies [12,18–20] still report underestimations of EEE. Responding to such findings, the original authors have argued that inappropriate usage of the concept could contribute to this underestimation [21]. Others have proposed alternatives to the original model [22]. Nevertheless, the validity of studies reporting EEE in intermittent sports based on tracking devices is ambiguous. This needs to be further investigated in female athletes and soccer players, as previous work on metabolic power is based on male athletes. Disparities in physiological factors between sexes such as work economy, efficiency, and body composition are also contradictory [23] and have not been addressed in the development of the metabolic power concept.

In a recent study [24] of female endurance athletes, only a slight caloric surplus of <200 kcal·d^{-1} in energy balance was associated with increased performance. However, failure to achieve a caloric surplus was associated with impairments to performance. This finding highlights the importance of accurately measuring EEE, as it may have direct consequences for the nutritional periodization of athletes, influencing performance, recovery, and health status. In summary, there is a need to identify the accuracy of current tracking devices being utilized to quantify EEE, offering implications for measuring LEA. Further, the investigation of female athletes is warranted to examine potential differences in estimating EEE and enable between study comparisons, regardless of sex.

Therefore, the aim of the present study was to examine the accuracy of three commonly used tracking devices utilizing metabolic power to quantify EEE during intermittent exercise in high-level female soccer players. Based on the current literature, we expected that the tracking devices underestimate the caloric expenditure as compared with indirect calorimetry as the criterion measure. we also examined whether EPOC during rest could be used to improve agreement and explain potential discrepancies in the results.

2. Materials and Methods

2.1. Study Design

Participants completed a single visit test protocol on artificial grass surface instrumented with a portable O_2 analyzer, and three different tracking devices. A pre-determined course consisting of walking, jogging, shuttle run/stride, and sprinting was designed to model the physical requirements in women's soccer [25,26]. The course length was 549.5 m and was repeated five times (total distance 2747.5 m) to ensure data sufficiency to measure movement and EEE (Figure 1). Participants were instructed to complete each part of the course at self-selected speeds, guided by movement descriptors. However, the 20 m sprint was instructed to be completed at maximal effort. Before starting the testing protocol, participants were instructed in how to use the Rated Perceived Exertion (RPE) [27] scale ranging from 0–10. All participants completed a standardized guided warm up, consisting of three rounds, corresponding to RPE 4, 6 and 8, respectively, the latter being the desired intensity for the completion of the 5-round protocol (self-selected speed corresponding to RPE 8). After each round, they rested standstill for 1 min. Here capillary blood lactate samples were collected, and the participants were asked to rate their RPE of the preceding round. Additionally, after the completion of the protocol, excess post exercise oxygen consumption (EPOC) was measured for 120 s to account for EEE derived work above VO_2 max and total session RPE was stated.

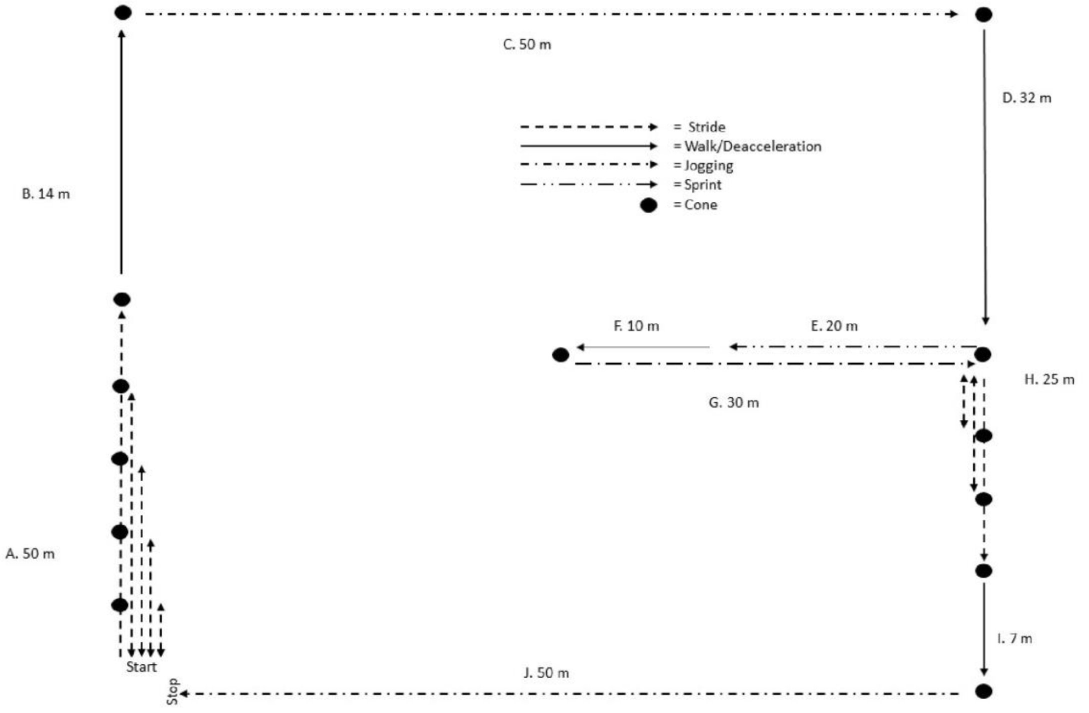

Figure 1. Illustration of the intermittent exercise protocol, indicating the type of movement and length of segment numbered A–J.

2.2. Participants

Eligibility criteria for the study were defined as (i) female competing at the highest level in Norway, (ii) >16 years of age, and (iii) absence of injuries or illnesses. In addition, participants were asked to abstain from caffeine intake on the day of testing, as well as ingesting their last meal approximately 2 hours before testing. A total of 13 professional female soccer players (age 20.5 ± 4.3 years; height 168.4 ± 5.1 cm; weight 64.1 ± 5.3 kg; fat free mass 49.7 ± 4.2 kg) completed the study. Two players declined the invitation to participate due to self-reported injury and time commitment. Following the Helsinki declaration, all participants were informed about the project both orally and in writing and signed an informed consent document. The project was approved by the Norwegian Center for Research Data (Reference: 807592).

2.3. Tracking Measures

Participants were equipped with an 18 Hz GPS device with 952 Hz tri-axel accelerometer, gyroscope, and magnetometer (GPS[1], Apex, StatSport, Newry, Northern Ireland, UK), a 10 Hz GPS device with 1 kHz tri-axel accelerometer, gyroscope and magnetometer

(GPS^2, Vector, Catapult innovations, Melbourne, Australia), and a 1000 Hz inertial sensor device, with accelerometer, gyroscope and multi-chip motion tracking module (IMU, Playermaker^{TM}, Tel Aviv, Israel). All devices were mounted and used according to manufacturers' guidelines. The GPS devices were securely positioned in a custom-made vest 2–3 cm apart, between the participants' scapulae. Both GPS devices were placed outside in record mode at least 20 min prior to testing, to ensure adequate satellite connection. The inertial sensor was mounted on the participants boots, using the manufacturers boot strap designed for this purpose. After completion, data were uploaded to the device-specific software and analyzed to calculate EEE for the whole period, before being exported to Microsoft Excel. After reaching out to the manufacturers of GPS^1 and GPS^2, both confirmed that the calculation of metabolic power builds on previous work by Osgnach et al. [16], utilizing acceleration and velocity data for the calculation. For the inertial measuring device (IMU), EEE calculations were done by the manufacturer as the software lacked this feature. The technical properties of this device is explained elsewhere [28] and it applies the same metabolic power method [16]. Specifically, speed and acceleration were calculated in 10 Hz, together with the formula and constants provided in the algorithm by [16]. All tracking data were also edited post hoc, by synchronizing the start and cessation of the protocol with the oxygen consumption (VO_2)-derived data, ensuring the same measurement time for the various devices.

2.4. Indirect Calorimetry

Indirect calorimetry (VO_2 Master Health Sensors Inc., Vernon, BC, Canada) was used to establish VO_2-derived EEE and served as criterion measure against the tracking systems. The VO_2 master have previously been validated [29] resulting in a difference ranging from 0.17–0.27 VO_2 (L/min) during different intensities, compared to the Parvomedics trueOne 2400 metabolic cart (Parvomedics, Inc., Salt Lake City, UT, USA). Participants wore the VO_2 master for the entire protocol, including rest periods, as well as 120 s following the last round to account for EPOC, following the intermittent exercise protocol. To establish resting energy expenditure, participants wore the VO_2 master for 10 min, after arriving at the facility, laying down in supine position. The mean VO_2 (L/min)-derived EE value from the last 5 minutes was subtracted from the total EE during the test post hoc for each individual, consistent with previous literature [20,30]. The VO_2 master was calibrated according to the manufacturer's guidelines prior to each testing session. After completion, breath by breath analysis of VO_2 (L/min) was analyzed in 30 s intervals, before being converted to kJ to establish VO_2 derived EEE using a respiratory exchange ratio (RER) of 1.00, indicating mainly glycolytic energy production [31]. This was done as the usage of RER assumes constant oxygen content and that CO_2 exchange in the lungs reflects that of the cells [32]. As this is not the case during intermittent exercise, lactate measurements served as confirmation of the appropriate RER level chosen.

2.5. Lactate Measurement

Blood lactate (mmol/L) was measured using the lactate plus (Lactate Plus, Nova Biomedical, Waltham, MA, USA), which have previously been validated [33]. Samples were taken at rest from the index finger, following resting energy expenditure measurement and after each round of the protocol, indicating the level of intensity for the completed work. Thus, blood lactate measures were used to verify the RER used to calculate EEE, as blood lactate is associated with substrate metabolism during exercise [34].

2.6. Statistical Analyses

The statistical analyses and preparation were conducted using SPSS 26 (IBM, Armonk, NY, USA) and Microsoft Excel (Microsoft corporation, Redmond, WA, USA). Descriptive statistics from the participants are given for total running distance, inter-device distance, percentage difference, as well as the lactate levels for the separate circuit rounds. All devices were directly compared to the criterion measure (VO_2 derived EEE) and intraclass correlation coefficients (ICC) were estimated to determine the level of agreement between the individual tracking devices and the criterion measure. We estimated two-way mixed ICC models with the subjects and method factors as random and fixed, respectively [35]. ICC estimates are presented based on the single measure formula since a single device score represents the EEE score. ICC estimates for both relative (consistency) and absolute agreement are given along with their 95% confidence intervals.

Paired sample t-tests were used to examine for mean differences between the measurement methods, thus indicating the general level of bias. Effect sizes (ES) for these differences were calculated by dividing on the standard deviation of the difference scores corrected for their correlation. We report Hedge's g, which additionally corrects bias related to smaller samples, thus reducing overestimation according to the formula [36]: $g = \frac{\overline{M_1 - M_2}}{\sqrt{(s_1^2 + s_2^2 - 2rs_1s_2)}\ /\ \sqrt{2(1-r)}} \times J$ (with J as the Hedge's g correction factor according to [37]. The a priori alpha level was set to $p < 0.05$. We added linear regression analyses to examine the level and direction of systematic biases between the methods. The tracking device in question was used as a predictor with VO_2 as the outcome. Unstandardized residuals, which represent the difference between the predicted and the actual VO_2 energy consumption score was saved and plotted on the y-axis against VO_2 data on the x-axis. Systematic biases would be present if the residual scores showed a non-flat increasing or decreasing pattern depending on the actual VO_2 levels. Lastly, we examined if adjusting the EEE calorimetry scores by subtracting the resting time or the EPOC scores could reduce bias and yield better agreement according to new paired sample *t*-tests, smaller residual scores and improved ICC estimates. All results are presented as mean ± SD, unless specified.

3. Results

The total distance and mean energy expenditure measured for the tracking devices GPS^1, GPS^2, and IMU are presented in Table 1. Compared to the manually measured track, results for distance displayed a percentage difference of 4.4%, 3.7%, and 0.7%, respectively. Mean lactate measurement was significantly elevated compared to baseline resting values (1.3 ± 0.4 mmol/L) during round 1–5 (Figure 2).

Table 1. Upper panel displays descriptive data for the criterion measure EEE^{VO2}, distance measured by devices, and total EEE for the individual tracking devices. Middle panel display ICC measures for absolute and consistency measures, percentage error (EEE), as well as paired sample *t*-test between specific devices and criterion measure for total energy expenditure. Lower panel of the table display the values adjusted, by removing EPOC measurement from the criterion measure value (VO_2), only analyzing moving time. Exercise energy expenditure = EEE; kilojoule = kJ; effect size = ES; post-exercise energy consumption = EPOC.

	GPS^1	GPS^2	IMU
N	13	11	11
VO_2^{EEE} (kJ)	1038 ± 183	1043 ± 198	1016 ± 191
Distance (% error)	2625 ± 25 (4.4%)	2644 ± 73 (3.7%)	2767 ± 207 (0.7%)
EEE (kJ)	933 ± 83	843 ± 73	879 ± 82
ICC^{ABS}	0.39	0.24	0.30
ICC^{CON}	0.48	0.44	0.42
Percentage error	10.7%	20.6%	14.5%
p value	0.022	0.002	0.017
ES	0.60	0.96	0.77
Values adjusted for EPOC			
VO_2^{-EPOC} (kJ)	868 ± 156	875 ± 168	847 ± 161
ICC^{ABS}	0.49	0.54	0.49
ICC^{CON}	0.54	0.53	0.48
Percentage error	7.2%	3.1%	3.7%
p value	>0.05	>0.05	>0.05
ES	0.44	0.15	0.21

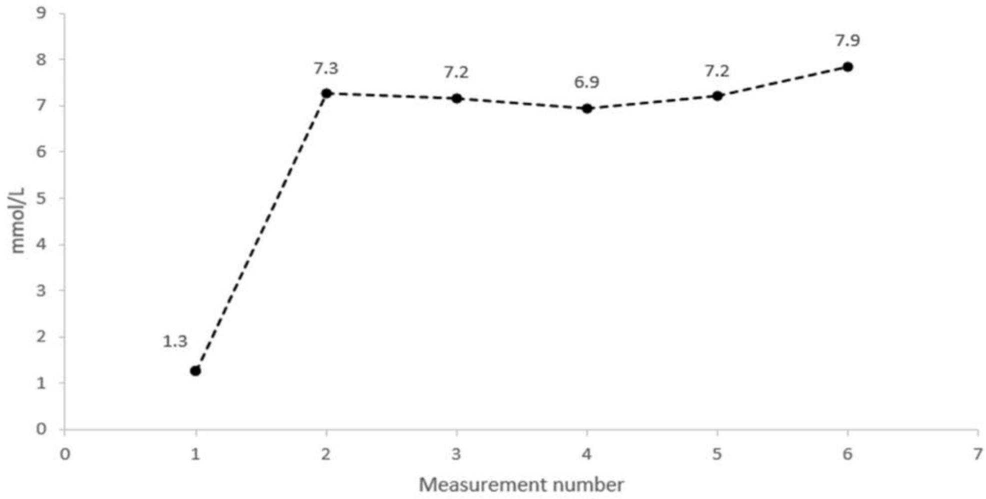

Figure 2. Time plot of the lactate measurement levels during the protocol, at baseline (rest) and the following each completed round.

EEE measured as indirect calorimetry (criterion measure) compared to EEE estimated by GPS^1, GPS^2, and IMU (EEE^{IMU}) is presented in Table 1. Compared to the indirect calorimetry, all tracking devices significantly underestimated the caloric expenditure during intermittent bouts of exercise (GPS^1, $p = 0.022$, ES = 0.60, GPS^2, $p = 0.002$, ES = 0.96 and IMU, $p = 0.017$, ES = 0.77). When adjusting EEE by subtracting EPOC (EEE measured during standstill resting periods) from the measurement, no differences were found ($EEEGPS^1$ $p > 0.05$, ES = 0.44, EEE^{GPS2} $p > 0.05$, ES = 0.15 and EEE^{IMU} $p > 0.05$, ES = 0.21) (Table 1).

The ICC values for total EEE ranged between 0.48 and 0.21 based on consistency estimation, and between 0.39 and 0.24 based on absolute agreement estimation, respectively. Adjusting calculations by excluding EPOC measurements, only analyzing moving time, ICC values ranged between 0.54 and 0.48 and between 0.54 and 0.49 based on consistency and absolute agreement estimation, respectively (See Table 1 for specific values).

Series of regression analyses with VO_2 as outcome and the specific tracking device as predictors are given in Table 2. An unstandardized beta coefficient above or below 1 indicates under- versus overestimation, respectively. Using mean centered values as predictors, the unstandardized coefficients for EEE^{GPS1} were 1.42. The GPS^1 device thus underestimated true calorimetry usage with 0.42 kJ per unit increase in the VO_2 measurement. For EEE^{GPS2}, the unstandardized coefficient was 1.82; hence, underestimating estimated caloric expenditure with a mean of 0.82 kJ, compared to the criterion measure. Lastly for EEE^{IMU}, unstandardized coefficient was 1.28, yielding a mean underestimation of 0.28 kJ.

Table 2. Display regression coefficients representing the mean change for specific devices as predicted by the regression model, compared against the criterion measure VO_2. The first part of the table displays the values for total EEE, with the second part showing values adjusted by removing EPOC measurements, only analyzing moving time.

	GPS^1	**GPS^2**	**IMU**
N	13	11	11
Intercept	1038.5	1043.1	1016.2
Beta	1.42	1.82	1.28
t	2.75	2.75	2.1
Absolute residual error (kJ)	112.3 ± 78.7	109.1 ± 89.4	121.6 ± 92.3
p value	0.019	0.022	0.077
95% CI	0.3–2.5	0.3–3.3	0–2.6
Values adjusted for EPOC are presented below			
Intercept	867.8	875.4	846.8
Beta	1.23	1.66	1.11
T	2.86	3.16	2.1
Absolute residual error (kJ)	97.3 ± 59.9	89.1 ± 67.2	103.6 ± 72.1
p value	0.015	0.011	0.69
95% CI	0.3–2.2	0.5–2.8	0–2.2

Adjusting the caloric estimation by removing EPOC from the estimated EEE_{VO2} score, produced lower unstandardized coefficients, hence yielding lower mean differences between estimated and true caloric expenditure values (0.23 kJ for GPS^1, 0.66 kJ for GPS^2 and 0.11 kJ for IMU respectively (Table 2). The regression analysis also displays increased disagreement between predicted EEE values for tracking devices, compared to the observed EEE, as caloric expenditure increases. Thus, increased bias is expected when the caloric expenditure increases during intermittent activity (Figure 3).

