



# Study protocol: prevalence of low energy availability and its relation to health and performance among female football players

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## ABSTRACT

Enduring low energy availability (LEA) is associated with several potentially serious physiological and mental conditions. LEA has been found highly prevalent among female elite athletes within endurance sports, thus hampering athletes' health and performance. The prevalence and the underpinning risk factors of LEA among female elite football players are less studied. One reason is that the existing self-report measures and technological devices to monitor energy intake and expenditure are inadequately adapted to capture the nature of the physical activity and energy expenditure among football players and are thus inaccurate.

The present paper outlines a study protocol addressing the prevalence of LEA, the measurement of LEA and the correlations of LEA in terms of health and performance in female football players. Four studies will be conducted with the following aims (1) to evaluate the accuracy of global positioning systems (GPS)-based devices to monitor energy expenditure with indirect calorimetry as the gold standard, (2) to assess energy intake, quantify energy expenditure and investigate energy availability through self-report instruments, double labelled water (DLW) and GPS monitoring devices, (3) to determine the point prevalence of LEA using self-report instruments, DLW, dual-X-ray-absorptiometry (DXA) to quantify muscle and bone mass distribution and density, and a battery of hormonal analyses, and (4) to explore whether the prevalence of LEA varies across a full football season.

Measures covering mental symptoms and psychological resources will be included, and a selection of biological measures derived from study 3.

Measurements of DXA and DLW are resource-demanding and will be collected from one professional club (N=20 women). In contrast, the remaining data will be collected from four professional clubs (N=60 women) located in Bergen, the largest city within the Western region of Norway. Overall procedures and biobank storage procedures have been approved for data collection that will end in December 2024.

## INTRODUCTION

### Energy availability and energy expenditure

An enduring imbalance between energy intake (EI) and exercise energy expenditure (EEE) may result in low energy availability

## Key messages

- ▶ The current protocol outlines studies that explore the point and 1-year prevalence of LEA among female elite football players using an extensive battery of technical, biological, and psychological measures.
- ▶ A critical point is the number of participants, and actions will be taken to limit drop-out and missing data.

(LEA). LEA is associated with a cluster of endocrine, cardiovascular, inflammatory, gastrointestinal and mental features. This has been labelled a 'relative energy deficiency in sports' (RED-S) within sports medicine. LEA can lead to the manifestation of RED-S, a condition that can result in irreversible health and performance impairments.<sup>1-3</sup> The prevalence and covariates of LEA have been heavily explored among female athletes representing weight-sensitive sports, but not among female football players on an elite level.

Some studies among female football players have reported a prevalence of LEA from 23% to 64%,<sup>4-6</sup> and elite players are in the upper range. Most of these studies have used the Low Energy Available in Females Questionnaire (LEAF-Q).<sup>7</sup> The LEAF-Q determines LEA risk from symptoms and is validated based on endurance sports. It might probably yield biased prevalence figures because it does not fully capture the football-specific characteristics such as the intermittent nature of the physical activity and the type and location of injuries.

A generic methodological problem is the measurement of EEE and EI (EA/EI).<sup>8</sup> Valid biological measures are quite reliable but costly, such as dual-energy X-ray absorptiometry (DXA) to quantify muscle and bone mass distribution and density and free fat mass (FFM), and the use of doubly labelled



water (DLW) to quantify the rate of energy elimination. High costs connected with logistic demands make such procedures unfeasible during matches, especially in larger study populations. Questionnaire-based methods are feasible alternatives, that is, procedures like food diaries, remote food photographic methods and 24 hours recalls in quantifying EI—but at the expense of more measurement errors. Yet, high reliability of self-reporting procedures against DLW has been found only for male football players.<sup>9</sup>

Another approach in measuring EEE is the use of microtechnological positioning devices. Activity monitors that combine accelerometry and measurement of heat production and skin conductivity show promising findings among ball game athletes with indirect calorimetry as the gold standard.<sup>10</sup> Still, the commercial sale of such devices has been terminated. Other devices used in the literature include heart rate, power metres and global positioning systems (GPS). Recent quality improvements<sup>11</sup> of GPS-based devices make them a feasible alternative to monitor player load.<sup>12</sup> However, in exploring the prevalence of LEA and RED-S, GPS and other microtechnological devices may fall short considering the intermittent nature of physical expenditure in football, which the GPS algorithms cover more poorly.<sup>13</sup> The theoretical concept of metabolic power has been proposed<sup>14</sup> to better account for the energetic cost of accelerations and decelerations. Still, metabolic power-based methods seem to underestimate the energetic cost of football-specific actions compared with the reference standard of indirect calorimetry.<sup>13</sup> Indeed, there is a need to investigate the accuracy of different devices in measuring EEE during intermittent activity to assess the EA among football players more accurately.

