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Review Article

Low Energy Availability in Female Athletes: From the Lab to the Field

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Abstract

Decades of laboratory research have shown impairments to several body systems after only 4-5 days of strictly controlled consistent low energy availability (LEA); where energy availability (EA) = Energy Intake (EI) – Exercise Energy Expenditure (EEE) / Fat-Free Mass. Meanwhile, cross-sectional reports exist on the interrelatedness of LEA, menstrual dysfunction and impaired bone health in females (the Female Athlete Triad). These findings have demonstrated that LEA is the key underpinning factor behind a broader set of health and performance outcomes, recently termed as Relative Energy Deficiency in Sport (RED-S). There is utmost importance of early screening and diagnosis of RED-S to avoid the development of severe negative health and performance outcomes. However, a significant gap exists between short-term laboratory studies and cross-sectional reports, or clinically field-based situations, of long-term/chronic LEA and no definitive, validated diagnostic tests for RED-S exist. This review aims to highlight methodological challenges related to the assessment of the components of EA equation in the field (e.g. challenges with EI and EEE measures). Due to the uncertainty of these parameters, we propose the use of more chronic "objective" markers of LEA (i.e. blood markers). However, we note that direct extrapolations of laboratory-based outcomes into the field are likely to be problematic due to potentially poor ecological validity and the extreme variability in most athlete's daily EI and EEE. Therefore, we provide a critical appraisal of the scientific literature, highlighting research gaps, and a potential set of leading objective RED-S markers while working in the field.

Keywords: low energy availability, relative energy deficiency in sport, diagnosis, female athletes

1. Introduction

The associations between low energy availability (LEA)/eating disorders, menstrual dysfunction, and impaired bone health in female athletes were established more than three decades ago when it was discovered that female athletes reporting with menstrual dysfunction appeared to also suffer from low bone density and underlying eating disorders [1]. These early discoveries were followed by an official recognition of the condition as the female athlete triad (the Triad) in the 1990's [2], which was further updated in 2007 to emphasize that it was a continuum from health to disease and that the presence of any one of the three symptoms poses a risk for the female athlete [3], of which LEA is the underlying etiology. Energy availability (EA) is defined as dietary energy intake (EI) minus exercise energy expenditure (EEE) corrected for fat-free mass (FFM) [4]. Subsequent strictly controlled laboratory interventions in sedentary females established links between 4-5 days of an EA of <30 kcal/kg FFM/d and impaired endocrine marker concentrations, thus defining this as a threshold for LEA [4]. However, while the concept of EA is scientifically sound in well-controlled laboratory settings, applying it directly in the field is challenged by several methodological considerations, mainly to do with the large variability and poor accuracy of field-based EI and EEE assessments.

In 2014, these identified LEA impairments originally recognized from Triad research were known to extend across other body systems and functions, and both females and males, identified as Relative Energy Deficiency in Sport (RED-S) [5, 6]. Here, RED-S was 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"[7]. Despite progress, there are still no validated tool(s) for early detection of LEA to prevent the development of more severe forms of RED-S.

Accordingly, this review will address some important differences and gaps between short-term (<7 days) laboratory-based investigations of LEA (Figure 1) and their application to the field where symptoms usually reflect underlying, medium-term (weeks to <3 months) or long-term (>3 months to years) LEA. Specifically, we will: 1) discuss the key methodological challenges of EA calculations; 2) summarize the current evidence of short-term, strictly controlled laboratory investigations on the effects of LEA, along with an evaluation of potential markers to use in the assessment of EA status; as well as 3) explore the challenges and risks of extrapolating laboratory-based findings to screening and monitoring of risk of LEA in the field. Although much progress has been made on LEA and RED-S in males [8], this review will focus primarily on females to leverage the extensive decades of Triad data, into the RED-S context. Taken together, we hope this review will illuminate significant progress in understanding of the impact of LEA and RED-S in females. We will also highlight our significant field-based experiences of working with athletes with LEA, to demonstrate the challenges in applying laboratory findings to the field, as well as explore future research opportunities.

2. Methodology and challenges of the energy availability equation

Broadly speaking, LEA status can be assessed: a) Directly, through algebraic calculations from dietary EI and EEE data; and b) Indirectly through assessment of potential symptoms indicative of short to long-term LEA.

2.1 Assessment of EA in the lab and field: methodology and challenges

Although the algebraic calculation of EA is simple ((EI-EEE)/FFM), the practical and validity challenges of accurately determining EI and EEE are often ignored, which can result in significant errors when assessing these in the field or with poor laboratory methods [4, 9].