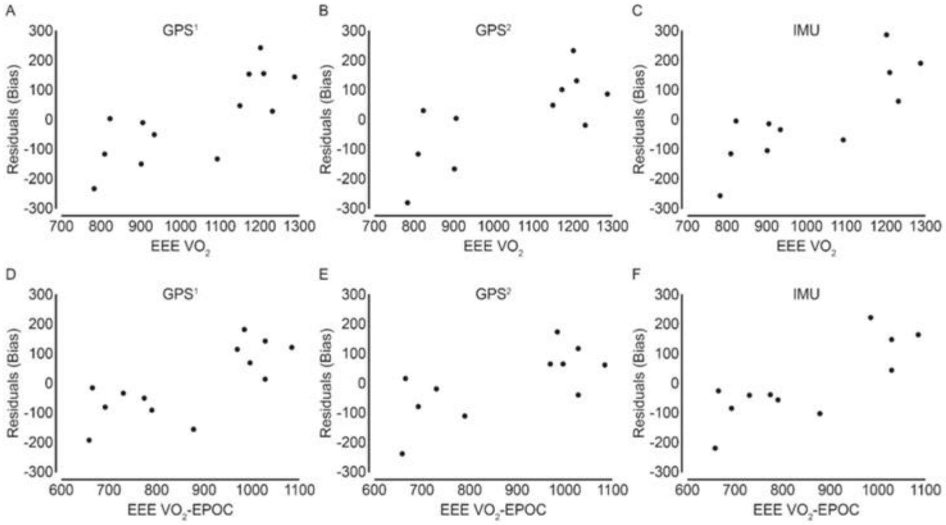

Figure 3. Residual plot indicating the disagreement between the predicted EEE (residuals) and measured EEE_{VO2} in the upper panel and EEE_{VO2}-EPOC in the bottom panel (values displayed in kJ). Negative values indicate overestimation and positive values indicate underestimation. Exercise energy expenditure = EEE; kilojoules = kJ; oxygen consumption = VO_2; excess post-exercise energy consumption = EPOC.

4. Discussion

This is the first study to quantify the accuracy of the latest tracking devices in female athletes. Our results show that all tracking devices underestimated the EEE and, thus, failing to adequately estimate caloric expenditure, yet GPS^1 provided the most accurate results. The level of underestimation shrunk for all devices when the criterion measure (indirect calorimetry) was adjusted by subtracting EPOC (the standstill rest periods) from the estimation of energy expenditure. Furthermore, the tracking devices displayed a systematic pattern of bias by overestimating EEE at lower levels of caloric expenditure and underestimating EEE at higher levels of caloric expenditure.

These findings align with several previous studies conducted among male athletes, reporting that GPS and accelerometer-based tracking devices fail to accurately estimate total EEE in intermittent team sports [12,18,20]. Albeit with older technology, Brown et al. [16] reported that GPS units displayed reasonable accuracy during steady-state jogging and running [20]. However, substantial underestimation was observed during intermittent movements and high-intensity actions. Stevens et al. [16] reported that metabolic power [16] overestimated EEE during continuous, steady-state running. Conversely, these authors also found that metabolic power underestimated EEE during aerobic shuttle running. As such, it is possible that the metabolic power algorithm systematically overestimate EEE during walking and jogging and underestimate during high-intensity circuit movements, resulting in total underestimation of EEE during high-intensity intermittent exercise. Recently, Savoia et al. [16] proposed an alternative metabolic power algorithm based on the original work [22]. Here the authors claim that previous studies demonstrating underestimation of EEE using metabolic power, utilize prolonged periods of rest, not reflecting the actual demands of the game. This may be of importance in the interpretation of the present, and the previous studies, as metabolic power primarily relies on locomotion when estimating EEE. Nevertheless, our results demonstrate that three of the latest and most-used tracking devices in modern soccer all underestimate EEE with its current technology.

In the present study, all three devices displayed total distances within 4.4% of the manually measured track. However, the metabolic power equation bases its calculations on velocity and accelerations; thus, the inability of tracking devices to accurately estimate this will influence the total estimated EEE. Previous research has found interindividual differences between devices utilizing metabolic power, although displacement measures were relatively similar, indicating disparities in the filtering of the GPS data [15]. Nonetheless, acceptable validity and reliability for GPS devices of 10 and 18 Hz, as used in this study, have been reported [38]. Further, the inertial sensor used have been compared against high sampling GPS units [28]. Although SD varied between the specific devices, this alone is unlikely to explain the discrepancy in EEE. Hence, the underestimation seen during intermittent exercise might be highly influenced by the algorithm applied by the devices, rather than inaccurate sampling rates or measurements of velocity/acceleration. This assumption is strengthened when investigating the results with and without EPOC measures. Several assumptions are made in the modeling of metabolic power, especially during high intensity running, including running efficiency [17,39]. Further, surface may also play an influential role on the energetic cost of running [40], together with individual running economy. As the metabolic power model is based on well-trained male endurance athletes and this study was done in female soccer players on an artificial surface, these factors may well be partially responsible for some of the observed discrepancies. Future studies could therefore consider tailoring the metabolic power equation for females. Further, practitioners may consider applying individual data for running cost to the equation responsible for the EEE output. This would require substantial testing of each athlete, as well as post session/match editing of the GPS derived data to calculate the EEE using the athlete specific data as constants in the metabolic power equation. Since individuals clearly differ in response to estimates of metabolic power based on average data, this could be of interest in athletes where accurate measurement of energy availability is of special importance for health and performance outcomes.

Our results show an inverse relationship as EEE^{VO2} increases. In addition, individual lactate measurements increase together with EEE^{VO2}. As such, it appears that the metabolic power estimates are somewhat correlated with exercise intensity. This is confirmed when adjusting the analysis by subtracting EPOC measurements, resulting in non-statistically significant differences between all devices and EEE^{VO2}. These results indicates that tracking devices are unable to sufficiently account for anerobic energy metabolism, manifested by elevated VO_2 levels during rest (e.g., replenishing substrate stores, repaying O_2 debt from the previous high-intensity action) [13], similar to previous research [18].

Several studies investigating LEA in female athletes have used devices relying on metabolic estimates for EEE, to calculate EA [9,10,41,42]. Nonetheless, based on the findings of our study, caution should be taken when interpreting results from studies utilizing algorithmical estimates to quantify EEE. Undoubtedly, the main challenge when identifying LEA in athletes is the definition of EA itself, as it relies heavily on measures of EI and EEE, both fragile for significant error [11]. As such, more objective physiological markers have been proposed going forward within the field of LEA [43]. These include the usage of hormonal data; however, more research is needed regarding the sensitivity and specificity of distinctive markers.

The sample size may raise concerns with response to statistical power. This could have been increased with repeated measures, which was not possible due to the heavy schedule of the players. Nevertheless, anthropometric characteristics are similar to those reported in international and high domestic leagues elsewhere [41]. As such, we will argue that the present results are applicable to other elite female soccer players. Moreover, power is not critical as the main aim of the study was not to test specific hypotheses, but to test the level of accuracy against indirect calorimetry for each tracking device. Conversely, power is a concern in the sense that increased power would have allowed us to test accuracy across the different tracking devices. We chose extended periods of rest between rounds and post activity, to account for EPOC. During actual gameplay, prolonged periods of total rest are sparser and, thus, may have contributed to the deviation between tracking devices and VO_2 measurements. We also acknowledge that 120 s of EPOC measurement post-test is not sufficient to return to baseline levels, hence, expanding the time would likely increase discrepancy. However, given the slope of EPOC [42], 120 s will encompass the majority of significant elevation in energy expenditure.

5. Conclusions

To our knowledge, this is the first all-female study exploring estimated EEE, as well as comparing the latest model of three widely used tracking devices. The GPS and accelerometer-based tracking devices tested generally underestimate the caloric expenditure during intermittent exercise in professional female soccer players. This is primarily because such devices cannot account for anaerobic energy production seen during high-intensity exercise. Furthermore, the observed differences between manufacturers could be of importance for practitioners and their choice of equipment. Therefore, caution should be taken when utilizing estimated EEE in calculations of EA and nutritional calculations. This is of special importance in training situations, where increased rest times between drills and play is likely to produce greater underestimation of total EEE. Nevertheless, as calculations based on EEE is the only method for assessing players EA at this point, the usage of devices applying metabolic power is presumably superior to standard GPS and heart rate measures. However, the deviations seen in caloric expenditure must be considered by practitioners and researchers depending on the need of accuracy. Future studies should also aim to include female players in the validation of the algorithms, as well as individualizing the algorithm. Despite generally underestimating EEE, the devices tested can still provide useful information in quantifying EA, taking the highlighted limitations in consideration.

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Informed Consent Statement: Written and verbal informed consent was obtained by all participants involved in the study.

Data Availability Statement: Data presented are available on request from the corresponding author.

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Energy expenditure, dietary intake and energy availability in female professional football players

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ABSTRACT

Objectives To quantify energy expenditure and intake in professional female footballers playing on a national and/or international level. Second, to determine the prevalence of low energy availability among these players, defined as <30 kcal/kg fat-free mass (FFM)/day.

Methods Fifty-one players completed a 14-day prospective observational study during the 2021/2022 football season. Energy expenditure was determined using the doubly labelled water method. Energy intake was assessed using dietary recalls, while global positioning system determined the external physiological load. Descriptive statistics, stratification and the correlation between explainable variables and outcomes were conducted to quantify the energetic demands.

Results The mean energy expenditure for all players (22±4 years) was 2918±322 kcal. Mean energy intake was 2274±450 kcal, resulting in a discrepancy of ~22%. Carbohydrate intake was below the recommended guidelines on match day at 4.5±1.9 g/kg. The mean energy availability was 36.7±17.7 kcal/kg FFM/day on matchday and 37.9±11.7 kcal/kg FFM/day on training days, resulting in a prevalence of 36% and 23% for low energy availability during the observational period, respectively.

Conclusion These elite female football players displayed moderate energy expenditure levels and failed to meet the recommended levels of carbohydrate intake. In conjunction with inadequate nutritional periodisation, this will likely hamper performance through inadequate muscle glycogen resynthesis. In addition, we found a considerable prevalence of low energy availability on match and training days.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Female international players show inadequate energy intake in relation to their energy expenditure.
- ⇒ Evidence on the prevalence of low energy availability in female football players is currently conflicting due to few high-quality studies.
- ⇒ No studies have investigated in-season energy requirements and practices in both national and international level players using doubly labelled water.

WHAT THIS STUDY ADDS

- ⇒ Considering current guidelines, domestic and international female football players display reduced carbohydrate intake independent of player position.
- ⇒ Female football players may be at risk for low energy availability; however, the prevalence is likely inflated due to under-reporting energy intake.
- ⇒ There were no positional differences in total daily energy expenditure.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ Players and team support staff should focus on nutritional periodisation to increase energy intake, notably through carbohydrates, to maximise performance and reduce negative health consequences associated with low carbohydrate and energy availability.

INTRODUCTION

Professional female football players generally cover 9–11 km during a match, with 22%–28% in high-intensity running and sprinting.[1 2] A typical 7-day in-season period may consist of 1–2 matches plus 4–6 additional training sessions.[3] Adequate energy intake (EI) (minimum above 30 kcal/kg fat-free mass (FFM)/day) is paramount to optimally performing and maintaining immune and metabolic functions.[4] Correct information about energy expenditure (EE) is essential for quantifying food requirements[4] to sufficiently fuel the energetic demands during matches and training. Although the physiological demands of female football are established in the literature,[2 5] studies regarding EE are scarce, and most estimates rely on accelerometers or global positioning systems (GPS).[3 6–8] In contrast, studies on male professional football players have used the gold standard method of doubly labelled water (DLW) and dietary information to quantify the daily energetic requirements.[9 10] Only one study using DLW in female football players exists to date.[11] Yet, this sample of players on international duty limits the findings, as it transfers less well to in-season national-level teams. Hence, scaled up DLW studies are needed to ascertain the energetic requirements of elite female football players.

During a football match, 60%–70% of the total energy requirements are supplied by carbohydrates (CHO),[12] making it the most important macronutrient for performance.[4 10] A recent expert group statement from UEFA recommends a daily CHO intake of 3–8 g/kg/day, depending on the intensity and volume of the activity (ie, training or match).[12] Previous studies among professional male[9 10] and female[11] players have shown that the total CHO intake is well below the recommended guidelines. In addition to CHO, protein is also essential to support skeletal muscle recovery and adaptation following exercise.[13] In contrast to CHO, the daily recommendations of 1.2–1.6 g/kg protein are generally met among female football players.[3 11]

There is an increasing focus on implementing female-specific research within sports medicine and nutrition.[14] This includes the female athlete triad and the Relative Energy Deficiency in Sports (RED-S),[15 16] in which low energy availability (LEA) is the main aetiological factor. As CHO constitutes the main macronutrient to meet total energetic needs in football players, the subsequent low intake reported in recent literature may not only reduce performance but also cause the development of negative symptoms described by the RED-S model.[3 11 17] To date, prevalence estimates of LEA among female football players are wide-ranging, between 20% and 80%,[3 6 11 18] and require more and larger high-quality studies. The primary aim of the present study was to quantify the total daily EE (TDEE) and EI in national and international female football players. Second, we aimed to estimate the prevalence of LEA in this sample.

MATERIAL AND METHODS

Participants

Fifty-one players from the Norwegian premier (two teams) and first division (one team) were included (table 1). Eight players were currently representing their national team, while seven players represented their designated youth national team. Using the recently published participant classification framework,[19] players were classified at tier 3 (national) or 4 (international) level. All players agreed to and signed a written consent form before partaking in the study. Inclusion criteria were as follows: (1) above 16 years of age, (2) no injury or illness affecting normal activity level and (3) being eligible for first-team competition matches. Seven players were excluded due to reported injury or illness during the study period (see online supplemental material for participant information).

Study design

Using a prospective observational study design, data collection occurred in three phases during the 2021 and 2022 Norwegian season (October–May). During the 14-day study period, players continued their regular living patterns, including training and match obligations.

Body composition

Body composition was measured using whole body fan beam dual-energy X-ray absorptiometry measurement (Prodigy, Encore, SP 4.1, V.18, GE medical systems, Madison, Wisconsin, USA) by a certified technician, following recommended guidelines.[20] All participants received a personal scale (Logic, London, UK) to which they were able to measure their body mass (BM) at home. BM was measured in the morning after the first voiding on days 1, 7 and 15 to the nearest±0.1 Kg. Before the data collection, participants were instructed to complete the measurement and send the results to a research team member following each weigh-in.

Training and match load

Training sessions and match performance were monitored using a GPS placed between the player's scapulae in a custom vest (Statsports, Newry, Ireland). Predetermined variables were recorded to quantify the total distance covered and metres in speed zones to determine the external physiological load. Standardised thresholds to determine workload intensity were used (table 2 specifies the various speed zones ranging from 1 to 5, coinciding with the measurement of EE by DLW). The coaching staff were familiar with the usage of the GPS; however, it was not applied to recovery sessions related to matchdays. Goalkeepers were excluded from the training and match load analysis.

Energy intake

EI was assessed by three 24-hour diet recalls, conducted using nutritional analysis software developed for research purposes with access to the Norwegian nutritional register (Myfood24, Leeds, UK). The software has previously been

Table 1 Participant characteristics

	Total (n=51)	Defender (n=15)	Midfielder (n=20)	Attacker (n=11)	Goalkeeper (n=5)
Age	22±4	23±4	21±3	21±5	20±3
Height (cm)	169±7	167±6	168±6	168±6	173±3
Body mass (kg)	63.9±6.6	62.4±5.9	62.9±5.2	63.4±5.8	75±6.4
Free fat mass (kg)	49.3±4.9	48.7±5.2	48.1±3.7	49.2±4.9	56.8±2.1
Body fat%	24.6±4.2	23.6±3.2	24.7±3.2	23.6±4.4	30.4±4.2

Table 2 Total distance and time in speed zone on training and match days

	Training (n=51)	Match (n=38)**
Total distance, meters (m)	5063±982	10309±1080
Speed zone 1, m (0–5.4 km/hour)	1975±360	2766±391
Speed zone 2, m (5.4–10.8 km/hour)	1867±432	3894±506
Speed zone 3, m (10.8–14.4 km/hour)	705±212	2072±345
Speed zone 4, m (14.4–19.8 km/hour)	379±146	1269±296
Speed zone 5, m (>19.8 km/hour)	90±91	323±169
Exercise energy expenditure (kcal)	422±83	826±98

Note: **p<0.01.
**p<0.001 for all comparisons.

validated against traditional dietary recalls and weighted dietary records in adults and adolescents, and details regarding the completion are explained elsewhere.[21 22] The recall method was chosen to ensure compliance. Before the data collection, the players were allowed to test the software, received general usage guidance and could ask questions to settle uncertainties. For players unable to conduct the diet recall in their native language (n=4), a registered dietician conducted the diet recall with the participants in English using video or in-person meetings. A registered dietician reviewed completed recalls. If any part of the registered EI was deemed insufficient or unclear, the dietician would validate this with the participant via telephone or text. The diet recalls were performed on random days corresponding to one match, training and rest days. Match days were considered valid if a player completed 60 min of the game. For players not fulfilling this requirement, two diet recalls were conducted on training days to ensure an equal number of completed diet recalls.