### Psychosocial correlates

While previous research has focused on the ‘triad’ between menstrual dysfunction, poor bone health and disordered eating as a marker of LEA, recent studies show a wide range of psychosocial correlates in terms of both indicators and consequences, like depression, irritability, impaired judgement and decreased concentration.<sup>1</sup> These factors may even outperform the triad factors in predicting LEA.<sup>15</sup> Other studies indicate an uncertain relation among subjects with a higher prevalence of depression and LEA, particularly among younger players in secondary divisions.<sup>16–18</sup> Moreover, this casts some uncertainty about the link between LEA and disordered eating as it is not depression but rather, anxiety disorders that stand out as a likely understanding of the spectrum of eating disorders<sup>19–21</sup> and the nature of excessive physical activity.<sup>22</sup> Furthermore, undereating and insufficient EI may arise from non-pathological reasons like poor awareness of appropriate sport-specific fuelling, lack of interest or time to prepare meals meeting refuelling requirements.<sup>1, 23–25</sup> Athletes are in general also characterised by positive attributes like resilience and well-being that may facilitate self-care and protect against

an inner or external drive to undereat.<sup>26–31</sup> Whether such attributes may lower the risk of LEA and RED-S remains uninvestigated.

The present protocol consists of four studies exploring the prevalence and correlations of LEA among female elite football players. **Table 1** provides an overview of study-specific aims, including information about the timeframe of the project and data collection related to self-report instruments, microtechnological devices, a gynaecological clinical assessment and biological measures of saliva, urine, blood and bone.

## METHODS

### Participants

For all studies (1–4), female players (age 16–34) are recruited from four high-level female football clubs, competing in the Norwegian first or second division. The four included teams are located in Bergen, the largest city in the Western region of Norway.

### Overall procedures

For all studies, information will be collected about age, educational/occupational status, training history, duration of team membership, training/competition volume, height, weight, FFM and position in the team, respectively. Data collection for study 1 will occur at the teams’ training facilities. Here, players will perform a football-specific circuit, mimicking the physiological demands in elite female football based on existing data from international games<sup>32</sup> and collecting GPS performance data. Additional data collection will primarily take place at outpatient healthcare facilities and certified physiological laboratories

### Measures of energy expenditure and intake

**Table 1** provides an overview of the microtechnological devices used in studies 1, 2 and 3, respectively. In study 1, these measures will be post-hoc compared against indirect calorimetry.<sup>33</sup> Oxygen uptake (VO<sub>2</sub>) will be measured continually throughout the test, using a portable gas analyzer (VO<sub>2</sub> Master Health Sensors). Due to oxygen deficit at exercise onset, the post-exercise oxygen consumption will be added to the overall oxygen cost of the physical exercise protocol, following rest periods within the protocol.<sup>34</sup> The results of study 1 will serve as a reference for measuring EEE during the data collection of the remaining studies, necessary to calculate EA. The data collection for studies 2 and 3 will be completed simultaneously for approximately 6 months. In study 2, total energy expenditure will be measured via DLW to quantify the total energy expenditure of this population, following the Maastricht protocol.<sup>35</sup> In studies 3 and 4, players will, during training and matches, wear a GPS device (based on study 1) for analysis of duration of the activity, total distance covered, average speed, time in different speed zones. For study 3, GPS measures using metabolic power will also aid to quantify EA through EEE, in addition to general EE provided by DLW, as the