EI can be estimated a number of ways, such as self-reports, interviews, and food frequency recalls [10]. All of these methods are prone to significant errors (up to ~600 kcal/d [9]) due to factors such as under/over/misreporting, poor athlete compliance as well as differences in data entry between experts. Furthermore, intakes are often recorded over a brief (3-7 days) time span which acts as a compromise to maintain compliance, while allowing for increased reliability and relevance of data. From an athlete perspective, data should be collected across different types of training days, and both weekdays and weekends, to gain reliable information of the overall practices [10].

The assessment of EEE in the field is equally challenging. While doubly labeled water is typically considered a gold standard for estimating total daily energy expenditure (TDEE), it provides average values over a longer time-period (usually 1-3 weeks) and does not differentiate between EEE and TDEE. There are currently no gold standard measurements for the estimation of EEE. At best, the use of laboratory-based measures where heart rate (HR) is plotted against indirect calorimetry data [11] can be used. In general, HR monitors or power meters (cycling, [12]) are likely to be superior to the use of metabolic equivalents (METs, [13]), but even these have the potential for errors in the magnitude of ~100-600 kcal/d [14]. Regardless, we note that net EEE describes the energy expended during exercise only, and the resting energy expenditure component should be subtracted from the overall EEE value prior to performing final EA calculations, based on the latest definition of EA [4, 5]. An additional challenge to the calculation of daily EEE is the consideration of what constitutes exercise versus activities of daily living (i.e. non-exercise activity thermogenesis (NEAT)). There is currently no consensus on when NEAT may become part of the EEE equation, and this is especially impactful for athletes who undertake exercise based commuting or physical labor employment situations. Finally, for optimal accuracy, FFM assessment requires specialized training and equipment (skinfold calipers, DXA, etc.), as well as careful preparation of the athlete (standardized measurement protocol) that are not readily available to all athletes [9]. Finally, we note that the assessment of the components of the EA equation requires specialized skills and training to optimize the accuracy and reliability of measures. For example, dietary analysis should ideally be done by a trained and experienced sports dietitian, assessment of EEE by an applied sports physiologist, while the measurement of body composition requires specialized training (for example, The International Society for the Advancement of Kinanthropometry certification or radiation safety training for use of Dual-energy X-ray Absorptiometry).

Considering the methodological challenges outlined above, it is not surprising that many primarily field-based studies have consistently shown that athletes divided into groups based on calculated EA show little differences in physiological outcomes related to LEA [15, 16], such as menstrual function [15, 17, 18], and that

impairments to physiological systems cannot be traced down to a single EA threshold [19]. Some of these mismatches may be explained by the fact that despite consistent findings in females in well-controlled laboratory studies, some of these outcomes may not apply to more elite athletes of all ages, sexes and training characteristics. Indeed, one significant gap in the literature is the lack of research around EA thresholds for adolescents or children. The adolescence period (~13 to 19 years of age) is a phase during which ~25% of adult total BMD is acquired during the 4-year period surrounding peak height velocity (puberty growth spurt), with ~90% of peak BMD being achieved by age 20 [20]. Further research is also required to better understand the impact of both gynecological age (age from first menses to current age) and accumulated eumenorrheic age (number of accumulated years with an eumenorrheic cycle) on EA thresholds and RED-S outcomes. Nevertheless, it is safe to assume that the EA thresholds for adolescents are likely to be very different to the ones applied to adults due to the different energetic requirements of growth, development and puberty. It is also possible that factors such as diet quality [21], timing of meals within-day and in relation to training [17], carbohydrate availability [22], the magnitude of changes in any of the EA components in any one time, overall training load and psychological stress may also impact on EA outcomes [9]. Furthermore, while studies on LEA have defined a threshold of EA below which impairments have been observed, we note that this threshold should be treated as a rough guideline while appreciating that several factors will eventually determine the threshold at/below which impairments occur and/or that impairments probably also occur at differing rates [23]. Overall, if one is to implement EA assessments, incredible care and gold standard methods should be implemented, which is challenging in most real-world situations. Accordingly, in the following sections we propose the use of objective LEA indicators for athlete screening and diagnosis (*section 3*).