Energy expenditure

TDEE was measured using DLW. The protocol used is developed by Maastricht university and described elsewhere.[23] In brief, individual doses were calculated from total body water, estimated from body mass index). The players collected a baseline urine sample at home, following training, in the evening before going to bed (day 0). After collecting the baseline sample, the participants consumed a weighted amount of ${}^2H_2O$ and $H_2{}^{18}O$, providing a body water enrichment of approximately 155 p.p.m. for H^2 and 235 p.p.m. for ${}^{18}O$. Following this, urine samples were collected on days 1, 7 and 14 from the second voiding in the morning. A second urine sample was collected in the afternoon or evening on the same days. Participants were instructed in urine sample collection and conducted this procedure at home using standardised urine cups. Urine samples were stored in the participants' homes refrigerators for no longer than 24 hours. A research team member then collected the urine sample within the given time frame. Urine samples were immediately taken to the lab, aliquoted to a 2 mL airtight glass vial and stored in a −20°C fridge until analysis. Urine samples were analysed with an isotope ratio mass spectrometer (Thermo Scientific Delta V Advantage; Thermo Fischer Scientific, Bremen, Germany). Carbon dioxide production was calculated from the difference between the elimination rates of ${}^2H$ and ${}^{18}O$ using the equation as recommended by the IAEA DLW database consortium.[24] TDEE was calculated from carbon dioxide production, assuming a respiratory quotient of 0.85. Exercise EE (EEE) was also collected from match and training GPS data to quantify the players' EA using the metabolic power equation calculated by GPS software.[25] Physical activity level (PAL) was calculated from TDEE (DLW) and measured resting metabolic rate (RMR) (Vyntus CPX, CareFusion, Hoechberg, Germany, Sentrysuit v. 2.21.4), as well as the Cunningham and Harris Benedict equations, for comparative purposes. This was possible as players completed RMR measurements during the data collection, following the best practice guidelines for RMR measurements,[26] as part of a larger project.[27] A more thorough methodological description is available elsewhere.[28]

Energy availability

EA on match and training days were calculated using the estimated EEE derived by the GPS using the formula (EA = [EI – EEE] / FFM (Fat-free mass)).[29] The estimation of the average EA during the 14 days was calculated using the method described by Morehen *et al.*[11] Specifically, the thermic effect of food (TEF) was assumed to be 10% across individuals,[30] estimating activity EE (AEE) possible through the formula (AEE=TDEE – [RMR+TEF]) and subsequently EA (EA=EI – [AEE/FFM]). LEA was defined as <30 kcal/kg FFM/day, consistent with previous literature.[15 29]

Statistical analyses

Descriptive statistics are presented separately for the total training load on match and training days. Average daily EI was calculated using the weighted mean from training, match and rest days. EI on different days was allocated a percentage weight based on their frequency during the

Table 3 Energy and macronutrient intake on training, match and rest days

		Training	Match	Rest
Energy intake	Kcal/day	2247±485	2468±834	2195±834
Carbohydrate	g	253±73	289±115	244±101
	g/kg	4.0±1.3	4.5±1.9	3.9±1.6
Protein	g	102±23	105±40	95±35
	g/kg	1.6±0.4	1.7±0.7	1.5±0.6
Fat	g	85.3±25	93±1.4	86±42
	g/kg	1.3±0.4	1.4±0.7	1.3±0.7

study period. The difference between TDEE and EI, physiological load, and the differences between energy and macronutrient intake on training, match and rest days were analysed using paired Student's t-tests, corrected for familywise error and the Holm's test. The mean EI and positional differences in TDEE and EI were assessed using one-way analysis of variance (ANOVA) or repeated measures ANOVA. Post hoc Holm's correction was made if a significant main effect was present. The relationship between TDEE and possible explanatory variables was tested using Pearson's r. The statistical analysis followed best practice guidelines[31] and was conducted with JASP (V.0.16.4). The alpha level was set to $p<0.05$, and all data are presented as mean±SD unless otherwise specified.

RESULTS

Training and match load

Table 2 presents the average physiological work completed by the players on match and training days. The mean number of match and training days were 1.7±1.5 and 10.7±0.9, respectively. For all speed zones (1–5), the intensity was higher ($p<0.001$, table 2) on the match vs training days. There was also a statistically significant difference in the total distance on the match vs training days ($p<0.001$).

Energy intake

The average weighted EI was 2274±450 kcal. Table 3 provides an overview of the energy and macronutrient intake on training, match and rest days. There was significantly higher EI on match (2468±834 kcal) vs rest days (2195±834 kcal, $p=0.046$), but not for training (2247±485) vs match ($p=0.094$) or training vs rest days ($p=0.647$). For CHO intake, there was a significant difference between training (4.0±1.3 g/kg) and match days (4.5±1.9 g/kg, $p=0.025$) and match vs rest days (3.9±1.6 g/kg, $p=0.004$). There was no significant difference between training and rest days ($p=0.429$).

We found no statistical differences in protein or fat intake on match (protein 1.7±0.7 g/kg, fat 1.4±0.7 g/kg), training (protein 1.6±0.4,) g/kg, fat 1.3±0.4) g/kg) and rest days (protein 1.5±0.6 g/kg, fat 1.3±0.7 g/kg) or in terms of energy and macronutrient intake according to player positions. Table 4 provides an overview of EI and macronutrient distribution for the different groups. For macronutrient and caloric distribution, see online supplemental figures 1 and 2, online supplemental file 1.

Energy expenditure

The average TDEE was 2918±322 kcal (45.4 kcal/kg). During the measuring period, the players' BM did not significantly change, with a mean of 65±7.9 kg and 64.7±7.8 kg for days 0 and 14 ($p>0.05$), respectively. Hence, the discrepancy between EE and EI indicates under-reporting nutritional intake by ~22%. The average PAL value was 2.0±0.3 (measured RMR), 1.98±0.2 (Harris-Benedict equation) and 1.89±0.2 (Cunningham equation), respectively, all displaying moderate PAL (table 4 and figure 1). There was a statistically significant positional difference in TDEE between goalkeepers and defenders ($p=0.010$), midfielders ($p=0.001$) and attackers ($p=0.008$), respectively (table 4). The analysis demonstrated statistically significant differences in BM between goalkeepers and defenders ($p<0.001$), midfielders ($p<0.001$) and attackers ($p=0.001$), respectively. Lastly, we

Table 4 Daily energy expenditure and macronutrient intake for the whole group and different player positions

TDEE	Kcal/day	2918±322	2926±274	2817±325	2874±325	3393±258
	kcal/kg	45.4	46.9	44.8	45.3	45.2
	$PAL_{Measured}$	2.0±0.3	2.1±0.3	1.9±0.3	2.0±0.2	2.0±0.4
	$PAL_{Harris\text{-}Benedict}$	1.98±0.2	2.0±0.2	1.9±0.1	2.0±0.2	2.1±0.2
	$PAL_{Cunningham}$	1.89±0.2	1.9±0.1	1.9±0.1	1.9±0.2	2.0±0.2
Daily EI	Kcal/day	2274±450	2393±516	2322±442	2137±321	1985±419
Carbohydrate	g	250±70	258±89	260±68	227±37	229±57
	g/kg	3.9±1.1	4.2±1.4	4.1±1.1	3.6±0.7	3.3±0.8
Protein	g	99±21	104±21	99±22	90±16	99±15
	g/kg	1.5±0.4	1.7±0.4	1.6±0.3	1.4±0.3	1.3±0.2
Fat	g	84±24	92±19	84±22	80±31	71±18
	g/kg	1.3±0.4	1.5±0.3	1.3±0.4	1.2±0.5	0.9±0.3

EI, energy intake; PAL, physical activity level; TDEE, total daily energy expenditure.

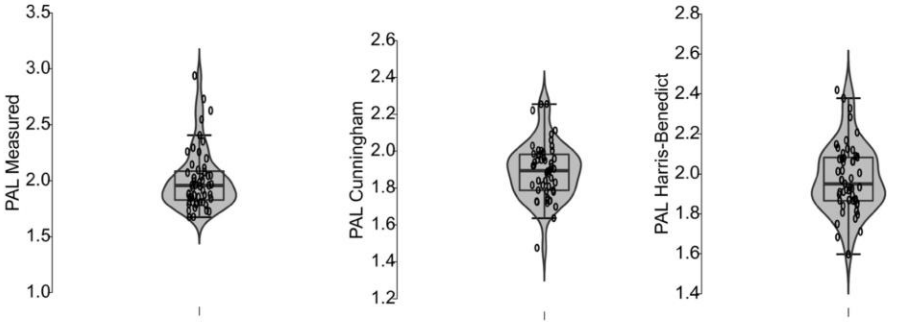

Figure 1 Mean and individual PAL based on measured RMR (A), the Cunningham equation (B), and the Harris-Benedict equation (C). PAL, physical activity level; RMR, resting metabolic rate.

found a significant linear relationship between TDEE-BM (r=0.64, p<0.001), TDEE-FFM (r=0.73, p<0.001), TDEE-height (r=0.49, p<0.001), TDEE-RMR (r=0.58, p<0.001) and TDEE -AEE (r=0.76, p<0.001) (figure 2). The corresponding non-linear quadratic effects were not significant.

Energy availability

EA derived from EEE (table 2) showed that the average EA was 36.7±17.7 kcal/kg FFM/day on matchday and 37.9±11.7 kcal/kg FFM/day on training days, with a prevalence of 36% and 23% for LEA, respectively. Average EA derived from AEE (TDEE – [RMR+TEF], was 21.6±10.7 kcal/kg FFM/day, indicating a prevalence of 74% for LEA.

DISCUSSION

This study aimed to determine TDEE through the DLW method, together with the quantification of EI and prevalence of LEA in professional female football players.

Energy expenditure

TDEE during the 14 days was 2918±322 kcal, which may be considered moderate.[32] Furthermore, the estimated PAL based on RMR demonstrates that the Harris-Benedict equation is more accurate for female players

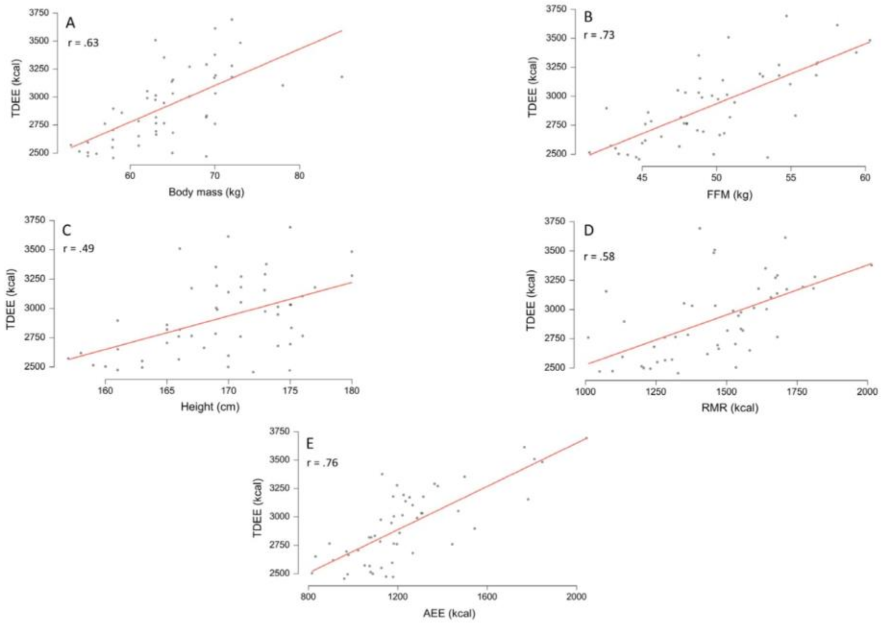

Figure 2 Correlation between TDEE (kcal) and explanatory variables body mass (A), FFM (B), height (C), RMR (D) and AEE (E). AEE, activity energy expenditure; FFM, fat-free mass; RMR, resting metabolic rate; TDEE, total daily energy expenditure.

than the Cunningham equation. Our results are similar to those of Morehen *et al*,[11] who speculated that energy requirements on the international level are greater than national-level teams. Our results contraindicate this statement and, as such, provide an evidence base for future nutritional guidelines, including both international and national level players. Our results also show that TDEE in elite female players ranges between 35.8 and 55.7 kcal/kg, analogous to elite male players.[9 10] These findings confirm that based on EE, nutritional recommendations should not differ between sexes.[12] Nevertheless, several mechanisms may affect substrate metabolism, and more research regarding potential sex differences is needed.[33] Although the energetic demand of female football seems moderate, the strain on muscles in terms of eccentric loading through repeated accelerations and decelerations is immense and must be accounted for.[34 35]

Energy intake

Given the mean EI of 2274±450 kcal, the use of DLW assessed TDEE as a reference, and the non-significant changes in BM, the level of under-reporting in EI (about 22 %), is comparable to other athlete studies.[36] Similar studies in elite female football players have reported estimated EI ranging from 1923 to 2387 kcal.[3 8 11 18] Thus, our findings converge with previous studies on the absolute level of EI and previous findings of under-reporting EI.[11] In the most recent UEFA expert group statement on nutrition in elite football, 6–8 g/kg of CHO is recommended on match day.[12] However, in our sample, only 24% of players met these recommendations. The same expert group statement recommends 3–8/kg on training days, depending on duration, intensity and player goals.[12] Our results show that over 50% of players had CHO intakes of 4 g/kg or lower on training days, which is likely inadequate for most of this cohort. If corrected for under-reporting by increasing EI by 22%, assuming equal distribution of macronutrient intake, the average CHO intake is still only 5.4 g/kg on match days, while over 30% of players still present with an intake of 4 g/kg or lower. These findings indicate that female elite football players generally consume inadequate amounts of CHO, which may hamper performance as muscle glycogen stores are unlikely to be adequately replenished for matches.[34 35] Our findings also provide evidence of minimal periodisation of caloric intake in relation to external work performed, contrary to current guidelines.[12 37] Conversely, protein intake was well within the recommendations for training, match and rest days. The disproportionately low CHO intake in the current study may be interpreted in light of recent findings among female professional football players,[38] stating that reduced adherence to current nutritional guidelines may be due to misconceptions about the impact of CHO on body composition, contributing to weight gain.

Energy availability

Using the classification by Loucks *et al*,[29] our findings that 23% of the players presented with LEA on training days and 36% on match days align with previous findings among tier 3 and 4 athletes,[3 18] in principle supporting the notion that female football players may be at risk for LEA during the season. Applying the same correction factor by increasing EI by 22%, the prevalence of LEA was 7% on training days and 29% on match days, suggesting a considerable reduction in actual incidence. In terms of the estimated average EA for the entire 14-day period, 76% of the players presented with LEA. However, this number was reduced to 45% when applying the correction factor. Again, these numbers are comparable to recent findings using similar methods but higher than those reported using EEE to quantify EA.[3 6 39] As our estimates converge with previous findings, this indicates that under-reporting may also have been present in previous studies.

Strengths and limitations

Although being the first study to provide measures of TDEE using DLW in a sample of national and international female football players, there are limitations that need consideration. The first concerns the use of the self-reported dietary method for assessing EI. However, this generally applies to studies using dietary assessments. In addition, the 24-hour diet recall method has been shown to provide estimates ranging between 8% and 30% of under-reporting, thus, providing better accuracy than most comparable methods.[40] By applying average weighted EI based on match, training and rest days, similar to previous studies,[10] we were able to provide assessments of EI with a high degree of compliance from the participants. Nevertheless, there appears to be some systematic error related to the measurement of EI in this study.[36] A second possible limitation concerns the estimation of EEE, which was calculated using metabolic power based on GPS, as this device has been shown to underestimate high-intensity bouts of intermittent exercise.[41] Regarding EA, we acknowledge the methodological difficulties of applying overall AEE. However, we believe it provides insight for comparisons between different measures of EA. Lastly, our classification of LEA (<30 kcal/kg FFM/day) is based on laboratory studies with high internal validity, whereas new evidence suggests that daily EA is more heterogeneous.[42] This increases some uncertainty in the estimates of LEA.

CONCLUSION AND IMPLICATIONS

In conclusion, in-season international and national female football players show moderate levels of TDEE, comparable to what has been reported in professional males. The fact that female players fail to meet the recommended nutritional demands (notably CHO) should also be addressed by team supports staff and players. Lastly, our data indicate that the prevalence of LEA among female football players may be inflated due to underreporting of EI.

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Contributors The study was designed by MSD, MK, JHR, GPe, JKSB and OF. Data collection and analysis was conducted by MSD, MK and GPl. MSD serves as guarantor. All authors have been involved in the manuscript preparation, revisions and they have all authorised the final version.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and was approved by the Regional Committee for Medical and Health Research ethics (2016/787) and Norwegian Center for Research Data (807592). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data may be obtained from a third party and are not publicly available. Sharing of anonymous data will be considered on request.

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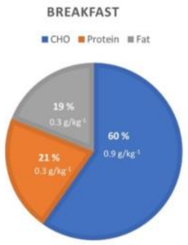
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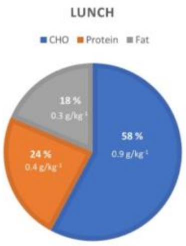
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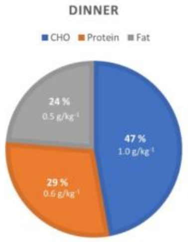
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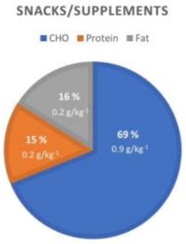
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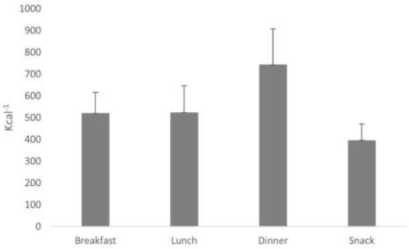
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Paper III

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Sports Medicine - Open

ORIGINAL RESEARCH ARTICLE **Open Access**

Evaluating the Suitability of the Low Energy Availability in Females Questionnaire (LEAF-Q) for Female Football Players

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Abstract

Background The Low Energy Availability in Females Questionnaire (LEAF-Q) is a screening tool developed to detect endurance athletes and dancers at risk for development of persistent low energy availability (LEA) and the female athlete triad (Triad). This study investigated the applicability of the LEAF-Q in a cohort of sixty professional female football players.