**Table 1** Overview of study specific aims, samples and data sources

Studies, samples, and purposes	Time frame	Self-report instruments	Microtecnological devices	Biological measures
Study 1 (n=13) Aim: To evaluate the accuracy of methods measuring exercise energy expenditure (EE) against the gold standard of indirect calorimetry.	March–April 2021. Baseline +7 days consecutively	RPE	GPS-tracking, foot mounted accelerometer/gyroscope Vo <sub>2</sub> (indirect calorimetry)	Blood lactate (mmol/L)
Study 2 (n=20) Aim: To quantify energy intake (EI), EE and its implications for nutritional recommendations	September 2021–April 2022. Baseline +14 days consecutively	24 hours diet recall × 3. Weekly weight measurement.	GPS tracking Activity monitor with integrated accelerometer	DLW and DXA
Study 3 (n=50) Aim: To identify the point prevalence of LEA and validity of subjective measure of LEA through objective physiological markers	September 2021–April 2022. Baseline +30 days	LEAF-Q +additional items EDE-Q core items. EI (food registration) and training intensity on food registration days		DLW RMR DXA Analysis of the following biochemical markers: TSH, free T3 free T4, glucose, insulin, C-peptide, cortisol, S-CTX-1 (collagen I), PINP, leptin, adiponectin, IGF-1, IGFBP3, estradiol, testosterone and SHBG. Serum and plasma collection for biobanking. Transvaginal Ultrasound (If indicated by testosterone levels)
Study 4 (n=50) Aim: To explore whether the prevalence of LEA varies across a full football season and its possible biological and psychological covariates	January 2022–January 2023. 1 year (four measure points during 3–5 testing days at baseline, in season, and post-season, respectively)	LEAF-Q +additional items EDE-Q core items CFQ (fatigue) GHQ-12 (mental distress) BIS (insomnia) SoC-13 (resilience) QoL-BREF (well-being)		See study 3. A selection will be made based on study 3-findings for a subsample (N=20). Physical performance (baseline and post season).

BIS, Bergen Insomnia Scale; CFQ, Chalder Fatigue Scale; CTX-1, type I collagen cross-linked C-telopeptide; DLW, double labelled water; DXA, dual-X-ray-absorptiometry; EDE-Q, Eating Disorder Examination Questionnaire; GHQ-12, General Health Questionnaire-12; LEA, low energy availability; LEAF-Q, Low Energy Availability in Females Questionnaire; PINP, Procollagen type I N-terminal propeptide; RMR, resting metabolic rate; RPE, Rated Perceived Exertion; SoC-13, Sense of Coherence Scale; TSH, thyroid stimulating hormone; VO<sub>2</sub>, oxygen uptake.

latter cannot specify the EE of single training sessions or matches. Measurement of EE through GPS devices will also be used during study 4 to quantify EA. A 24-hour diet recalls distributed randomly throughout the data collection will quantify EI for studies 2,3 and 4, respectively.

### Biological data collection and storage

Blood samples for all hormonal data will be collected after an overnight fasting period (8–10 hours). A transvaginal ultrasound will be performed and examined in conjunction with the serum testosterone where necessary to screen for potential polycystic ovarian symptoms. The gynaecological examination will be conducted at an

outpatient clinic if required. Other biochemical parameters that will be measured are presented in [table 1](#). Moreover, serum and plasma samples for biobanking will be obtained. Using the Maastricht Protocol,<sup>35</sup> urine from the DLW-samples will be stored in airtight vials and frozen until analysis to determine the isotopic disappearance rate and thus energy expenditure. Data from DXA and resting metabolic rate (RMR) based on DLW and physical performance will be completed at a certified physiological laboratory at Western Norway University of Applied Science. Serum and plasma blood samples will be collected and stored in an existing biological bank. All

hormonal contraceptive agents used by participants will also be registered for analytical purposes.

### Self-Report Questionnaires

The set of self-report questionnaires (table 1) includes a measure of LEA<sup>7</sup> with additional football-related items inspired by previous work<sup>36</sup> to capture football-specific injuries (studies 2–4). Also, we will measure mental and physical fatigue (study 4),<sup>37</sup> a brief version<sup>38</sup> of a measure of eating problems (Eating Disorder Examination Questionnaire)<sup>39</sup> to capture weight and shape concerns (studies 3 and 4). In study 4, we also measure psychological distress,<sup>40</sup> insomnia and sleeping difficulties,<sup>41</sup> resiliency and sense of coherence<sup>42</sup> as well as the overall quality of life and well-being.<sup>43</sup>

### Statistical approach

Using a within-study design with unevenly spaced-out repeated measures, a linear mixed modelling approach is preferred as it supports the use of time-variant predictors, variations in the repeated time codings, including superior flexibility in adjusting the fixed coefficients for complex error correlation structures related to repeat measures or within team dependencies. The restricted maximum likelihood method ensures the use of all available information, thus being more lenient toward missing data in estimating marginal mean changes over time.

