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Note: This article will be published in a forthcoming issue of the *International Journal of Sport Nutrition and Exercise Metabolism*. This article appears here in its accepted, peer-reviewed form; it has not been copyedited, proofed, or formatted by the publisher.

Section: Original Research

Article Title: Low Energy Availability is Difficult to Assess But Outcomes Have Large Impact on Bone Injury Rates in Elite Distance Athletes

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Running Head: RED-S in elite female and male endurance athletes

Journal: *International Journal of Sport Nutrition and Exercise*

Acceptance Date: November 27, 2017

DOI: https://doi.org/10.1123/ijsnem.2017-0313

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Word count: 4136

Abstract count: 249 words

Number of references: 35

Number of figures and tables: 5 tables and 1 figure

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ABSTRACT

We aimed to (1) report energy availability (EA), metabolic/reproductive function, bone mineral density (BMD) and injury/illness rates in national/world-class female and male distance-athletes; and (2) investigate the robustness of various diagnostic criteria from the Female Athlete Triad (Triad), Low Energy Availability in Females Questionnaire (LEAF-Q) and Relative Energy Deficiency in Sport (RED-S) tools to identify risks associated with low EA. Athletes were distinguished according to benchmarks of reproductive function (amenorrheic [n=13] vs eumenorrheic [n=22]; low [lowest quartile of reference range, n=10] vs normal testosterone [n=14]) and EA calculated from 7-day food and training diaries (< or >30 kcal.kg^{-1}FFM). Sex hormones ($p<0.001$), triiodothyronine ($p<0.05$) and BMD (females, $p<0.05$) were significantly lower in amenorrheic (37%) and low testosterone (40%; 15.1±3.0 nmol/L^{-1}) athletes and bone injuries were ~4.5-fold more prevalent in amenorrheic (ES=0.85; large) and low testosterone (ES=0.52; moderate) groups compared to others. Categorization of females and males using Triad or RED-S tools revealed that higher risk groups had significantly lower T3 (female and male Triad and RED-S:$p<0.05$) and higher number of all-time fractures (male Triad:$p<0.001$; male RED-S and female Triad:$p<0.01$) as well as non-significant but markedly (up to 10-fold) higher number of training days lost to bone injuries during the preceding year. Based on the cross-sectional analysis, current reproductive function (questionnaires/blood hormone concentrations) appears to provide a more objective and accurate marker of optimal energy for health than the more error-prone and time-consuming dietary and training estimation of EA. This study also offers novel findings that athlete health is associated with EA indices.

Key words: bone health, metabolic hormones, reproductive hormones, RED-S, Triad

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INTRODUCTION

Low energy availability (EA) is the failure of athletes to consume sufficient energy to cover the energy cost of exercise as well as energy required for optimal metabolic function and health (Loucks et al., 2011). Low EA has been reported in both female (Melin et al., 2015) and male (Viner et al., 2015) endurance athletes and is emerging as one of the most significant factors associated with athlete illness/injury (Tenforde et al., 2017). Original recognition of this syndrome in females, the *Female Athlete Triad* (Triad), identified the inter-relatedness of low EA, menstrual dysfunction and poor bone health (Nattiv et al., 1994; Joy et al., 2014). Indeed, there is ample evidence of the high prevalence (63%, Melin et al., 2015) and negative effects of low EA on hormonal function (Loucks & Thuma, 2003), bone health (Ihle & Loucks, 2004) and injury risk (Tenforde et al., 2017) in females. On the contrary, until recently, research on low EA in male athletes was lacking almost completely. Indeed, it was not until 2014, when the concept of *Relative Energy Deficiency in Sport* (RED-S) was introduced to include both sexes and a broader spectrum of health and performance-related concerns that the existence of low EA in males was acknowledged (Mountjoy et al., 2014). Since then, several reports have confirmed that male athletes do indeed suffer from the negative consequences of low EA (Tenforde et al., 2016), including low testosterone (Gomez-Merino et al., 2002) and metabolic hormone (Koehler et al., 2016) levels, albeit likely at a lower threshold than females (20-25 kcal·kg FFM·day^{-1}, Fagerberg, 2017). Furthermore, it is noteworthy that despite significant reductions (10-40%) in testosterone levels due to low EA (Tenforde et al., 2016) have been observed, the values have remained within the normal clinical range. This is important as the use of non-athlete clinical cut-off values in assessing suppressed reproductive function in male athletes may lack sensitivity, at least based on current literature.