Methods The participants were classified as *at risk* (≥ 8) or *not at risk* (< 8) for persistent LEA and the Triad according to their LEAF-Q score, before being compared. Receiver operating curves were then conducted to examine the ability of the overall LEAF-Q and subcategories to correctly determine the presence of clinically defined markers of the Triad. Additionally, Youden's index was calculated to determine the best fitting cut-off values.

Results Thirty-two percent of participants were classified as *at risk* by the LEAF-Q. We found no statistically significant differences between the two groups for any markers associated with persistent LEA. Except for acceptable accuracy in determining menstrual status, all other LEAF-Q components exhibited poor accuracy and predictive values. Youden's index scores imply that increasing the overall and injury cut-off values to ≥ 10 and ≥ 5 respectively, would yield increased performance.

Conclusions Our findings do not support the use of the LEAF-Q for the purpose of detecting LEA and Triad conditions among female football players.

Key points

- The LEAF-Q is a screening tool developed to identify female endurance athletes and dancers at risk for development of low energy availability and the female athlete triad. The questionnaire has been used in a wide range of athlete populations, including female football players, without sufficient knowledge about its applicability.
- The prevalence of acute and impact injuries in football, compared to endurance sports and dancing, may introduce substantial bias, leading to artificially inflated scores on the questionnaire.
- Our findings do not support the use of the LEAF-Q for the purpose of detecting symptoms of the female athlete triad and LEA among female football players.

Keywords Low energy availability, Football, Soccer, Female, Relative energy deficiency in sports, Female athlete triad, Athlete health

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Introduction

Energy availability (EA) is defined as the difference between energy intake (EI) and exercise energy expenditure. It is expressed relative to an individual's fat-free mass (FFM) and represents the residual amount of energy available to sustain all physiological functions [1]. Consequently, low energy availability (LEA) denotes the state where the body receives insufficient energy to optimally perform these functions. Despite emerging evidence indicating individual variation in response to LEA, it is commonly defined as < 30 kcal/kg^{-1} FFM/day^{-1} [2]. Persistent or severe LEA are recognized as the etiological underpinning of both the female athlete triad (Triad) and Relative Energy Deficiency in Sport (REDs) [3, 4]. The Triad encompasses three interrelated conditions: EA, menstrual function and bone health, all ranging on a continuum from health to disease, with LEA as a causal factor of Triad dysfunction [5]. The REDs model, however, describes a broader range of potential consequences affecting both health and sports performance among males and females, caused by LEA [3]. Despite discussions regarding the scientific rigor and causal evidence supporting the REDs model [6], it now contributes to the International Olympic Committee's (IOC) consensus statement and guidelines for supporting athletes' health [3].

The Low Energy Availability in Females Questionnaire (LEAF-Q) is a screening tool developed for endurance athletes and dancers, and is validated against clinical markers of the Triad and persistent LEA [7]. The LEAF-Q consists of 25 items that assesses three subcategories: injuries, gastrointestinal symptoms, and reproductive/menstrual function, respectively. The original study recommended that additional validation of the questionnaire is necessary before utilizing it beyond the intended population [7]. The clinical utility of the LEAF-Q was recently examined in a mixed-sport cohort of athletes within individual sports, as well as netball and water polo [8]. It was concluded that the LEAF-Q was suitable to "rule out" LEA-related conditions in athletes who scored below the originally published cut-off value, however, it failed to identify athletes "at risk" of the Triad or LEA with its associated symptoms.

Football is characterized by a mixture of high- and low-intensity efforts and actions, and is played on a relatively large pitch (90–120 m length, 45–90 m width) [9]. High-level female players usually cover between 9–11 km during a game [10]. As such, football sets itself apart from the majority of contemporary individual and team sports. Although LEA and its associated symptoms are thought to be most prevalent in endurance and weight-sensitive sports [3], studies have reported a wide range of prevalence estimates among female footballers, depending on the measurement methods applied [11–16]. This includes studies employing the LEAF-Q to screen for the Triad and LEA, despite insufficient evidence for its application in this population [12, 13, 17]. As LEA and subsequently REDs may have profound ramifications for athletes' health and performance, there is a need to investigate the suitability of commonly used measurement instruments among female football players. Therefore, the purpose of this study was to evaluate the applicability of the LEAF-Q as a screening tool for female football players. Using a cohort of professional female football players, we examined the capacity of the LEAF-Q to identify markers associated with the Triad and persistent LEA and to correctly classify players *at risk* for these conditions.

Methods

Study Design

In the present cross-sectional study, we conducted a comprehensive analysis of multiple clinical markers related to the Triad [7] and previously published literature on the subject [18–21]. The data collection was conducted between October 2021 and May 2022. Within two weeks each participant completed all measurements across two subsequent days.

Participants

Sixty female football players from three Norwegian teams were included in the study. Eight players were currently representing the Norwegian senior national team, while another eight players represented their designated youth national team. The participants were classified as tier 3 (national level) or 4 (international level) according to the athlete classification framework [22].

Body Composition and BMD

Body composition (% fat mass, FFM) and bone mineral density (BMD) were assessed in a fasting state using Dual-Energy X-Ray Absorptiometry (DXA; Prodigy, Encore, SP 4.1, version 18, GE medical systems, Madison, Wisconsin, USA), according to best practice guidelines [23]. Before completing the scan, body weight (Seca 869, Hamburg, Germany) ± 0.1 kg and height (Seca, Hamburg, Germany) was recorded. Participants, dressed in minimal attire (i.e., tights and t-shirt), were situated in the supine position, ensuring their body was properly aligned with the central longitudinal axis of the scan table. Both arms were positioned alongside the body, in neutral position to minimize overlapping of anatomical structures. Participants first underwent a total body scan for assessment of body composition. This was followed by an anteroposterior scan of the lumbar spine (L1-4) and bilateral hip densitometry to evaluate BMD. Automatic analysis was performed using the manufacturer's software (Encore, SP 4.1) and manually adjusted if indicated. All measurements and analyses were conducted by the same certified technician to avoid inter-rater variability and error.

Energy Availability

Measures of EA were estimated through resting metabolic rate (RMR). Specifically, RMR < 30 kcal/kg^{-1} FFM/day^{-1} or RMR$_{\text{Ratio}}$ < 0.90 using the measured value, and the Cunningham equation was considered indicative of LEA [24, 25]. RMR is considered a viable option for estimating EA, when used in combination with other markers, as well as being strongly correlated with energy deficiency and amenorrhea in exercising women [26, 27]. Due to significant challenges associated with the direct measurement of EA, and the lack of a gold standard method, it was deemed appropriate to utilize this surrogate marker [28, 29].

RMR was measured with the participants arriving at the test facility by motorized transportation between 06 and 09 a.m. in an overnight fasted state. Participants were placed in a silent room, in the supine position for 5 min, before a ventilated canopy hoodie (Vyntus CPX, CareFusion, Hoechberg, Germany, Sentrysuit v. 2.21.4) was positioned. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were then measured for 25 min, where the average value for the last 20 min was used to assess RMR.

Screening Instruments

Participants filled out the LEAF-Q after completion of RMR and DXA measurement, at the testing facility. The questionnaire was administered with a portable tablet, using a digital encrypted platform (Nettskjema, University of Oslo, Norway). Participants were classified as *at risk* (total score ≥ 8) or *not at risk* (< 8) for the Triad, according to the LEAF-Q scoring system [7]. Further, in accordance with the original publication, the LEAF-Q cut-off values associated with increased risk for Triad dysfunction were applied in the same manner (Injuries(≥ 2), gastrointestinal symptoms (≥ 2) and menstrual function (≥ 4)). As LEA may be present with or without disordered eating [30], the participants also completed the Eating Disorder Examination Questionnaire (EDE-Q 11). This has been extensively used to assess self-reported eating behavior pathology [31]. Information on the history of stress fractures, which has been strongly linked with LEA [32], was obtained through a custom-made question that specifically inquired about the injury position and frequency.

Menstrual Function

The LEAF-Q was used to determine menstrual status, i.e., eumenorrheic or amenorrheic (oligomenorrhea was considered as amenorrhea). Menstrual status could not be determined in participants who reported usage of hormonal contraception (55%). As it was not considered ethically acceptable to request cessation of hormonal contraceptive usage, the menstrual status was only classified in 27 of the participants (45%).

Blood Samples

After an overnight fasting period (8–10 h), blood was collected for both plasma and serum samples [33]. These samples were stored in Biobank Haukeland, Laboratory Medicine and Pathology, Haukeland University Hospital, Bergen, Norway, in 3.5 ml serum/gel vacutainers before analyses. Analyses that have either been directly linked to or associated with LEA were assessed. This included glucose, insulin, thyroid stimulating hormone (TSH), free triiodothyronine (T_3), free thyroxine (T_4), insulin-like growth factor 1 (IGF-1), and leptin, which were analyzed at the Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway. The laboratory is accredited in compliance with ISO 15189:2012. Glucose was analyzed using Cobas 8000, TSH, free T_3 and free T_4 were measured with Cobas e801. Insulin and IGF-1 were analyzed using Immulite 2000 XPi, whereas leptin was determined using an enzyme-linked immunosorbent assay kit (Mediagnost Cat#E07, RRID: AB_2813737)(non-accredited analysis).

Statistical Analyses

The statistical analyses were conducted using SPSS 28 (IBM, Armonk, NY, USA). Variables being non-normally distributed according to the Shapiro-Wilks test were described using median and range and between group variables examined with nonparametric tests (Mann–Whitney U). Otherwise, parametric tests (Welch's test for unequal sample size) and mean ± standard deviation (SD) were reported.

Descriptive statistics are provided for the whole sample, as well as separately for participants classified as *at risk* versus *not at risk* for symptoms of the Triad, as defined by their LEAF-Q scores. The alpha level was set to < 0.05.

We used receiver operating curve (ROC) analyses to examine the ability of the LEAF-Q to correctly determine the presence of clinically defined markers of the Triad. For this purpose, we report the area under curve (AUC), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), as well as the highest Youden's index [33] locating the best cut-off value for the overall and the subcategory LEAF-Q scores. These sample-derived discriminatory properties and cut-off scores were compared to the original overall and subcategory cut-off scores, as published by the original

Table 1 Descriptive data for all participants and stratified into groups (at risk/low risk of LEA) based on LEAF-Q score. Brackets indicate number of participants in the different groups

Measure	Total (60)	At risk (LEAF-Q ≥ 8) (19)	Low risk (Leaf-Q < 8) (41)	*P*
Age (Years)	22.5 [16, 32]	21.0 [17, 32]	22.0 ± [16, 32]	0.69
Weight (kg)	64.1 ± 6.3	64.3 ± 5.2	64 ± 6.7	0.88
Height (cm)	168.9 ± 6.0	169.1 ± 6.3	168.8 ± 5.8	0.86
Fat mass (%)	24.4 [16, 37]	25 [18, 33]	23 [16, 37]	0.39
BMI (kg/m^2)	22.4 [19, 29]	22.5 [19.6, 25.6]	22.4 [19.0, 29.1]	0.69
Weekly training volume (h) [a]	12.5 ± 3.2	12.5 ± 3.2	12.7 ± 3.1	0.82
RMR (kcal)	1464 ± 225	1460 ± 237	1467 ± 224	0.91
RMR$_{Ratio}$	0.96 ± 0.12	0.96 ± 0.15	0.95 ± 0.10	0.65
BMD lumbar spine (Z-score)	1.2 ± 1	1.0 ± 0.9	1.3 ± 1.0	0.45
BMD hip (Z-score)	2.1 ± 0.1	1.9 ± 0.8	2.1 ± 1.0	0.49
EDE-Q-11	0.8 [0, 3.8]	1 [0. 3.8]	0.6 [0, 3.4]	0.13
Leptin (ug/L)	6.5 [2.6, 32.4]	6.3 [2.7, 32.4]	6.7 [2.7,32.3]	0.83
Free T$_3$ (pmol/L)	4.9 ± 0.7	4.8 ± 0.8	5.1 ± 0.6	0.24
Free T$_4$ (pmol/L)	15.8 ± 1.9	15.1 ± 1.8	16.2 ± 1.8	0.05
TSH (mIU/L)	1.6 [2.8, 6.8]	1.6 [0.6, 5.0]	1.6 [0.6, 4.3]	0.98
Glucose (mmol/L)	4.6 [2.8, 6.8]	4.7 [3.8, 5.0]	4.5 ± [2.8, 6.8]	0.65
Insulin (mIU/L)	8.8 ± 8.7	7.5 ± 6.8	9.4 ± 9.5	0.45
IGF-1 (nmol(L)	29.1 ± 7.6	29.6 ± 7.2	28.9 ± 7.9	0.75

a = self-reported training volume excluding matches

BMI = body mass index, RMR = resting metabolic rate, EDE-Q = eating disorder examination questionnaire, T3 = triiodothyronine 3, T4 = thyroxine, TSH = thyroid-stimulating hormone, IGF-1 = insulin-like growth factor 1

authors [7]. The AUC estimates the overall capacity of the LEAF-Q to correctly discriminate Triad from non-Triad cases. The AUC value ranges between 0 and 1, with higher values indicating better discrimination. A non-discriminatory test has an AUC of 0.5 (50%), while higher AUC values represent better than random classification with AUC = 1.0 (100%) being perfect. AUC values > 0.70 (70%) are considered as fair, > 0.80 (80%) as good, and above > 0.90 (90%) as excellent. AUC values below 0.5 indicate reciprocal discrimination that is opposite of expected [34].

In the event of significant AUC values, precision recall curves (PRC) were additionally calculated as ROC analyses may be misleading in case of severely imbalanced data sets (e.g., skewed numbers of positive or negative cases) [35]. Since the majority of athletes generally are expected to be non-symptomatic, we were also interested in the ability of the LEAF-Q score to positively predict individuals with markers of LEA. As such, the PRC may provide additional information regarding the questionnaire's tenability through measures of *precision* (identical to PPV in ROC) $= \frac{\text{true positives}}{(\text{true positives+false positives})}$ and *recall* (identical to sensitivity in ROC) $= \frac{\text{true positives}}{\text{true positives + false negatives}}$. As none of these formulas involve the number of "true negative" (TN) cases, which is expected to constitute most cases in the present sample, the PRC curves are likely less biased due to the extreme skewness the true negative cases represent in the ROC curve.

Two participants were excluded from the respective analyses; one who was not able to provide blood samples (did not meet for the scheduled appointment), and one who could not provide a measure of RMR due to illness.

Results

There were no statistically significant differences between the two groups for any of the clinical markers associated with LEA, however, free T$_4$ had a P-value of 0.05. Descriptive statistics of the sample, which separates the women classified as *at risk* (LEAF-Q ≥ 8) and *not at risk* (LEAF-Q < 8) is presented in Table 1.

The overall LEAF-Q score had a mean value of 7.0 ± 3.0, whereas the mean values for the subcategories were 3 ± 2.3 (injury), 2 ± 1.7 (gastrointestinal symptoms) and 2 ± 2.4 (menstrual symptoms), respectively. Moreover, 32% were classified as *at risk* for the triad (LEAF-Q total ≥ 8). For the subcategories, 68%, 55% and 15% scored above the LEAF-Q cut-off score which are associated with LEA in the original publication [6] for injury (≥ 2), gastrointestinal symptoms (≥ 2) and menstrual symptoms (≥ 4), respectively.

For the overall LEAF-Q, the AUC index was poor for the clinical markers RMR$_{Ratio}$, BMD$_{lumbar}$, BMD$_{hip}$ and

Table 2 Diagnostic performance of the LEAF-Q overall score to identify individuals with clinical indicators of the Triad

Measure	AUC [95% CI]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RMR_{Ratio} (< 0.90)	0.46 [0.31, 0.62]	24	63	26	60
RMR_{Ratio} (< 0.90)[a]		14	90	50	66
BMD (Z-score < – 1)					
Hip[b]	–	–	–	–	–
Lumbar spine	0.53 [0.37, 0.68]	0	68	0	98
Lumbar spine[a]		100	44	3	100
Hip	0.44 [0.28, 0.61]	0	10	0	5
Hip[a]		45	100	100	44
Lumbar spine	0.65 [0.40, 0.91]	40	69	11	93
Lumbar spine[a]		40	95	40	95
RMR < 30 kcal/kg^{-1} FFM/day^{-1}	0.44 [0.29, 0.59]	28	66	37	56
RMR < 30 kcal/kg^{-1} FFM.day^{-1} [a]		12	91	50	59
Amenorrhea [27]	0.86 [0.69, 1.03]	75	79	60	88
Amenorrhea[a]		75	95	86	90

Cut-off values are based on consensus values derived in the literature

a = Best performing cut-off value based on Youden's index, b = indicates that no participant presented with the condition variable, not allowing for statistical analysis

EA = energy availability, LEA = low energy availability, AUC = area under the curve, CI = confidence intervals, RMR = resting metabolic rate, FFM = fat free mass, PPV = positive predictive value, NPV = negative predictive value, BMD = bone mass density

RMR < 30 kcal/kg^{-1} FFM/day^{-1} (AUC = 0.44 – 0.53), whereas detection of amenorrhea had a good AUC = 0.86. Table 2 provides estimates of sensitivity, specificity, PPV and NPV, respectively. The PRC for amenorrhea showed a precision of 67% and a recall of 75%, indicating a reduction in actual precision compared to the ROC analysis in terms of identifying athletes at risk. Furthermore, the Youden's index implies that a cut-off score ≥ 10 as opposed to ≥ 8 would be more appropriate for this cohort.