### Power calculations

The use of double labelled water (DLW) and full-body DXA scans are highly resource-demanding; hence, these data are collected from a single team consisting of at least 20 women. Despite numerous studies on the use of DLW, we only located a single study examining the relationship between DLW-based RMR (representing chronic energy imbalance) and the LEAF-Q questionnaire, which indicates a Pearson  $r$  of approx. 0.45. As football is physiologically more intensive, we consider a stronger association, likely between 0.45 and 0.60. Using an  $\alpha=0.05$ ,  $r=0.50$ , and a lower 95% CI of  $r>0.30$ , requires ~50 independent observations. Assuming an intraclass correlation of 0.25 requires 5 repeated measures from 20 women to achieve an effective sample size of  $N=50$ , given a statistical power of 0.80. For study 3 (prevalence), assuming a prevalence of ~0.35 (reported between 0.15 and 0.55 in the literature), power of 0.80,  $\alpha$  of 0.05 and a finite population (max 200 elite players in Norway), requires a minimum of approx. 60 or 120 observations for a precision of  $\pm 0.10$  and  $\pm 0.05$ , respectively. We, therefore, recruit a minimum of 60 women from 3 elite clubs. Adding 4 repeated measures throughout the full football season (assuming ICC=0.25) yields an effective sample size of approximately 137 independent observations that we regard as adequately sensitive to study point precision and variation in prevalence.

### User involvement and participation

User involvement enhances the relevance and quality of research.<sup>44</sup> Hence, 3–5 elite female football players from

clubs that do not participate in the study will cooperate with the research group, notably in the implementation of the protocol and the dissemination of the results.

## DISCUSSION

Previous studies have indicated that LEA is present among ball game female athletes. Yet, methodological issues and limited accuracy of measurements may underpin the huge range in prevalence figures across studies. The main purpose of the present protocol is to establish the technological and psychometric basis for exploring the prevalence and correlates of LEA among female elite football players, and hence, contribute to the existing literature within this area which is quite limited for female football players.

With the heavy logistics connected with data collection, storage and analysis of biological data, the number of football clubs involved had to be restricted to a particular region in Norway. The statistical power may thus be a critical issue in this study. The COVID-19 pandemic restrictions and the nature of the finite population provide few options to enlarge the project to other national regions, which could entail an increased risk concerning lowering feasibility related to non-participation drop-out or missing data. In this project, feasibility was weighted highly due to the allegiance, commitment and adherence to the overall aims and the procedures for data collection among the female players, the club leaders and the overall supporting teams. Moreover, to ensure compliance, a research team member will be present to respond to queries and secure optimal data quality during the data collection procedures. In the present COVID-19 situation with heavy travel restrictions, a similar allegiance and commitment might not have been possible with a national, multisite implementation approach. Given that we consider the effect size estimations as fair and the compensation that repeated measures offer in terms of statistical power and offering future interesting study possibilities, the current project may yield new and high-quality knowledge to this emerging research field.

User involvement may enhance the relevance and quality of research.<sup>44</sup> Hence, a group of end-users (2-3) /elite female football players (who are not participating in the project) will cooperate with the research group, notably in implementing the protocol and disseminating the results. Another asset of user involvement is related to the development of items in addition to the LEAF-Q to capture football-related injuries. It takes a considerable amount of time and access to a large group of project-independent players to develop and validate a football-specific new instrument to capture the proxies of LEA. However, the present user engagement procedure is, in our opinion, satisfactory and will provide a basis for future validation studies.

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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** Not applicable.

**Ethics approval** The protocol has in July 2021 been approved by the Norwegian Centre for Research Data (ref. no. 807592). The Regional Committee for Medical and Health Research (REK) judged that while the study did not fall under the Norwegian Health Research Act (ref. no. 257695), ethical approval was needed for the biobank storage of serum and plasma blood samples. A REK-approval was given in July 2021 (ref. no 29081) for storage in previously approved biobank (2016/787).

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**Data availability statement** No data are available. This is a study protocol article and hence, no data are available at present.

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