2.2 Considerations of the time frame of EA assessment

Most research has not considered the ecological validity of reporting daily EA over the short-term (e.g. within-day or <1 week) period as a true indicator of the EA over medium to long-term (weeks to months or years). Laboratory-based studies tend to implement a homogenous/consistent LEA over the course of 3-6 days [5]. However, athletes show large natural fluctuations in EI and EEE (training or competition) within [24] and between days [12]. Preliminary analyses of unpublished data from our team show for the first time that in free-living professional male road cyclists in pre-season training, assessed over 7 consecutive days, there is a strong inverse relationship between daily EEE and EA (r^2 = -0.78), with spontaneous daily EI failing to match the energy with increasing EEE resulting in very low EA on days with high EEE (*Areta JL, manuscript in preparation*). While these observations are in male cyclists, we believe that the same inverse relationship between EEE and EA would be evident in females and that EA is likely to vary between training days, largely as a function of EEE, as there are no reasons to believe there would be a sex-based difference for this phenomenon. This is in line with what we have previously shown in elite male cyclists, where alternating days (e.g. intermittent) of LEA (~15 kcal/kg FFM/d) on race days were reported in comparison to optimal EA (~57 kcal/kg FFM/d) on days in-between racing [12]. Here, despite extreme LEA on race days, there was no effect on physique outcomes or concentrations of testosterone, T3, IGF-1 or cortisol over an 8-d period, perhaps due

to the fact that the average EA over this observation period was ~36 kcal/kg FFM/d. This suggests that brief intermittent periods of extreme energy deficits, at least in males, may be manageable if overall EA across a time-period of ~1 week remains within acceptable limits; however, these outcomes remain to be established in female athletes. This study also challenges the notion that matching EI to EEE within every 24 hr block is crucial, and instead suggests that perhaps it is more important to maintain an overall, optimal EA over a rolling average of days.

In contrast to this idea, some research shows that small to moderate within-day periods of LEA over a single 24 hr observation period are associated with negative health symptoms despite adequate daily total EA [17, 24]. For example, female athletes with a greater number of hours of energy deficit of >300 kcal and with the largest daily energy deficits reported to have greater body fat percentage compared to those with less severe energy deficits within-day [24]. These transient within day deficits were also correlated with lower RMR_{ratio} and estradiol and higher cortisol concentrations in females with menstrual dysfunction [17]. Although over a single 24 hr period, these findings may suggest that EA should be matched within-day, perhaps around exercise, to maintain optimal health. However, since this data is cross-sectional and thus makes it impossible to draw conclusions on causality, as well as determine whether within-day EA deficiency was short-term or long-term, more research is warranted around this topic.

Furthermore, it is likely that the duration, depth and gradient of LEA all play a role in the "dose" of the LEA ([5]; Figure 2) and thus, the potential outcomes of an intervention. The challenge with strictly controlled laboratory studies is that it is often impossible to monitor athletes for longer time periods. As such, our current knowledge relies largely on extrapolations or field-based reports. Therefore, we recommend that the athlete aims to maintain an overall EA level that meets the specific goals and requirements of their individual situation. That said, we also suggest that athletes focus on the timing of meals in relation to exercise, as this may have an additive effect on athlete health.

3. Measurement and interpretation of blood markers as an alternative option to assess LEA

The early detection of LEA in athletes is imperative as it represents the gateway to long-term or repeated exposure to the deleterious outcomes of prolonged LEA. It is well established that long-term (>3 months to years) LEA can ultimately result in important negative clinical outcomes such as functional hypothalamic amenorrhea and low bone mineral density (BMD), as well as potentially several other impairments to health and performance [6, 7, 25]. However, identification of early markers is still in its infancy.

Clinical diagnosis of long-term LEA is typically determined through assessment of bone health and menstrual function [25], but these markers have important shortcomings. In relation to bone, indications of LEA (structural changes) are often detected only after months or years of long-term/chronic LEA, where the effects of low BMD and related stress fractures can be irreversible. In relation to menstrual function, alteration of normal menstrual bleeding —an easily identifiable clinical marker of prolonged LEA— lies at the end of a

continuum anteceded by luteal phase defects and anovulation [26, 27] and it may not become evident, or it can become masked, due to the use of oral contraceptives [28], despite LEA [19, 26, 29].

Given that there is currently no validated tool to integrate potential objective physiological markers for detection of short-and medium-term (days to weeks) LEA, this section aims to identify measurable parameters that have been shown to be sensitive and responsive to LEA in laboratory-based studies (Table 1). We believe that these parameters may allow for objective identification of athletes at risk of LEA independent of other stressors, and help overcoming the challenges of assessing EA in the field based on EI and EEE.