For the subcategories, the AUC index performed poorly in detecting clinical markers of LEA for BMD_{hip}, EA, and stress fracture (AUC = 0.43–0.47). AUC to detect amenorrhea was excellent (0.93), but fair (0.78) in detecting BMD_{lumbar}, indicating overall good performance for these subcategories (Table 3). The PRC for amenorrhea showed a precision of 70% and a recall of 88%, while BMD_{lumbar} had a precision of 2% and recall of 100%. The Youden's index implies that an increase in cut-off score to ≥ 5 as opposed to ≥ 4 yield a better accuracy for the injury subcategory.

Discussion

The current study aimed to examine the applicability of the LEAF-Q to identify markers associated with the Triad and persistent LEA and correctly identify players *at risk* for these conditions. In terms of broad indicators associated with LEA, no statistically significant differences were observed between the groups. While T_4, an important metabolic regulator, approached significance, this tendency was not resembled by other hormones, such as T_3. It is notable that T_3, more closely associated with LEA in the literature, is derived from T_4, yet did not follow the same trend [36, 37]. It is possible that further reduction in T_4 levels could induce changes in T_3 within the *at risk* group, thereby exacerbating their risk for metabolic alterations associated with LEA. Melin et al. found significant differences between groups for several LEA related hormones, including leptin, T_3 and glucose [7]. Consequently, our findings align more closely with those of Rogers et al. who found minimal variation in LEA indicators between mixed sport athletes, categorized by the LEAF-Q [8]. Pertaining to our results, the observed uniformity in LEA indicators across the groups is consistent with the general performance of the LEAF-Q in this study.

The overall performance of the LEAF-Q in detecting menstrual dysfunction was commendable, evident by an AUC of 0.86, suggesting that the original cut-off value of ≥ 8 is appropriate for this indicator. However, the questionnaires effectiveness in identifying players presenting with clinical symptoms of the Triad was suboptimal, rarely performing better than guessing by random. Further, among the recalculated cut-off scores, only amenorrhea boasted a Youdens's index above 50%, demonstrating poor performance for the recalculated cut-off scores as well. This underscores the apparent disconnect between the perceived risk, as determined by the LEAF-Q assessment, and the tangible manifestation of Triad and LEA indicators. It is important to note that

Table 3 Diagnostic performance of the LEAF-Q subcategories (injury, gastrointestinal symptoms, and menstrual function) to identify individuals with the associated indicators of the Triad, based on the original publication

Cut off-score	AUC [95% CI]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Injury ≥ 2					
BMD (Z score < -1)					
Lumbar spine	0.78 [0.61, 0.96]	100	32	2	100
Lumbar spine[a]		100	66	5	100
Hip		–	–	–	–
History of Stress fracture	0.43 [0.25, 0.62]	54	28	18	68
BMD (Z score < 0)					
Lumbar spine	0.77 [0.59, 0.95]	80	33	10	95
Lumbar spine[a]		80	69	19	97
Hip	0.47 [0.01, 0.92]	50	31	2	95
Hip[a]		50	66	5	97
Gastrointestinal symptoms ≥ 2					
RMR < 30 kcal/kg/FFM[b]	0.45 [0.31, 0.60]	56	46	42	59
Menstrual function ≥ 4					
Amenorrhea (27)[b]	0.93 [0.83, 1.03]	88	90	78	94

LEA Low energy availability

a = best performing cut-off score based on Youden's index; b = indicates that the original cut-off score is the best performing based on Youden's index

the prevalence of positive indicators for Triad and LEA was relatively low in this cohort, which is consistent with previous assumptions [38]. This could partly explain the poor diagnostic performance relative to previous investigations [7, 8]. Despite this, the LEAF-Q was still unable to accurately identify individuals without signs of LEA, strengthening the overall weak performance observed.

For the injury subcategory, the LEAF-Q demonstrated a fair AUC, as well as excellent sensitivity. Nevertheless, the specificity and PPV was very poor, showing that the LEAF-Q would fail to identify individuals with compromised markers, given a higher prevalence of the condition. The mean injury score was 3.0 ± 2.3 in the context of an overall mean score of 7.0 ± 3.8, resulting in a 68% prevalence above the ≥ 2 cut-off value. This indicates a systematic bias toward elevated injury scores among the participants. It is important to note that the LEAF-Q was originally validated for endurance athletes and dancers [7], who primarily experience overuse injuries [39, 40]. Football, on the other hand, is a high-impact sport with potential for both acute and overuse injuries [41]. This crucial distinction is not accounted for by the LEAF-Q, consequently leading to skewed scores and biased results. Furthermore, the gastrointestinal subcategory performance in detecting athletes with Reduced RMR_{Ratio} or $RMR < 30$ $kcal/kg^{-1}$ FFM/day^{-1} (LEA) was very poor, with an AUC of 0.45. The original LEAF-Q included gastrointestinal symptoms as this have been reported in female athletes suffering from disordered eating and or eating disorders [42]. It is possible that these disorders are underrepresented in football, thus making gastrointestinal symptoms inappropriate as a clinical marker among female footballers.

Although the prevalence of LEA and REDs is equivocal in female football players, access to a quality screening tool is necessary. A recent investigation revealed that English female football players exhibit insufficient nutritional knowledge and express apprehension regarding carbohydrate consumption [43]. Moreover, the available literature indicates that female football players may not ingest adequate energy amounts to support optimal performance and recovery [11, 15]. Nonetheless, the existing version of the LEAF-Q lacks the necessary predictive capacity for usage among female footballers. The questionnaire was also developed before recent advancements related to REDs, primarily focusing on the causal relationship between LEA, menstrual disorders, and BMD [4, 20]. As such, resources should be allocated to further exploration of reliable surrogate markers in line with future developments. Connected to this, a recent debate has also emerged about the BMD Z-score thresholds for high-impact sports like football. As athletes experiencing high amounts of mechanical loading are expected to have elevated BMD compared to controls, utilizing the same threshold of < -1 might mask potential consequences of persistent LEA in football players [44]. Nevertheless, increasing the BMD threshold to Z-score < 0 did not significantly change or increase the prevalence estimates in our cohort.

A number of participants in the present study were unable to provide direct assessment of EA. Currently, there is no recognized gold standard method to quantify EA and there are significant constraints associated with direct measurement, particularly in intermittent sports [28, 45]. Hence, we decided to apply a surrogate marker to quantify EA, diverging from the approach of the original study. RMR may be prone to confounding factors such as energy status and recent training intensity/volume [46]. Together with assumptions related to RMR_{Ratio}, this could potentially affect our results. As a cross-sectional study, outcome variables will reflect the training and match load at the time of testing. The study included several teams, which were tested at different periods during the year (October-May). Limits of the cross-sectional design may therefore, to some extent, be counterbalanced by catching variability of physiological load across seasons and teams. Lastly, due to contraceptive usage among the participants, information about menstrual irregularities could not be attained by all. This raises the risk of underestimating the prevalence of actual amenorrhea in the cohort. This is, however, reflective of the situation in real world settings [47, 48].

Conclusion

In a diverse array of athletic cohorts, the utilization of the LEAF-Q screening tool persists, despite the fact that its validation remains restricted to endurance athletes and dancers. The poor predictive power of the LEAF-Q does not support its use for the purpose of detecting symptoms of the Triad and LEA with its associated symptoms among female football players. Consequently, the present study may serve to reconsider the interpretation of previous findings where the LEAF-Q has been used to estimate the prevalence of the Triad and LEA in populations for which it has not been validated. Future development of health screening tools for football players should consider the impact and injury mechanisms, as compared to non-contact sports.

Abbreviations

EA	Energy availability
EI	Energy intake
FFM	Fat free mass
LEA	Low energy availability
REDs	Relative energy deficiency in sport
TRIAD	The female athlete triad
LEAF-Q	Low energy availability in female questionnaire
BMD	Bone mass density
DXA	Dual-energy X-ray absorptiometry
RMR	Resting metabolic rate
T_3	Triiodothyronine
T_4	Thyroxine
ROC	Receiver operator curve
AUC	Area under the curve
PPV	Positive predictive value
NPV	Negative predictive value
PRC	Precision recall curve
TSH	Thyroid stimulating hormone
IGF-1	Insulin-like growth factor 1
EDE-Q	Eating disorder examination questionnaire
BMI	Body mass index

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Author Contributions

MSD, OF, GP, MK, JSB and JHR prepared and designed the study. MSD and MK completed the data collection. OF, MSD and JVS completed the data analysis. MSD and OF prepared manuscript draft. MSD, OF, MK, GP, JSB, JVS and JHR edited and approved the final version of the manuscript.

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Availability of Data and Materials

Any data requests can be directed to the corresponding author upon reasonable request.

Declarations

Ethics Approval and Consent to Participate

The study was approved by the Norwegian Center for Research Data (NSD 807592) and was conducted according to the declaration of Helsinki. All handling and storage of biomedical material was approved by the Regional Committee for Medical and Health Research Ethics (REK 2016/787). All players signed a written consent form before partaking in the study.

Consent for Publication

Not applicable.

Competing Interests

The authors declare no conflict of interest.

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Publisher's Note

Paper IV

Prevalence of indicators associated with Relative Energy Deficiency in Sport (REDs) among professional female football players

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Abstract

High prevalence of low energy availability has been reported in female football players, however, the measurement of energy availability is problematic as it relies on inaccurate measurement instruments. The present study aimed to investigate the prevalence of indicators associated with Relative Energy Deficiency in Sport (REDs), as this may provide a better understanding of individuals suffering from health and performance detriments. Indicators of REDs classified as primary, secondary, or associated based on their strength of evidence were collected. We included 60 participants (mean age 22.5, mean body mass 64.1 kg) from three Norwegian football teams. The group was analyzed as one and further stratified based on player position and menstrual status. In total, absence of any primary, secondary, and associated indicators ranged from 33-55%. By contrast, 3% presented with three of six markers of the primary indicator, 25% presented with two of the five markers of the secondary indicator while 5% presented with three of the eight markers for the associated marker. Moreover, 22% presented with clustered primary and secondary indicators. Amenorrhea as a principal marker of low energy availability was reported by 30% among non-contraceptive users (n=27). The prevalence of indicators was unrelated to player positions or whether participants were classified as eumenorrheic or amenorrheic. In total 42% reported not increasing energy intake in response to match and hard training. Teams should focus on universal, health promoting strategies to prevent the development of symptoms associated with REDs, notably by a focus on sufficient energy availability and nutritional periodization.

Highlights

- Nearly half of the participants presented with at least one primary indicator associated with REDs.
- Amenorrhea was reported by 30% of the non-contraceptive users.
- Amenorrheic athletes displayed significantly higher number of cumulative REDs indicators, compared to Eumenorrheic athletes.

- Practitioners and team-support staff should prioritize universal interventions (e.g., nutritional periodization) to reduce exposure to low energy availability.

Keywords: Relative energy deficiency in sport, Low energy availability, Female athlete, football

Introduction

Relative Energy Deficiency in Sport (REDs) is a syndrome that describes impairments of several bodily systems, and where its main etiological factor is low energy availability (LEA) [1,2]. LEA is further defined as inadequate energy intake (EI) relative to exercise energy expenditure, resulting in insufficient support for maintaining physiological homeostasis [3]. LEA and subsequently REDs can manifest itself with or without disordered eating (DE) behaviors and have severe health and performance consequences affecting e.g., metabolic, bone, reproductive, psychological and endocrine functions [2,4]. Controlled laboratory studies have shown that energy availability < 30/kcal^{-1} kg/FFM^{-1} may induce physiological impairments, including secondary amenorrhea which is considered the principal indicator of REDs, and this threshold have generally been adopted in the majority of studies [3]. However, concerns regarding the ecological validity of this universally applied threshold have been expressed [5].

A recent study on a mixed cohort of athletes reported that the majority of participants exhibited at least some impaired physiological function, consistent with the REDs model [6]. Further, the prevalence of anxiety and depressive mood was high. Contrary, a study on Kenyan male and female distance runners reported few symptoms of REDs, despite a low EI [7]. Overall, REDs seem to be present in diverse athletic populations, however, its manifestation may depend on for instance psychosocial, and cultural factors [6,8,9].

Women's football is rapidly evolving, and players are exposed to increased physiological demands, which may vary significantly depending on player position [10]. Professional players will generally play 1-2 games per week, in addition to 4-6 training sessions, emphasizing the importance of "fueling for the work required" [11,12]. Studies that have investigated LEA among female football players report that the prevalence ranges between ~ 20-80% [11,13,14].However, direct measurement of LEA is intrinsically problematic due to the high risk of for measurement error, particularly in intermittent sports like football [15]. Therefore, the proportion of female football players factually suffering from health or performance detriments, caused by LEA, is unknown. In response to these limitations, broad identification of symptoms indicative of REDs provides a compelling approach when assessing female athlete health, because outcome variables described by the REDs model is what ultimately compromises physiological processes, with LEA being the causing factor [2].

Previous studies indicate that the prevalence of LEA in female athletes is extensive [11,13,14], but the accuracy of methods used to quantify EA is questionable [15–17]. Alternatively, measurement of outcomes defined by the REDs model can offer information about the true prevalence of indicators, thus informing the need for universal prevention of REDs within this population. In accordance with the IOC's request for additional research in this domain [2], the primary aim of this study was to assess the prevalence of indicators associated with REDs in a cohort of professional female football players. Secondary, we aimed to explore if the prevalence of indicators of REDs varied across player positions, as well as between eumenorrheic and amenorrheic women.

Methods

Study design and participants

The study was designed as a cross-sectional observation study, where data collection took place between October 2021 and May 2022 [18]. Planning and data collection were done in agreement with the coaching staff, to avoid interference with the team's normal schedule. In total, 60 participants from three Norwegian teams competing in the national premier league and first division were recruited. Eight participants were currently representing their senior national team, while another eight participants represented their designated youth national team. Participants were classified as tier 3 (national level) or 4 (international level) [19].

Ethics

The study was approved by the Norwegian Center for Research Data (807592). All aspects concerning sampling and storage of biochemical material were additionally approved by the Regional Committee for Medical and Health Research Ethics (2016/787). All players signed a written consent form before partaking in the study.

Clinical measures

The clinical measures were conducted over two days and a schematic overview of the protocol is presented in Figure 1. All testing was done in an overnight fasted state between 06-10 a.m. Each team conducted all testing during a 14-day period coinciding with a concurrent data collection process, which is explained in detail elsewhere [14].

Insert Figure 1 approximately here.

Resting metabolic rate

Participants conducted an indirect calorimetry protocol, using a ventilated canopy hoodie (Vyntus CPX, CareFusion, Hoechberg, Germany, Sentrysuit v. 2.21.4). They were instructed to arrive at the laboratory facility using motorized transportation, providing minimal physical strain. On arrival, participants were placed in a silent room, in a supine position for 5 minutes before the canopy was positioned. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured over a 25-minute period, where the last 20 minutes were used to assess resting metabolic rate (RMR).

Body composition and bone mineral density

Body composition including bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry (DXA) (Prodigy, Encore, SP 4.1, version 18, GE medical systems, Madison, Wisconsin, USA). The Z-score values were determined from the lumbar spine (L1-4) and hip for all participants and followed the recommended guidelines for best practice [20]. All measurements and analyses were conducted by the same certified technician.

Hormonal markers

After an overnight fasting period (8-10 hours), blood was collected for both plasma and serum samples. These samples were stored in Biobank Haukeland, Laboratory Medicine and Pathology, Haukeland University Hospital, Bergen, Norway prior to analyses. All analytes were assayed at the Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway. The laboratory is accredited in compliance with ISO 15189:2012. Glucose, Total-cholesterol, and low-density lipoprotein (LDL) were analyzed using Cobas 8000 c702, whereas thyroid stimulating hormone (TSH), free triiodothyronine (FT_3), free thyroxine (FT_4), and ferritin was assayed using Cobas

8000 e801. C-terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-propeptide (P1NP) were assayed using Cobas c602. Insulin and insulin-like growth factor 1 (IGF-1) were analyzed using Immulite 2000 Xpi, whereas leptin was assayed using an enzyme-linked immunosorbent assay kit (Mediagnost Cat#E07, RRID: AB_2813737) (not accredited analysis). Serum cortisol was analyzed using an in-house-developed high-performance liquid chromatography-tandem mass spectrometry [21].

Self-reported physiological and psychological outcomes

After completing the RMR and DXA measurement, participants were given breakfast and instructed to complete an electronic questionnaire administered on a portable tablet (iPad pro, Apple, California, USA). Participants completed a survey consisting of several previously validated questionnaires. These included the Low Energy Availability in Females Questionnaire (LEAF-Q) [22], the 11-item Eating Disorder Examination Questionnaire (EDE-Q 11) [23], the Bergen Insomnia Scale [24], the Chalder Fatigue Scale (CFS) [25], the 12-item General Health Questionnaire GHQ) [26], as well as an adapted version of the Oslo Sports Trauma Research Questionnaire (OSTRCQ) [27]. Further, customized questions regarding diet and EI on match, training and rest days were administered, as well as questions specifically inquiring about history of stress fracture, including injury position and repetitiveness. All questionnaires were completed using an encrypted digital platform (Nettskjema, University of Oslo, Norway).

RED-S indicators and pooling

Indicators were categorized as primary, secondary or associated based on the strength of evidence associated with LEA and REDs [2,6,28]. Primary indicators included secondary amenorrhea (based on self-reported menstrual status from the LEAF-Q) [29], low levels of FT_3 [30], elevated score on the EDE-Q-11 [23], BMD Z-score at the hip or lumbar spine (L1-4) below -1 [1], and history of stress fracture, respectively [31]. The secondary indicators comprised low levels of IGF-1 [30], blood glucose [32], TSH [33]; elevated LDL [34], major time loss from both training and match participation caused by illness/sickness measured by the OSTRCQ [27]. Associated indicators included low RMR (defined as < 30kcal/kg^{-1} FFM/day^{-1} [35]), ferritin [36], leptin [30], FT_4 [30], P1NP [37]; elevated total cholesterol [34], CTX-1 [37],and cortisol [30] respectively.