3.1 Short-term effect of LEA on blood parameters

Most laboratory-based studies in females have tested the causal effects of short-term (3-6 days) LEA, usually at <30 kcal/kg FFM/d, on a range of hormonal and metabolic parameters in blood [4, 23, 30]. Based on these studies, with a relatively brief exposure to LEA, there is a clear disruption in the hormonal milieu and metabolism, suggesting various parameters that could be used in clinical practice (Table 1).

Leptin. Leptin is an important regulator of energy metabolism and when reduced under normal levels, it exerts a modulatory effect peripherally on a range of tissues and centrally in the hypothalamus [31]. Circulating leptin has been shown to unequivocally be reduced with LEA [22, 23, 32-34]. An EA of 30 kcal/kg FFM/d for 5 days was sufficient to decrease circulating leptin by ~35%, and further decreases of ~70% were seen with an EA of 10 kcal/kg FFM/d [23]. It must be kept in mind that many commercial laboratories do not routinely measure for leptin, but it is a parameter that may be available to be measured on request, but at high cost.

Thyroid hormones. Thyroid hormones modulate energy expenditure through central and peripheral pathways [35] and are the main regulator of resting metabolic rate (RMR) [36]. Circulating levels of thyroid hormones are regulated through integration of energy-sensing inputs in the hypothalamus [35]. While T4 has not been measured in most experimental research on EA, free and total T3 has been shown to consistently be downregulated with LEA [23, 32-34, 37-39]. A threshold EA of ~19-25 kcal/kg FFM/d for 5 days has been reported to decrease T3 in a dose-response study [40] and it appears that as little as 2 days of EA of ~11 kcal/kg FFM/d may reduce circulating T3 [36]. Group average reductions from baseline values have been shown to range from ~6% at an EA of 30 kcal/kg FFM/d [23] to ~20-25% at EA of 10-19 kcal/kg FFM/d [23, 32, 33, 37, 39, 40]. On the contrary, some literature suggests that LEA may increase T4 [36, 40], which could be related to reduced conversion of T4 to T3.

The hypothalamic-pituitary-ovarian axis. The negative effects of LEA on female reproductive endocrinology and physiology have been thoroughly researched [41]. Available evidence suggests that LEA is the main cause of functional hypothalamic amenorrhea and disrupts normal endocrine responses during the early follicular phase, such as reducing pulse frequency and increased pulse amplitude of luteinizing hormone (LH) [23, 32, 37, 39]. However, follicle stimulating hormone (FSH) and oestrogen (E2) morning values, which are important hormones in the clinical diagnosis functional hypothalamic amenorrhea [28] and clear candidates as early

markers of LEA, are not affected by short-term LEA [23, 32, 37-39]. Therefore, changes in these hormones may become evident only after prolonged (weeks to months) LEA, but more research should address if short-term LEA affects their normal concentration in the luteal phase. Additionally, about 50% of female athletes use hormonal birth control, which makes the use of these hormones as reference unsuitable [42].

Insulin-like growth factor 1 (IGF-1) and growth hormone (GH). IGF-1 is an important hormone for protein synthesis and cell proliferation [43]. Pituitary release of GH exerts the majority of its effects through regulating IGF-1 release from the liver [44]. LEA induces a state of *GH resistance*, in which normal GH effect on liver IGF-1 release is impaired, resulting in increased circulating GH and reduced circulating IGF-1 [44]. LEA induces a state of GH resistance within days, making both GH and IGF-1 good candidates as clinical markers of short-term LEA. Three studies in females have reported GH concentrations above resting levels with 4-5 days of EA ≤20 kcal/kg FFM/d [23, 32, 39]. The 24 h average GH concentration has been shown to increase by 23-120% with an EA of 10 to 20 kcal/kg FFM/d [23, 32], while morning values increased by ~26% with an EA ~13 kcal/kg FFM/d [39], with no effect with an EA of 30 kcal/kg FFM/d [23, 32]. IGF-1 has been shown to consistently be reduced with similar amounts of LEA in studies with female participants [23, 32-34, 38, 39]. The reduction of morning circulating IGF-1 values has been on average ~34% for short-term EA 10-20 kcal/kg FFM/d [23, 32, 33, 38, 39], while no effect was seen with an EA of 30 kcal/kg FFM/d [23]. Based on the evidence, IGF-1 seems to have significant support to be used as a morning marker of short-term LEA, while GH is unclear. IGF-1 and GH are typically not routine tests but can be easily accessed on demand.

Bone formation and resorption markers. Low BMD and increased prevalence of stress fractures are the result of medium to long-term negative balance of bone matrix synthesis that is an outcome of an imbalance between bone resorption and bone formation. Acute changes in bone turnover can be detected by measuring blood circulating markers of bone resorption and bone formation that are sensitive to exercise and nutrition interventions [45] and change within days of LEA. The acute effect of LEA on markers of bone metabolism has been limited to a few studies which collectively seem to support markers of bone formation are more sensitive than on markers of bone resorption [33, 34, 46]. For example, bone formation marker carboxy-terminal propeptide of type 1 procollagen (P1CP) has been shown to decrease linearly in one study titrating EA from 30 to 10 kcal/kg FFM/d from 10 to 29% of baseline values, respectively [46]. It is beyond the scope of this review to elucidate all bone metabolism markers, and the interested reader is directed here [45]. However, we should highlight that currently commercial labs do not offer analysis of these markers and validated established norms do not yet exist.

3.2 Urine luteinizing hormone surge

Functional hypothalamic amenorrhea sits at the end of a continuum of endocrine regulations anteceded by luteal phase defects and anovulation, which are observed in proportion to markers of energy conservation [26, 27]. Although studies assessing the endocrine effects of LEA have not investigated changes in LH surge during the menstrual cycle, this represents another potential practical parameter that could be used in the field with

non-hormonal contraception users [47], before more severe menstrual disturbances become evident. It is important to highlight though, that one study failed to find a relationship between the level of energy deficit and severity of induced menstrual disturbances during three consecutive menstrual cycles in exercising women [48], making unclear how sensitive this parameter can be to LEA.

3.3 On using indirect markers to assess LEA in female athletes

We believe that some of the biomarkers outlined above could potentially help in identifying short-term LEA in female athletes (Table 1). However, we note that changes in these markers may not exceed the threshold for clinical reference range. It is also unclear whether general population norms can be directly applied to the athletic population, but to date no athlete specific reference values have been established. Within-individual variations as well as assay reliability should be considered as sources of random error that must be taken into account to determine whether a change in a parameter represents a real and meaningful change indicative of LEA. Additionally, these markers may only indicate the acute presence of LEA, without currently having the capacity to distinguish exactly what level of LEA and/or for what duration. Potentially, markers that have been shown to be strongly related to menstrual dysfunction but have not shown changes in acute LEA studies — such as E2 and FSH— [5] could be investigated as markers of medium-long term exposure to LEA. Further complexity to the interpretation of these markers comes from the fact that some of the hormones (for example, GH and cortisol) may be sensitive to the effects of acute or chronic exercise [49]; it may therefore be challenging to determine whether abnormalities in a marker are due to exercise training or underlying LEA. Ideally, we recommend collection for these blood tests to be performed in the overnight fasted state, first thing in the morning prior to exercise and to have the last high-intensity (hard training) session at least 48 hr prior, to minimize the potential effects of exercise and normalize circadian rhythms on these markers. Finally, it is important to emphasize that the blood biomarkers are based on a range of well-controlled laboratory studies determining the effect of LEA **only** on very specific populations (often sedentary women) and need to be validated in wider athlete populations, specific timing of sample collection in relation to LEA exposure, and they may only be used as a broad guidance for further investigation of energetic status of athletes to avoid potential negative effects of prolonged LEA.

4. Risks and flaws of extrapolating findings from the lab to the field – a identifying key differences between the two different settings

While strictly controlled, short-term laboratory investigations of LEA are essential to characterize the physiological outcomes of acute LEA, their applicability to the field is challenged by several factors, which will be outlined below.

4.1 Length of exposure

Without exception, laboratory studies focusing on LEA implement very brief (3-5 days) protocols [11, 22, 23, 33, 36, 38-40, 46]. Accordingly, these investigations are often unable inform us about the long-term effects of LEA. Even if a specific marker does not show a change after 5 days, it may be affected with increasing duration

of LEA (for example, total cholesterol and E2 appear to be influenced only after prolonged LEA; [23, 32, 37-39, 50]). Furthermore, some markers that have showed a change in short-term laboratory studies appear to be within the reference range in cross-sectional comparisons of amenorrheic vs eumenorrheic females (for example, insulin and IGF-1 rarely differ between the two [15, 16]). Indeed, long-term exposure seems to reduce the circulating level of at least some of these markers, even if the values remain within the clinical reference value [51]. Regardless, since athletes may spend a considerably longer time-period (often weeks to months; ≥10 to 20 times longer than laboratory studies) in LEA [52], extrapolating observations from the lab to field settings appears inappropriate.