The clinical findings from the screening process were scored dichotomously as positive (1) or negative (0) similar to the outline described elsewhere [38,39]. Symptoms consistent with REDs and cut-off values were determined based on previously published literature and expert opinions [2,6]. FT_3 and IGF-1 have displayed attenuated serum levels within the reference range in LEA and amenorrheic athletes, compared to controls [29,39,40]. Therefore, these markers were considered positive if values were within or below the lowest quartile of the reference range. Other blood markers applied were considered positive if the values fell outside of the clinical reference range. Since there may be variation in laboratory reference ranges and absolute values, depending on factors such as pre-analytic conditions and instrumentation [41], we consequently applied the clinical reference values of the testing laboratory [42]. As for leptin, our laboratory did not provide a standardized reference range. The cut-off value was therefore determined based on the effect of induced LEA on this hormone demonstrated in previous literature [30].

Statistical analyses

Data analyses were conducted using the open software R (version 4.2.2). The cohort was analyzed as one, then further stratified into groups and compared for differences based on player position with the Welch's t-test, analysis of variance (ANOVA), Chi square test, or Pearson's correlation coefficient. The point prevalence of each RED-S criteria was calculated for the whole group and subgroups (point prevalence = $\frac{number\ of\ cases}{population\ size\ for\ assessment}$) using individual criteria for each condition. Data are presented as mean ± standard deviation (SD).

Since amenorrhea is considered the principal indicator of LEA [1], the sub-group of participants not using hormonal contraception (n=27) were also analyzed separately and divided into an eumenorrheic (EUM) and amenorrheic (AME) group, based on menstrual status retrieved from the LEAF-Q [22]. The two groups were compared using the Welch's test.

Results

The 60 female football players' mean age was 22.5 (± 3.7), with a mean height 168.9 cm (± 6.0), a mean body mass of 64.1 kg (± 6.3), body mass index 22.4 kg/m^2 (± 1.7), percentage fat mass (FM) 24.7% (± 4.2), and a fat free mass (FFM) of 49.3 kg (± 4.7). The self-reported weekly training volume was 12.5 hours (± 3.2), excluding games.

Insert figure 2. approximately here

In total, 55% of the players presented with no primary indicators. Moreover, 33 %, 9% and 3% presented with one, two, and three primary indicators, respectively. In terms of secondary indicators, 33% of the players presented with no indicators, 42% with one indicator, and 25% with two indicators. Lastly, for the associated indicators, 33% of the players presented with none, 50% with one, 12% with two and 5% with three, respectively. We found a 22% prevalence of participants with clustered primary and secondary indicators. A distribution of all REDs indicators is presented in Figure 2. Notwithstanding a positive correlation between RMR and TSH values ($r = 0.31, p = 0.021$) non-significant correlations were found between the number of primary and secondary ($r = 0.10, p = 0.437$), primary and associated ($r = 0.24, p = 0.057$) or secondary and associated ($r = 0.07, p = 0.592$) indicators. On the other hand, significant positive correlations were found between the eating disorder symptoms (EDE-Q 11) and sleep disturbances (BIS) ($r = 0.33, p = 0.010$) as well as between, general health (GHQ-12) ($r = 0.43, p < 0.001$), and fatigue (CFI) ($r = 0.040, p = 0.002$). Additionally, 42% reported they do not increase EI on match and hard training days, while 22% reported deliberately decreasing their energy intake on rest days and easy training days.

Insert table 1. approximately here

The prevalence of primary, secondary, or associated indicators of REDs was unrelated to players' position. We found a significant main effect (ANOVA) for body mass ($p <0.001$), FFM ($p < 0.001$), and BMD ($p = 0.035$), with goalkeepers having significantly higher values compared to other player positions for all these measures (Table 2). There was also a significant difference in cortisol between defenders and all other player positions ($p = 0.034$).

Insert table 2. approximately here

We found no differences in anthropometric measurements or age between the AME and EUM groups (AME: age 20.0 ± 4.4, height 171.5 ± 5.3 cm, body mass 68.1 ± 4.5 kg, FM 24.7 ± 4.3 %, FFM 52.7 ± 5.8 kg; EUM: age 22.6 ± 4.2, height 167.7 ± 6.8 cm, body mass 63.6 ± 8.1 kg, FM 25.5 ± 5.1 %, FFM 48.8 ± 5.6 kg). Excluding menstrual dysfunction as an indicator, the AME group displayed significantly higher number of cumulative REDs indicators (2.9 ± 1.4) compared to the EUM group

(1.6 ± 0.6); ($p < 0.005$). There were no significant differences for any individual REDs indicators, although the AME group consistently displayed unfavorable outcomes, compared to EUM group (Figure 1-3 supplementary materials). In addition, the AME group reported higher incidence of stress fracture history (AME 38% vs EUM 16 %, $p > 0.005$), while the proportion of major time loss due to illness/sickness was 0% and 26% in the AME and EUM group, respectively ($p > 0.005$)

Discussion

The present study is the first one to report on the prevalence of a wide range of indicators outlined by the REDs model in female football players, quantifying the impact of prior estimates of LEA.

REDs indicators

One third of the participants presented with a singular primary indicator of REDs. Despite no correlation between cumulative incidence of primary, secondary, and associated indicators, a significant proportion of the cohort presented with either two or three groups of indicators. Our findings confirm that female football players may be at risk of developing REDs, which is in concurrence with prior prevalence estimates of LEA in this population [13,14]. Recent evidence suggests that adverse health outcomes associated with LEA exhibit individual variability along a spectrum, challenging the conventionally accepted threshold criteria [5]. This could explain why previous estimates of LEA in female football players are higher than the proportion of players deemed at high risk for development of REDs in the current study [5,43]. This underscores the importance of adopting an individualized approach when addressing the health and nutritional needs of athletes. It is also interesting to note that goalkeepers had significantly elevated BMD, together with %FM compared to other player positions, which on a physiological level, may be regarded as a protection for development of REDs.

The 30% prevalence of amenorrhea among participants not using hormonal contraceptives, are akin to prevalence figures reported among elite endurance athletes [38]. This was unexpected, as endurance athletes generally are seen as more susceptible to menstrual disturbances, compared to team-sport athletes [1]. Similar to the findings from Rogers et al., other features of the female athlete triad (Triad) were less prevalent, i.e., disordered eating (10%) and BMD (2%) than several of the broader symptoms of REDs.

Contrary to previous studies on elite athletes, we found no statistically significant difference between the AME and EUM group for any indicators of REDs [38,44]. Despite this, the AME group consistently demonstrated unfavorable outcomes concerning the REDs indicators compared to EUM participants. As REDs is diagnosed clinically, based on the relative weight of symptoms, this difference could be of clinical importance. It is also important to note that the AME group was significantly smaller in size compared to the EUM group, attenuating the statistical power of the analysis.

Blood markers

Impairments to the hormonal milieu following LEA is described in detail by the REDs model [2]. Nonetheless, few large scale studies conducted outside of controlled laboratory settings have provided causal evidence for the broad range of hormonal markers linked to the syndrome [30]. The current cohort exhibits considerable prevalence of attenuated FT_3, the hormone most strongly associated with REDs, at 13%. Elevated LDL levels had a prevalence of 22 % in the current study, however, no difference between the AME and EUM group was observed. Rickenlund et al. reported that AME was associated with unfavorable lipid profiles in female endurance athletes, analogous to findings reported among individuals with anorexia nervosa [34]. Nonetheless, the difference in body composition between the present investigation and that study is substantial. It is therefore unlikely that the relatively high occurrence of elevated LDL observed can be directly attributed to LEA, but rather is normally distributed throughout this cohort. Leptin, another important metabolic regulator associated with LEA has been highlighted as a promising marker for REDs [17]. Our findings seem to coincide with those of Rogers et al., who observed no relationship between increased risk of REDs indicators and attenuated levels of leptin [6]. Regarding cortisol, the fact that 25% of participants presented with high levels should be interpreted with caution, as this hormone is known to be sensitive to several factors, including stress, which may explain the high occurrence in the present study. We observed a statistically significant difference between defenders and other player positions for this hormone but cannot attribute this difference to any specific reason, highlighting the probability of a spurious finding.

Overall, the cohort exhibited low prevalence of broad alterations in blood markers. Nevertheless, several individuals had compromised hormonal values, including FT_3, likely increasing their risk of developing the REDs syndrome.

Bone health

Severe LEA is shown to have direct effects on BMD in female athletes [2]. Bone remodeling is affected by the interplay between osteoblasts, osteocytes, and osteoclasts, which among other factors are influenced by mechanical loading and nutrition [45]. A Z-score of < - 1 is usually applied when utilizing BMD as an indicator of REDs [1]. However, the universal application of this threshold, regardless of sport and consideration of mechanical loading has been under scrutiny. Football is characterized as a high-impact sport, potentially making the usage of this threshold unsuitable. In this cohort, only one player had a Z-score of < -1. In total, seven participants fell below the set threshold, if applying a Z-score of < 0, as proposed by Jonvik et al [46]. As several of these participants also elicited other signs of REDs, these findings could imply that a Z-score of < 0 is more appropriate for detecting low BMD in football players. This is supported by the fact that thirteen of the participants in this cohort reported a previous history of stress fractures. DXA does not distinguish between cortical and trabecular bone mass, and bone morphology may also play a role in the development of stress fractures [47]. Hence, the application of bespoke Z-score thresholds for impact sports such as football warrants further investigation. For markers of bone remodeling, the prevalence of compromised levels of P1NP and CTX-1, associated with bone remodeling and resorption was zero and 8%, respectively. Thus, regardless of Z-score threshold applied, the bone markers confirm the low prevalence of compromised BMD. This also prompts the question of whether BMD is sensitive enough as an indicator of REDs, in sport with high amounts of mechanical loading.

Psychological factors

In total, 10% of the athletes were categorized with DE, measured by the EDE-Q-11. This is consistent with recent findings by Abbot et al. ; however, it is comparatively lower than the results reported by Sundgot-Borgen & Torstveit, using clinical interviews measuring eating disorders [48,49]. This considered, along with the recent findings related to nutritional literacy and body image among female football players, it is possible that clinical/qualitative interviews may contribute in fully encapsulating the manifestation of DE in this population [8]. Several studies have reported that female football players exhibit insufficient energy and carbohydrate intake [13,14]. Merely 58% of the participants in the current study reported intentionally augmenting their EI in response to rigorous training sessions and matches. These findings reinforce the idea that the actual occurrence of individuals exhibiting problematic attitudes and behaviors towards food may exceed the figures reported in this study. On the other hand, this lack of nutritional periodization may also be unintentional, highlighting the need for nutritional education. The present study revealed a generally low prevalence of psychological factors associated with the syndrome. However, consistent with the REDs model we found a strong correlation between measures of DE, depressive symptoms, and sleep disturbances. This finding highlights the importance of thoroughly assessing mental well-being when screening for REDs among female football players.

Methodological considerations

Although several teams testing at different times of the season were included in the study, our data only provides a "snapshot" of the physiological and psychological profile of the participants. The prevalence of markers associated with REDs may change during a season, and this should be acknowledged when interpreting our findings. However, most of the symptoms outlined by the REDs model are hypothesized to result from prolonged or severe LEA [1,2], raising the probability of being captured even in a cross-sectional design. On the other hand, because of our design, determination of subjective markers may have been less accurate due to a memory recall bias. Future prevalence studies should therefore be prospectively designed, using repeated measures to capture the evolution of symptoms throughout the year.

Conclusion

The prevalence of indicators associated with REDs may be considered low to moderate. However, a considerable proportion of participants presented with clustered primary and secondary indicators. This shows that female football players may be susceptible for development of REDs. Hence, team support staff should be aware of indicators and their varying degree of association to REDs to enable individualized follow-up, when needed.

Implications

We propose that incorporating universal health promotion strategies, as opposed to selective screening procedures, could be advantageous for female football players. Such strategies may include efforts to ensure adequate supply of carbohydrate intake before, during and after matches or hard training sessions, as female footballers typically consume inadequate amounts of carbohydrates, also evident in this cohort [13,14]. Given that insufficient nutritional intake and periodization can increase the risk of developing LEA, addressing these measures will likely reduce exposure to risk factors associated with REDs.

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Contributions

The study was designed by MSD, MK, JHR, OF, JSB, GP and MKT. Data collection was conducted by MSD and MK, while analyses were done by MSD, OF and JVS. MSD, JHR and MKT drafted the first

version of the manuscript. All authors were involved in revision and further development of the manuscript and have all authorized the final version.

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Conflict of Interest

The authors declare no conflict of interest

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Tables

Table 1. Prevalence of indicators associated with REDs among participants.
Note: a = prevalence is based on non-contraceptive users; b = age specific reference ranges

Primary indicators	**Prevalence % (n)**
Amenorrhea[a]	30 (8)
$FT_3 < 4.0$ pmol/L [b]	13 (8)
EDE-Q > 2.5	10 (6)
BMD hip Z-score < -1	0
BMD lumbar Z-score < -1	2 (1)
History of stress fracture	22 (13)
Secondary indicators	
IGF-1 < 20.7 nmol/L [b]	10 (6)
Blood glucose < 4 mmol/L	7 (4)
LDL ≥ 3 mmol/L	22 (13)
TSH < 0.40 mlU/L [b]	0
Time loss	10 (6)
Associated indicators	
RMR < 30 kcal/kg^{-1} FFM/day^{-1}	42 (25)
Ferritin < 18 µg/L	3 (2)
Leptin < 3.7 ng/mL	10 (6)
$FT_4 < 9.5$ pmol/L	2 (1)
CTX-1 > 0.69 µg/L [b]	8 (5)
P1NP < 94 µg/L [b]	0
Cortisol > 600 nmol/L	25 (15)
Total cholesterol > 6.1 mmol/L [b]	0

Table 2. Overall and Position specific characteristics and mean values. P values represent the ANOVA analysis.

Note. * = <0.005, ** = < 0.001

Measure	**Overall (60)**	**Defender (20)**	**Midfielder (21)**	**Attacker (13)**	**Goalkeeper (6)**	**P value**
Height	168.9 ± 6.0	168 ± 6	169 ± 6	170 ± 7	173 ± 3	0.371
Body mass	64.1 ± 6.3	62.4 ± 5.6	62.9 ± 5.2	63.6 ± 5.3	74.7 ± 5.8	**<0.001****
%Fat mass	24.7 ± 4.2	23.4 ± 3.2	25.2 ± 3.5	23.8 ± 4.2	29.6 ± 6.3	0.009*
FFM	49.3 ± 4.7	48.7 ± 4.9	47.9 ± 3.8	49.2 ± 5.0	56.5 ± 2.0	**<0.001****
RMR (kcal.kg/ FFM/day)	29.2 ± 5.3	27.6 ± 7.2	29.3 ± 3.1	30.7 ± 5.5	30.4 ± 2.8	0.382
RMR_{Ratio}	0.96 ± 0.3	0.93 ± 1.0	0.94 ± 0.2	0.98 ± 0.2	1.1 ± 0.1	0.282
BMD hip Z-score	2.1 ± 1.0	2.2 ± 0.9	1.7 ± 1.0	2.1 ± 0.7	2.9 ± 1.1	0.035*
BMD lumbar Z-score	1.2 ± 1.0	1.3 ± 0.9	0.9 ± 1.0	1.1 ± 0.9	2.1 ± 0.9	0.061
Blood glucose mmol/L	4.6 ± 0.5	4.8 ± 0.7	4.6 ± 0.5	4.5 ± 0.4	4.4 ± 0.3	0.251
TSH mIU/L	1.9 ± 1.1	1.9 ± 0.9	1.8 ± 1.1	1.8 ± 1.2	2.0 ± 1.3	0.983
FT_3 pmol/L	4.9 ± 0.7	4.9 ± 0.5	4.9 ± 0.5	5.1 ± 1.1	4.8 ± 0.6	0.873
FT_4 pmol/L	15.8 ± 1.9	16.6 ± 2.0	15.8 ± 1.9	15.3 ± 1.7	14.8 ± 0.6	0.133
Ferritin µg/L	50.8 ± 30.5	50.1 ± 30.0	57.8 ± 36.5	36.2 ± 10.0	61.2 ± 33.9	0.192
LDL mmol/L	2.5 ± 0.6	2.4 ± 0.4	2.4 ± 0.6	2.7 ± 0.7	2.3 ± 0.6	0.448
Leptin ng/mL	7.8 ± 5.5	7.4 ± 3.6	8.3 ± 6.2	6.2 ± 2.6	10.7 ± 10.8	0.389
Total cholesterol mmol/L	4.3 ± 0.6	4.2 ± 0.8	4.3 ± 0.5	4.5 ± 0.8	4.0 ± 0.7	0.285
Cortisol nmol/L	473.1 ± 231.2	599 ± 189	412 ± 227	408 ± 190	418 ± 140	**0.034***
IGF-1 nmol/L	29.1 ± 7.6	28.8 ± 9.4	29.6 ± 6.1	28.5 ± 9.4	29.1 ± 5.0	0.979
CTX-1 µg/L	0.75 ± 0.3	0.74 ± 0.4	0.76 ± 0.2	0.69 ± 0.3	0.81 ± 0.2	0.872
P1NP µg/L	103.9 ± 56.4	105.5 ± 78.0	104.8 ± 35.7	94.8 ± 48.5	115.7 ± 24.0	0.897

Figures

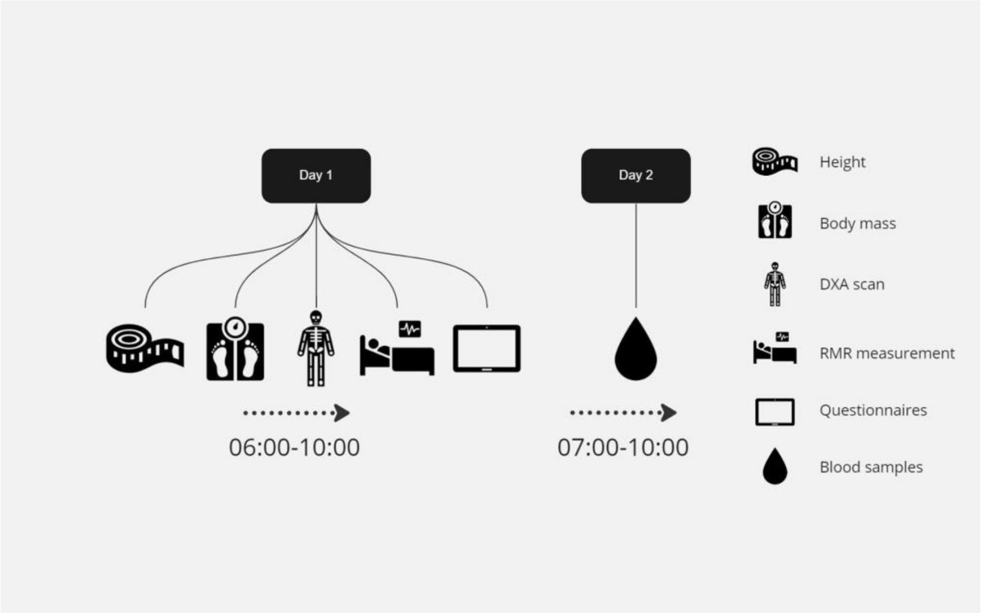


Figure 1. Schematic overview of the testing protocol completed by the participants.