In addition, currently very little is known of the interactions between the magnitude of change required for a single biomarker (e.g. a decrease in PN1P or a decrease in T3) to induce a subsequent, meaningful functional impairment (e.g. with P1NP, a decrease in BMD and development of a stress fracture; or with the case of T3, a reduction in RMR). This is obviously a key consideration as an isolated change in a biomarker is irrelevant unless a functional outcome follows. Unfortunately, the literature lacks mechanistic data on the time-course or threshold for which a functional change can be expected. Nevertheless, an investigation in female physique competitors reported endocrine impairments (reductions in T3, E2, leptin and testosterone) as a result of a 4-month preparatory period (and LEA) for a competition but a rebound in most hormones except T3 and testosterone at 3-4 months post-competition [53]. The length of exposure is an important consideration as the reversal of impairments may be dependent on this factor [54]. Based on available literature, we hypothesize that reversal is rapid following brief (<1 to 3 months) periods of LEA [53], whereas there could be potential for the development for much slower reversal of biomarkers, and some irreversible symptoms (including persistent amenorrhea or low BMD), with increasing duration of LEA [15, 55, 56].

4.2 Ecological validity of daily EA and dietary macronutrient composition

Another challenge in the application of laboratory-based results into the field is the use of sudden drops in EA (for example, from 45 to 15 kcal/kg FFM/d) that remain constantly low, without between-day fluctuations in EA. This is likely not representative of what the majority athletes may experience in the field, in which athletes may be exposed to LEA in different patterns (Figure 2). Most laboratory-based studies induce a consistent/homogeneous LEA (often ~10-20 kcal/kg FFM/d), while we have observed daily fluctuations in EA which can be inversely related to EEE [13; Areta JL, *manuscript in preparation*], which is line with the recent findings of fluctuation of EA across training/competition seasons being related to EEE [57]. These protocols are the opposite of what is currently considered best practice for weight loss for athletic populations, where some evidence supports the use of gradual, small changes in EI with weight-loss interventions [58]. Meanwhile, more research is required to determine the optimal periodization of EA "dose" to prevent deleterious health outcomes, while optimizing potential performance outcomes (Figure 2). Furthermore, laboratory investigations tend to implement significantly lower EA values for the LEA intervention arms than what has been reported in the cross-sectional research [15, 50]. Therefore, it is possible that some of the changes reported in laboratory-based LEA investigations after only 5 days of LEA are more a result of a sudden

change and/or the application of an extreme LEA, or potentially, a combination of both, and may poorly translate into the field where more conservative approaches are likely to be implemented.

In addition to EI, the macronutrient composition deserves a brief discussion. Surprisingly and against the recommendations for optimized weight-loss approach to athletes [59], most studies on LEA have implemented a static macronutrient ratio for all levels of EA. This not only results in absolute reduction of EA, but also in a decrease in all macronutrients. Here, carbohydrates and proteins are of special interest as both have important roles in the management of the metabolic rate and physique outcomes during energy restriction. Indeed, studies have shown that some of the effects of LEA are mediated via reduced CHO availability [22], which appears to have effects on especially leptin and T3 concentrations [60, 61]. These in turn affect RMR, thus facilitating or suppressing metabolism during periods of LEA [62]. Meanwhile, increased protein intake in the face of LEA allows maintain increased muscle protein synthesis [63] lean mass and health during weight loss [59]. Therefore, it could be argued that the poor emphasis on optimized CHO and protein intake, coupled with more extreme levels of LEA, in many LEA investigations may potentiate the negative outcomes and is likely to decrease the ecological validity and applicability of the results into the field practice when following current dietary recommendations.

Finally, strict laboratory-controlled investigations are often conducted in sedentary or only moderately trained individuals, where applications to the elite female athlete are troublesome. For example, there are genetic, anthropometric and physiological differences between untrained, trained and elite athletes [64]. In addition, and as mentioned earlier, elite athletes engage in high training loads which can be 2- to 4-fold greater compared to recreational athletes (for example, [65]).

5. Summary and future research considerations

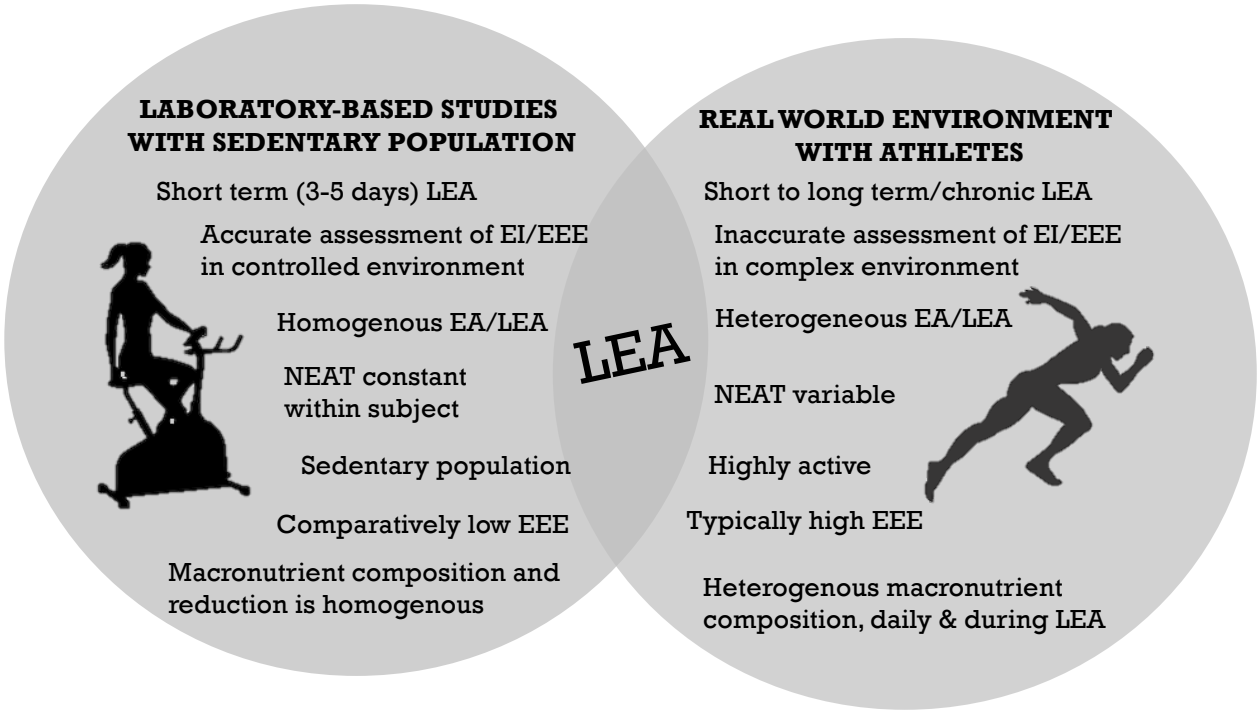
Decades of laboratory research have shown that impairments to several body systems can be seen in as little as 4-5 days of strictly controlled LEA. Meanwhile, a large body of cross-sectional reports exists on the interrelatedness of eating disorders, menstrual dysfunction and impaired bone health in females. These findings have increased the awareness of LEA as the key underpinning factor behind a broader set of health and performance outcomes recognized in models of ‘Relative Energy Deficiency in Sport’ and the ‘Triad’. However, a significant gap exists between short-term laboratory studies and cross-sectional reports of long-term/chronic LEA; as such, no consistent and reliable tools exist to screen athletes for LEA. Here we have highlighted several methodological challenges related to the assessment of the parameters of EA equation on the field (namely, EI and EEE). In light of the uncertainty of these parameters, we propose the use of more “objective” parameters as ‘end point’ markers of LEA (i.e. blood markers). These suggestions are mostly based on short-term laboratory research which supports the use of several hormone markers in identifying LEA in female athletes. However, we note that direct extrapolations of laboratory-based outcomes into the field are likely to be problematic due to several challenges relating to ecological validity. Therefore, we provide a critical appraisal of the scientific literature and a set of potential set of tools to utilize on the field. Further work

366 is warranted to gain a more sophisticated understanding of the best biomarkers to use for assessment of
367 medium- and long-term LEA. This includes the development of athlete specific standard ranges and an
368 understanding of what constitutes a real meaningful change from baseline (if repeat measures are done) as well
369 as of how to interpret isolated (single time point) measures. Future studies should aim to more systematically
370 assess and define the best markers of LEA to detect early signs of LEA and thereby, avoid the long-term
371 impairments to health and performance of the female athlete.

Figure legends

Table 1. Summary of parameters that are likely to provide an objective measure of short or moderate (days/weeks) exposure to low energy availability (LEA) in females. For blood parameters assessed in a biochemical laboratory it is important to consider that within-individual changes should be considered provided that values may be affected by LEA within individual without falling outside the accepted clinical range. Ideally, we recommend blood tests first thing in the morning prior to exercise and to have the last high-intensity (hard training) session at least 48 hours prior, to minimize the potential effects of exercise and normalize circadian rhythms on these markers. Overall, it is important to standardize measurements and consider the typical variation for an individual and error of measurement to determine likelihood of real change. Rather than considering one parameter in isolation, it is likely a stronger indicator to note trends in several parameters in combination.