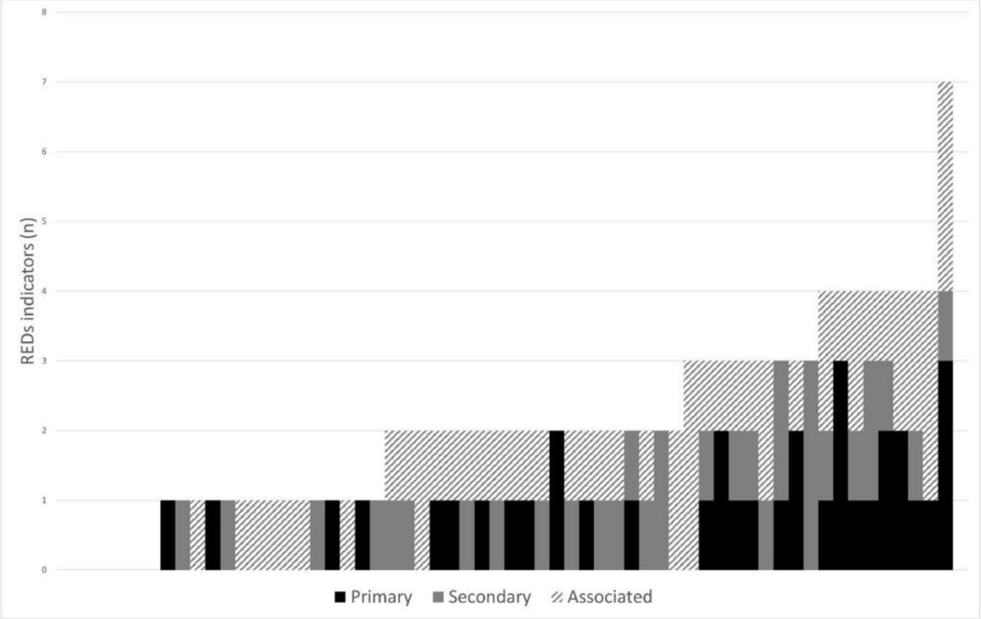
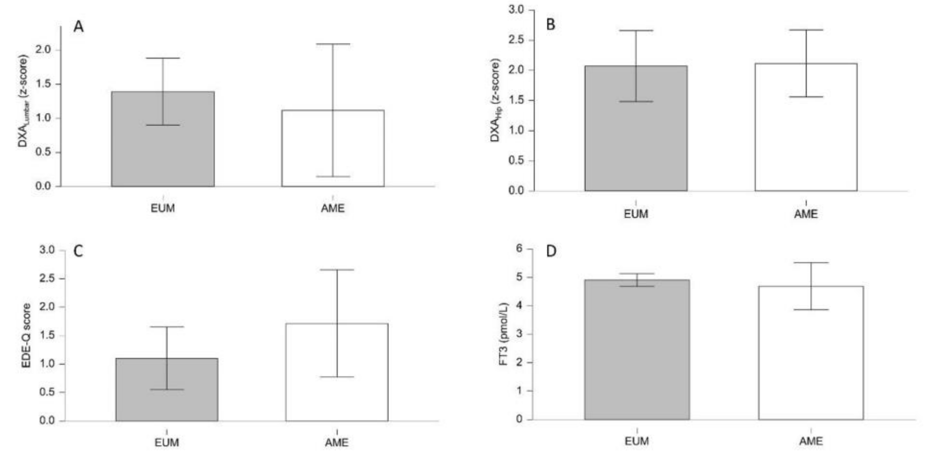
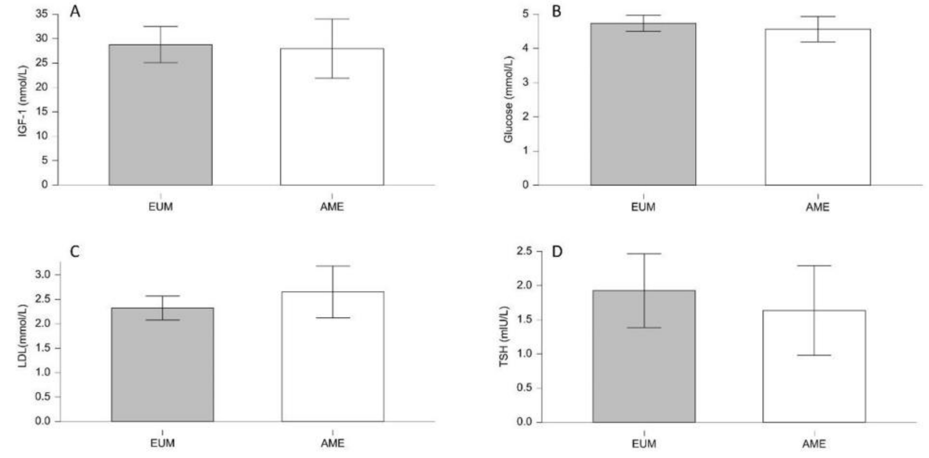


Figure 2. Distribution plot in the order from minimum to maximum, exhibiting the prevalence of primary, secondary, and associated symptoms of REDs.

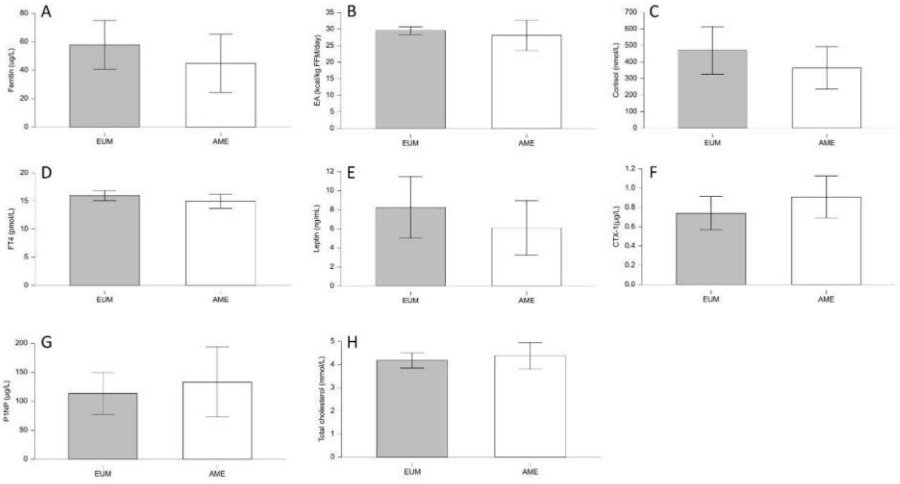
Supplementary materials



Supplementary Figure 1. Comparison of primary indicators between the AME and EUM group



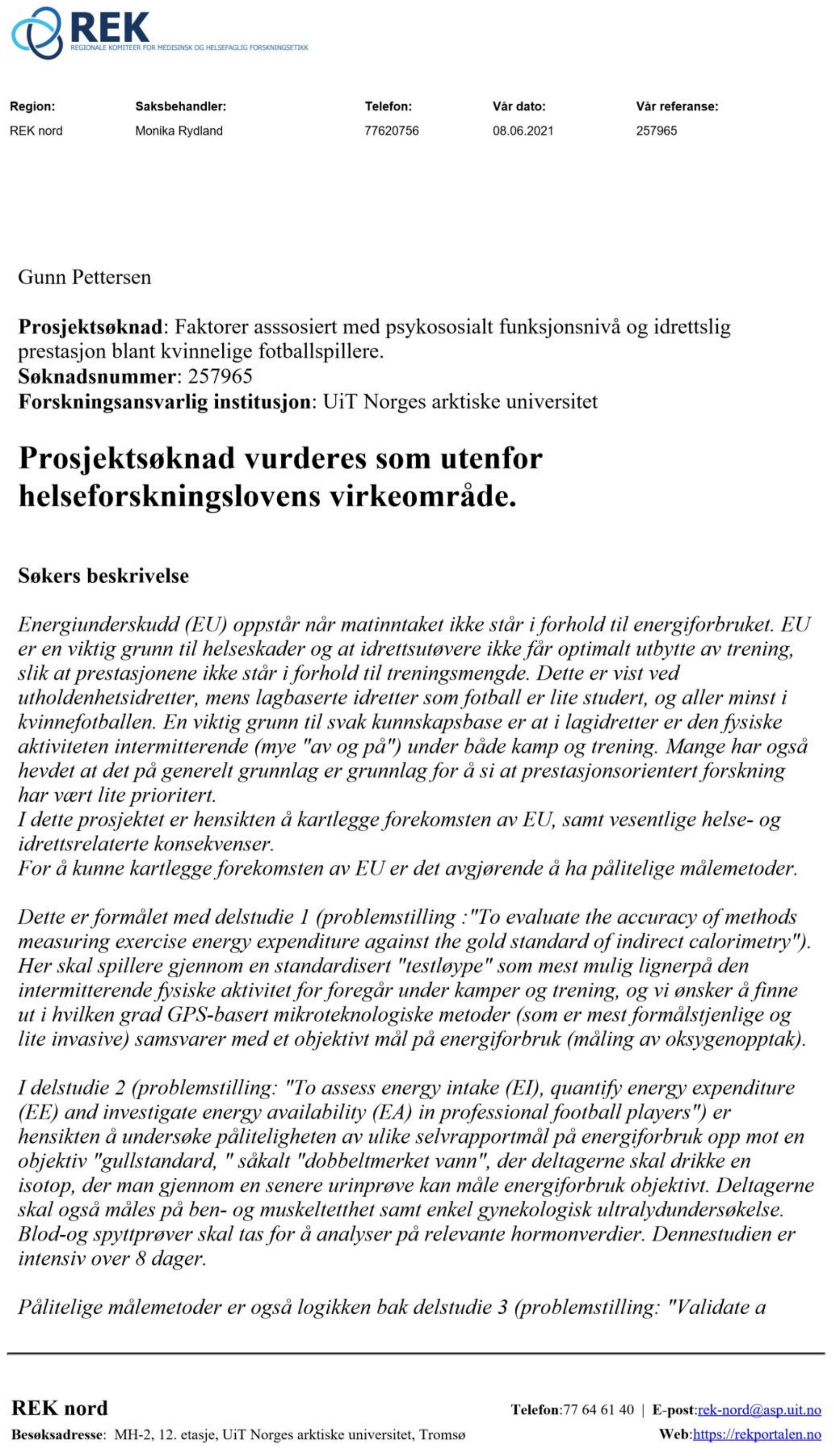
Supplementary Figure 2. Comparison of secondary indicators between the AME and EUM group



Supplementary Figure 3. Comparison of associated indicators between the AME and EUM group

Appendix I

Decision letters from the Regional Committee for Medial and Health Research Ethics and Norwegian Center for Research Data and informed Consent.



REK

REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK nord	Monika Rydland	77620756	08.06.2021	257965

Gunn Pettersen

Prosjektsøknad: Faktorer asssosiert med psykososialt funksjonsnivå og idrettslig prestasjon blant kvinnelige fotballspillere.
Søknadsnummer: 257965
Forskningsansvarlig institusjon: UiT Norges arktiske universitet

Prosjektsøknad vurderes som utenfor helseforskningslovens virkeområde.

Søkers beskrivelse

Energiunderskudd (EU) oppstår når matinntaket ikke står i forhold til energiforbruket. EU er en viktig grunn til helseskader og at idrettsutøvere ikke får optimalt utbytte av trening, slik at prestasjonene ikke står i forhold til treningsmengde. Dette er vist ved utholdenhetsidretter, mens lagbaserte idretter som fotball er lite studert, og aller minst i kvinnefotballen. En viktig grunn til svak kunnskapsbase er at i lagidretter er den fysiske aktiviteten intermitterende (mye "av og på") under både kamp og trening. Mange har også hevdet at det på generelt grunnlag er grunnlag for å si at prestasjonsorientert forskning har vært lite prioritert.
I dette prosjektet er hensikten å kartlegge forekomsten av EU, samt vesentlige helse- og idrettsrelaterte konsekvenser.
For å kunne kartlegge forekomsten av EU er det avgjørende å ha pålitelige målemetoder.

Dette er formålet med delstudie 1 (problemstilling :"To evaluate the accuracy of methods measuring exercise energy expenditure against the gold standard of indirect calorimetry"). Her skal spillere gjennom en standardisert "testløype" som mest mulig lignerpå den intermitterende fysiske aktivitet for foregår under kamper og trening, og vi ønsker å finne ut i hvilken grad GPS-basert mikroteknologiske metoder (som er mest formålstjenlige og lite invasive) samsvarer med et objektivt mål på energiforbruk (måling av oksygenopptak).

I delstudie 2 (problemstilling: "To assess energy intake (EI), quantify energy expenditure (EE) and investigate energy availability (EA) in professional football players") er hensikten å undersøke påliteligheten av ulike selvrapportmål på energiforbruk opp mot en objektiv "gullstandard, " såkalt "dobbeltmerket vann", der deltagerne skal drikke en isotop, der man gjennom en senere urinprøve kan måle energiforbruk objektivt. Deltagerne skal også måles på ben- og muskeltetthet samt enkel gynekologisk ultralydundersøkelse. Blod-og spyttprøver skal tas for å analyser på relevante hormonverdier. Dennestudien er intensiv over 8 dager.

Pålitelige målemetoder er også logikken bak delstudie 3 (problemstilling: "Validate a *football adapted LEAF-Q to identify potential LEA among female football players"). LEAF-Q er et spørreskjema om indikasjoner på energiunderskudd, men dette er validert kun for utholdenhets- og estetiske idretter. Hensikten med delstudie 3 er å validere LEAF-Q for lagbaserte idretter, og ha et empirisk kunnskapsgrunnlag for eventuelt å foreta nødvendige justeringer i spørsmål eller spørsmålsformuleringer.*

Det metodologiske kunnskapsgrunnlaget for delstudie 4 ligger i delstudie 1-3. I delstudie 4 er problemstillingen "Explore the prevalence and seasonal variations in low energy availability and its biological and psychological covariates. Her følges fotballspillere opp i fem målepunkter gjennom en hel sesong. På hvert målepunkt vil vi da kunne måle forekomsten av EU, og de mulige psykologiske korrelatene. Korrelatene er både antatt helsemessig ugunstige, men også helsemessig gunstige. Prediktoranalyser er dermed egnet til ikke bare å besvare spørsmålet om EU er forbundet med helserisiko, men like så mye om helsemessig gunstige faktorer svekker eller eliminerer betydningen av de ugunstige faktorene.

Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK) i møtet 27.05.2021. Vurderingen er gjort med hjemmel i helseforskningsloven § 10.

REKs vurdering

Komitémedlem Oddgeir Friborg er medarbeider i prosjektet og deltok derfor ikke ved behandlingen av søknaden.

Om prosjektet

Prosjektet er et helsevitenskapelig Ph.d-prosjekt hvor man vil kartlegge forekomsten av energiunderskudd og helse- og idrettsrelaterte konsekvenser av dette.

De prosjektene som skal framlegges for REK er prosjekt som dreier seg om *«medisinsk og helsefaglig forskning på mennesker, humant biologisk materiale eller helseopplysninger»*, jf. helseforskningsloven § 2. *«Medisinsk og helsefaglig forskning»* er i § 4 a), definert som *«virksomhet som utføres med vitenskapelig metodikk for å skaffe til veie ny kunnskap om helse og sykdom»*. Det er altså formålet med studien som avgjør om et prosjekt skal anses som framleggelsespliktig for REK eller ikke.

Av prosjektbeskrivelsen fremgår det at man vil finne ut i hvilken grad GPS-basert mikroteknologiske metoder samsvarer med et objektivt mål på energiforbruk, man vil undersøke påliteligheten av ulike selvrapportmål på energiforbruk opp mot en objektiv "gullstandard", og man vil se i hvilken grad EU er forbundet med helserisiko.

Av søknaden fremgår det at det er spesifikke faktorer rettet mot matinntak og energiforbruk med formål å øke prestasjonsnivå blant kvinnelige fotballspillere som ligger til grunn for studien.

Selv om prosjektet har helsemessige elementer og indirekte vil kunne gi helsemessige gevinster faller ikke prosjektet inn under definisjonen av de prosjekt som skal vurderes etter helseforskningsloven.

Prosjekter som faller utenfor helseforskningslovens virkeområde kan gjennomføres uten godkjenning av REK. Det er institusjonens ansvar å sørge for at prosjektet gjennomføres på en forsvarlig måte med hensyn til for eksempel regler om taushetsplikt og personvern

Vedtak

Etter søknaden fremstår prosjektet ikke som et medisinsk og helsefaglig forskningsprosjekt som faller innenfor helseforskningsloven. Prosjektet er ikke framleggingspliktig, jf. helseforskningsloven § 2.