Parameter	Sensitivity to LEA	Feasibility of test in real world	Best time of menstrual cycle to test	Ideal testing conditions	Refs
Blood Leptin	High	High; Not a routine biochemical assessment	Concentrations independent of cycle phase? (studied in early follicular non-OC users)	Standardized morning fasted; completed in the same laboratory	[22, 23, 32-34]
Blood IGF-1	High	High			[23, 32-34, 38, 39]
Blood Bone formation and resorption markers	Formation likely more sensitive than resorption	Low; Not a routine biochemical assessment			[33, 34, 46]
Blood total and freeT3	High	High			[23, 32-34, 36, 37, 39, 40]
Urinary LH surge	?	High; Affordable home kit	Between the follicular and luteal phase (non-OC users)	Collection of urine at 11-15 hr	[26, 27, 47]



382

383 **Figure 1.** Visual summary of the main differences between laboratory-based studies assessing the endocrine, metabolic and physiological effects of low energy
384 availability (LEA) in females (left) and the real-world environment (right) when working with athletes likely exposed to LEA. It is important to consider these
385 differences to understand the limitations and potential errors in extrapolating laboratory-based studies to field observations. These considerations put on evidence
386 that the typically considered LEA 'threshold' of 30 kcal/kg FFM/d —which has been determined in laboratory-based studies of ~5 days in duration— may not
387 necessarily represent a true indicator of LEA when assessed on the field. EA, energy availability; EI, energy intake; EEE, exercise energy expenditure; NEAT,
388 Non-exercise activity thermogenesis.

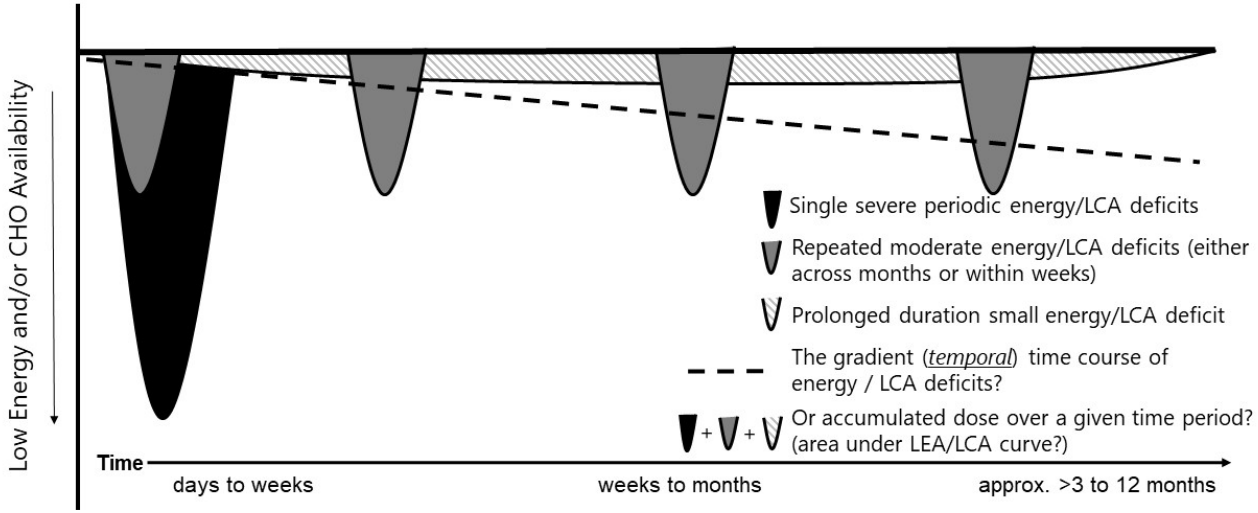


Figure 2. Visual representation of different ways in which low energy availability (LEA) or low carbohydrate availability (LCA) doses may be achieved in real-world scenarios. While laboratory-based studies tend to induce single severe LEA *(black area*), it is likely that on the field there are different scenarios, such as subtle LEA over prolonged time (*dashed area)* or a more heterogenous exposure to LEA during periods interspersed with adequate energy availability (*gray areas*). In addition, the gradient of change in LEA (e.g. sudden vs gradual drops; *dashed line*) is likely to play a role.

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