Vi gjør oppmerksom på at etter personopplysningsloven må det foreligge et behandlingsgrunnlag etter personvernforordningen. Dette må forankres i egen institusjon.

Klageadgang
Du kan klage på REKs vedtak, jf. forvaltningsloven § 28 flg. Klagen sendes på eget skjema via REK portalen. Klagefristen er tre uker fra du mottar av dette brevet. Dersom REK opprettholder vedtaket, sender REK klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag (NEM) for endelig vurdering, jf. forskningsetikkloven § 10 og helseforskningsloven § 10.

Med vennlig hilsen

May Britt Rossvoll
sekretariatsleder

Kopi til:

UiT Norges arktiske universitet

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK vest	Camilla Gjerstad	55978499	02.07.2021	29081
			Deres dato: /	

Lise Bjørkhaug Gundersen

Generell biobank: Generell forskningsbiobank: Idrett, Helse og Funksjon: Biomarkører
Søknadsnummer: 2016/787
Forskningsansvarlig institusjon: Høgskulen på Vestlandet

Generell biobank: Endring godkjennes med vilkår.

Søkers beskrivelse

Dette er en generell forskningsbiobank for lagring av biologisk materiale til fremtidig forskning om idrett og helse. Formålet med forskningen vil være å undersøke hvordan ulike former for trening og kosthold kan påvirke molekylære og cellebiologiske parametre i humane kroppsvæsker. Deltakerne er friske forsøkspersoner over 16 år.

REK vest viser til tilbakemelding mottatt 29.06.21, i forbindelse med ovennevnte generelle forskningsbiobank. Tilbakemeldingen er behandlet av komiteleder for Regional komité for medisinsk og helsefaglig forskningsetikk (REK vest) på delegert fullmakt fra komiteen, med hjemmel i forskningsetikkforskriften § 7, første ledd, tredje punktum. Vurderingen er gjort med hjemmel i helseforskningsloven § 11.

REKs vurdering

Biobankendring (innsendt 25.03.21)
Søkers beskrivelse av og begrunnelse for endringen:
Det søkes om endring av generell biobank 2016/787 Idrett, Helse og funksjon: Biomarkører. Årsak til dette er at det skal innhentes nytt biologisk materiale (spytt, blod, urin) i prosjektet «Female Football Center» i Bergen, som krever ny samtykkeerklæring (se vedlegg). Tidligere fremleggsvurdering er sendt til REK (referansenummer: 257984), hvor det ikke foreligger nødvendig REK godkjenning (Meldt til NSD). Da tidligere søknad for etablering av generell biobank omfatter «forskning på hvordan ulike former for trening og kosthold kan påvirke molekylære og cellebiologiske parametere i humane kroppsvæsker», så faller prosjektet «Female Football Center» sådan inn under dette, da formålet for prosjektet er å undersøke hvordan ernæringsstatus påvirker biologiske markører i kroppsvæsker (se vedlegg prosjekt og protokoll).

REK vest ba om tilbakemelding (brev 23.06.21)
REK vest utsatte saken og uttalte følgende:

Vi noterer at REK nord har vurdert studien 257965 der det biologiske materialet samles inn og har konkludert med at studien ikke er omfattet av helseforskningsloven.

REK vest oppfatter det likevel slik at det biologiske materialet nå vil bli lagres til fremtidig forskning som vil kunne bringe ny kunnskap om sykdom og helse. Fremtidige forskningsprosjekter som skal benytte materialet vil derfor være omfattet av helseforskningsloven. Det biologiske materialet må derfor lagres i en godkjent generell forskningsbiobank og deltakerne må gi et informert samtykke som oppfyller helseforskningsloven § 14. REK vest forutsetter at deltakerne mottar et eget informasjonsskriv om den generelle forskningsbiobanken, og får informasjon om hvor de vil kunne får tilgang til jevnlig informasjon om forskningen. Når det biologiske materialet i fremtiden skal tas ut av biobank for å inngå i nye forskningsstudier, må dette forelegges for REK via skjemaet "Prosjektsøknad" i REK-portalen.

I tidligere innsendt skriv om biobanken har ansvarshavende oppgitt at "*Vi har derved en offentlig nettside www.hib.no/biobank, der vi legger ut informasjon om hvilke forskningsprosjekter som har fått utlevert materiale fra biobanken."* Denne nettsiden virker ikke. REK vest ber om tilbakemelding på dette. Vi ber også om at godkjent biobankskriv med korrekt nettadresse sendes til REK vest.

Tilbakemelding fra søker

Vedrørende utsettelsesvedtak endringsmelding (Ref 29081) - Generell forskningsbiobank: Idrett, Helse og Funksjon: Biomarkører (søknadsnummer 2016/787). REK vest har bedt om en tilbakemelding på den offentlige nettsiden med tilgjengelig informasjon om forskningsbiobanken vedrørende utlevering av materiale fra biobanken (forrige adresse oppgitt i tidligere generelt biobankskriv virket ikke).

Dette har nå blitt iverksatt der webredaktør ved HVL har fått informasjon (27/6-21) om hvor denne nye nettsiden skal plasseres på HVL nettsidene (https://www.hvl.no/forsking/gruppe/idrett-helse-og-funksjon/). Det vedlagte oppdaterte informasjonsskrivet om generell biobank, med korrekt nettadresse, er herved lagt ved endringsmeldingen. Etter muntlig samtale med REK 29/6-21 var det greit å sende inn igjen på nåværende tidspunkt, under forutsetning av at nettsiden ble oppdatert.

Vurdering

REK vest ved komitéleder har vurdert tilbakemeldingen og godkjenner lagring av det innsamlete biologiske materialet i biobanken. Vi gjør oppmerksom på at en generell biobank kun har tillatelse til å lagre biologisk materiale og metadata om donor (f.eks. dato, kjønn, alder o.l). Øvrige opplysninger og analyseresultater fra det biologiske materialet er ikke en del av selve biobanken og krever annen godkjenning. Slik godkjenning kan være godkjenning fra REK i konkrete helseforskningsprosjekter. Ta kontakt med REK for veileding dersom det behov for mer informasjon.

Godkjenning av endringen gis på vilkår av at nettsiden oppdateres jevnlig slik at deltakerne kan motta informasjon om forskningen som foregår på materialet. Vi forutsetter også at det sendes prosjektsøknad til REK dersom materialet tas ut av biobanken og benyttes i fremtidige helseforskningsprosjekter.

Vedtak

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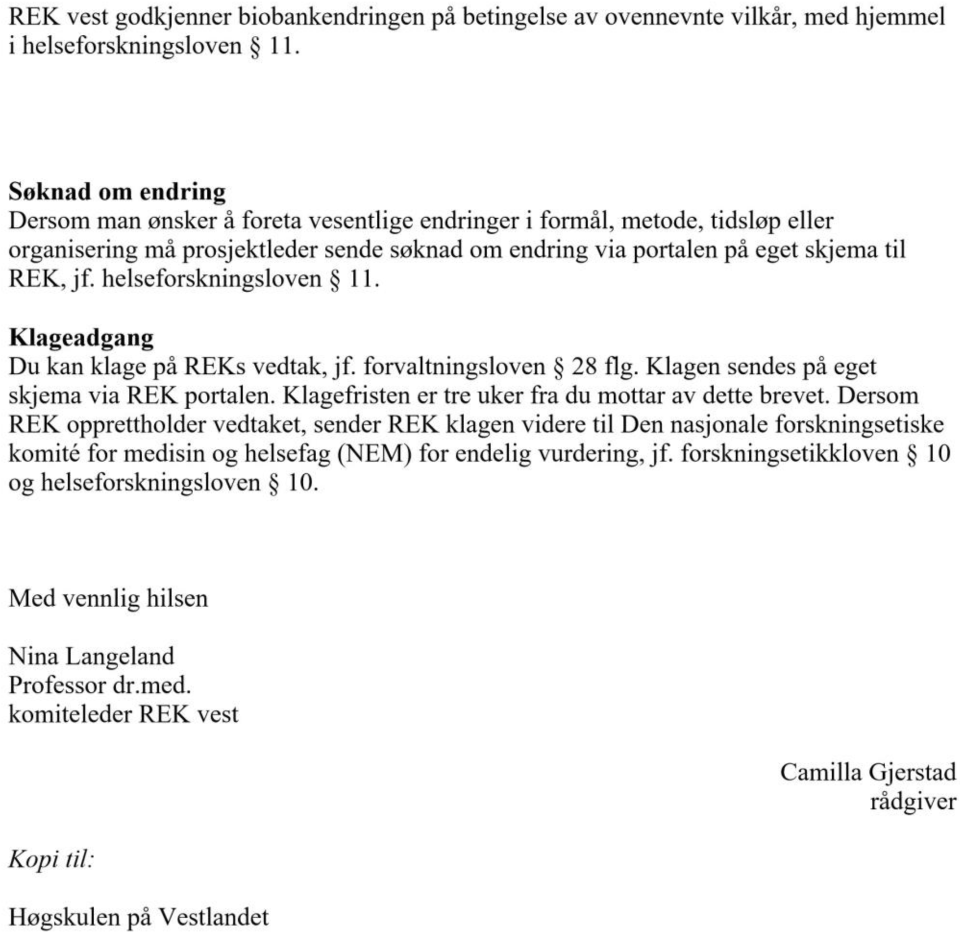

REK vest godkjenner biobankendringen på betingelse av ovennevnte vilkår, med hjemmel i helseforskningsloven § 11.

Søknad om endring

Dersom man ønsker å foreta vesentlige endringer i formål, metode, tidsløp eller organisering må prosjektleder sende søknad om endring via portalen på eget skjema til REK, jf. helseforskningsloven § 11.

Klageadgang

Du kan klage på REKs vedtak, jf. forvaltningsloven § 28 flg. Klagen sendes på eget skjema via REK portalen. Klagefristen er tre uker fra du mottar av dette brevet. Dersom REK opprettholder vedtaket, sender REK klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag (NEM) for endelig vurdering, jf. forskningsetikkloven § 10 og helseforskningsloven § 10.

Med vennlig hilsen

Nina Langeland
Professor dr.med.
komiteleder REK vest

Camilla Gjerstad
rådgiver

Kopi til:

Høgskulen på Vestlandet

NSD NORSK SENTER FOR FORSKNINGSDATA

Vurdering

Referansenummer

807592

Prosjekttittel

Female Football players: determinants of health and performance

Behandlingsansvarlig institusjon

UiT Norges Arktiske Universitet / Det helsevitenskapelige fakultet / Institutt for helse- og omsorgsfag

Prosjektansvarlig (vitenskapelig ansatt/veileder eller stipendiat)

Jan H. Rosenvinge, jan.rosenvinge@uit.no, tlf: 95280836

Type prosjekt

Forskerprosjekt

Prosjektperiode

01.01.2021 - 31.12.2024

Vurdering (1)

12.07.2021 - Vurdert

BAKGRUNN
Prosjektet er vurdert av Regionale komiteer for medisinsk og helsefaglig forskningsetikk (REK) i vedtak av 08.06.2021, deres referanse 257965 (se under Tillatelser). REK vurderer at studien framstår som forskning, men ikke som medisinsk eller helsefaglig forskning. Prosjektet er følgelig ikke omfattet av helseforskningslovens saklige virkeområde, jf. helseforskningslovens §§ 2 og 4. Prosjektet trenger derfor ikke godkjenning fra REK.

Det er også søkt om tillatelse fra REK til å lagre biologisk materiale til fremtidig forskning på idrett og helse. Det biologiske materialet skal lagres uten identifiserende opplysninger (kun metadata som alder og kjønn). Lagringen medfører en endring i tidligere godkjent generell biobank ved Haukeland Universitetssjukehus tidligere godkjent med referanse 2016/787. REK har godkjent endringen med hjemmel i helseforskningsloven § 11 (deres referanse: 29081)

Det er vår vurdering at behandlingen vil være i samsvar med personvernlovgivningen, så fremt den gjennomføres i tråd med det som er dokumentert i meldeskjemaet den 12.07.2021 med vedlegg, samt i meldingsdialogen mellom innmelder og NSD. Behandlingen kan starte.

TYPE OPPLYSNINGER OG VARIGHET
Prosjektet vil behandle alminnelige personopplysninger og særlige kategorier av personopplysninger om helseforhold frem til 31.12.2024.

LOVLIG GRUNNLAG

Prosjektet vil innhente samtykke fra de registrerte til behandlingen av personopplysninger. Vår vurdering er at prosjektet legger opp til et samtykke i samsvar med kravene i art. 4 nr. 11 og 7, ved at det er en frivillig, spesifikk, informert og utvetydig bekreftelse, som kan dokumenteres, og som den registrerte kan trekke tilbake.

For alminnelige personopplysninger vil lovlig grunnlag for behandlingen være den registrertes samtykke, jf. personvernforordningen art. 6 nr. 1 a.

For særlige kategorier av personopplysninger vil lovlig grunnlag for behandlingen være den registrertes uttrykkelige samtykke, jf. personvernforordningen art. 9 nr. 2 bokstav a, jf. personopplysningsloven § 10, jf. § 9 (2).

PERSONVERNPRINSIPPER

NSD vurderer at den planlagte behandlingen av personopplysninger vil følge prinsippene i personvernforordningen:

- om lovlighet, rettferdighet og åpenhet (art. 5.1 a), ved at de registrerte får tilfredsstillende informasjon om og samtykker til behandlingen
- formålsbegrensning (art. 5.1 b), ved at personopplysninger samles inn for spesifikke, uttrykkelig angitte og berettigede formål, og ikke viderebehandles til nye uforenlige formål
- dataminimering (art. 5.1 c), ved at det kun behandles opplysninger som er adekvate, relevante og nødvendige for formålet med prosjektet
- lagringsbegrensning (art. 5.1 e), ved at personopplysningene ikke lagres lengre enn nødvendig for å oppfylle formålet.

DE REGISTRERTES RETTIGHETER

NSD vurderer at informasjonen om behandlingen som de registrerte vil motta oppfyller lovens krav til form og innhold, jf. art. 12.1 og art. 13.

Så lenge de registrerte kan identifiseres i datamaterialet vil de ha følgende rettigheter: innsyn (art. 15), retting (art. 16), sletting (art. 17), begrensning (art. 18) og dataportabilitet (art. 20).

Vi minner om at hvis en registrert tar kontakt om sine rettigheter, har behandlingsansvarlig institusjon plikt til å svare innen en måned.

FØLG DIN INSTITUSJONS RETNINGSLINJER

NSD legger til grunn at behandlingen oppfyller kravene i personvernforordningen om riktighet (art. 5.1 d), integritet og konfidensialitet (art. 5.1. f) og sikkerhet (art. 32).

Nettskjema UiO er databehandler i prosjektet. NSD legger til grunn at behandlingen oppfyller kravene til bruk av databehandler, jf. art 28 og 29.

For å forsikre dere om at kravene oppfylles, må prosjektansvarlig følge interne retningslinjer/rådføre dere med behandlingsansvarlig institusjon.

MELD VESENTLIGE ENDRINGER

Dersom det skjer vesentlige endringer i behandlingen av personopplysninger, kan det være nødvendig å melde dette til NSD ved å oppdatere meldeskjemaet. Før du melder inn en endring, oppfordrer vi deg til å lese om hvilken type endringer det er nødvendig å melde:
https://www.nsd.no/personverntjenester/fylle-ut-meldeskjema-for-personopplysninger/melde-endringer-i-meldeskjema
Du må vente på svar fra NSD før endringen gjennomføres.

OPPFØLGING AV PROSJEKTET

NSD vil følge opp underveis (hvert annet år) og ved planlagt avslutning for å avklare om behandlingen av personopplysningene er avsluttet/pågår i tråd med den behandlingen som er dokumentert.

Kontaktperson hos NSD: Jørgen Wincentsen

Lykke til med prosjektet!

Appendix II

Non validated questionnaire

Nettskjema

Spørreskjema

Har du de siste 6 månedene pådratt deg fotballrelaterte skader?

Ja
Nei

Hvis ja, i hvilken grad har trenings- og kampmengde endret seg i denne perioden?

Kamp

Ingen endring
Noe endring
Stor endring

Trening

Ingen endring
Noe endring
Stor endring

Har du de siste 6 månedene hatt helseproblemer som har oppstått utenfor fotballen?

Ja
Nei

Hvis ja, i hvilken grad har trening- og kampmengde endret seg i denne perioden?

Kamp

Ingen endring
Noe endring
Stor endring

Trening

Ingen endring
Noe endring
Stor endring

Hvor fysisk sliten har du vært etter kamp og trening de siste 4 ukene sammenlignet med hva som er vanlig for deg

Kamp

Mindre enn vanlig
Som vanlig
Mer enn vanlig
Betydelig mer enn vanlig

Trening

Mindre enn vanlig
Som vanlig

Mer enn vanlig
Betydelig mer enn vanlig

Hvor mentalt sliten har du vært etter kamp og trening de siste 4 ukene sammenlignet med vanlig?

Kamp

Mindre enn vanlig
Som vanlig
Mer enn vanlig
Betydelig mer enn vanlig

Trening

Mindre enn vanlig
Som vanlig
Mer enn vanlig
Betydelig mer enn vanlig

Har du tidligere hatt tretthetsbrudd

Nei
Ja
Usikker

Hvis ja, beskriv hvor og antall ganger

Har du for tiden en diett- eller matplan) (eksempelvis: lavkarbokost, vegandiett, fasteperioder)

Ja
Nei

Hvis ja, beskriv hva

Inntar du for tiden med hensikt mer mat/energi/energidrikk i forbindelse med kamp eller ekstra harde treningsøkter?

Ja
Nei
Vet ikke

Inntar du med hensikt mindre mat/energi på kampfrie dager og på dager med lite eller ingen trening)

Ja
Nei
Vet ikke