Despite growing awareness of the causes and outcomes of low EA, several issues related to its detection and practical management remain difficult. This includes the lack of a

single sensitive tool to diagnose the phenomenon, particularly in field settings and with application to male athletes. We studied two cohorts of national to world-class distance female and male athletes with two primary purposes: (1) to provide a cross-sectional report on measurements of EA, metabolic and reproductive hormonal function, bone mineral density (BMD), injury/illness rates and body composition during a high-volume/intensity pre-competition training period; and (2) to investigate the robustness of risk-assessment of the Triad and RED-S diagnostic tools to identify athletes with these symptoms typically associated with low EA.

METHODS

Subjects

We used word of mouth and online advertisements to recruit national to world-class female and male middle- and long-distance runners and race walkers from the Finnish Athletics Federation (FIN; n=12 females and 10 males, at sea-level) as well as Athletics Canada, Australia and United States runners/race walkers at altitude in Flagstaff, Arizona (FLAG; n=27 females and 21 males). Inclusion criteria were: 18–40 years, events from 800 m-50 km walk, International Association of Athletics Federations (IAAF) score within the last 2 years of ≥880 (FIN; corresponding to 14:37.17 and 17:36.39 min for 5000 m for males and females, respectively) or ≥1050 (FLAG; 13:45.20 min and 16:00.04 min for 5000 m, respectively). Participants were informed about the study design before signing an informed consent. The studies were approved by the ethics committees of Helsinki University Hospital and University of Helsinki (BMD) and University of Jyvaskyla (other measurements). Study drop-outs included 11 participants (FIN: n=1, poor compliance (insufficient detail); FLAG: n=5, poor compliance, n=5 injuries during the recording week).

Study design

In a cross-sectional study design, we examined EA via 7-day EI (via dietary recording) and EEE (via training recording), metabolic and reproductive hormonal function, BMD (via Dual-energy X-ray Absorptiometry (DXA)), injury and illness rates and body composition of identified endurance athletes during sea-level pre-competition training in FIN during Spring 2015 (5% Olympians) and during a pre-competition altitude training camp in FLAG during Spring 2016 (27% Olympians).

Specifically, we divided female and male athletes into groups of apparently sub-optimal and normal EA status based on two different criteria: 1) an acute objective measure of reproductive hormone function/menstrual status coupled with assessments using Triad and RED-S tools or 2) a single time-span calculation of EA from food (energy intake; EI) and training (exercise energy expenditure; EEE) diaries over 7-days.

Blood samples

Fasted blood samples were obtained from an antecubital vein for analysis of insulin, testosterone (TES, males), estradiol (E2, females), triiodothyronine (T3), and insulin-like growth-factor-1 (IGF-1). FIN blood samples were drawn into a 5ml Vacuette gel serum tube (Greiner-Bio-One GmbH, Kremsmünster, Austria) and centrifuged at 3600 rpm for 10 min to collect serum; this was frozen at -20°C, before the measurement of IGF-1, E2, TES and insulin by chemiluminometric immunological analysis and T3 by electrochemiluminescence (Immulite 2000 XPi analysator, Siemens Healthcare, United Kingdom). The assay sensitivities were 0.5 $nmol \cdot L^{-1}$ (TES), 0.26 $nmol \cdot L^{-1}$ (IGF-1), 2 $mIU \cdot L^{-1}$ (insulin), 55 $pmol \cdot L^{-1}$ (E2) and 0.6 $pmol \cdot L^{-1}$ (T3). Reliability (CV) for between-day measurements was 9.1% (TES), 5.4% (IGF-1), 5.9% (insulin), 6.7% (E2), and 7.2% (T3). Eumenorrheic (EUM) females were measured

on days 3-7 during the follicular phase during a hard training period. Males and amenorrheic (AME) females were measured on an arbitrary day during a typical training week.

FLAG blood samples were collected into 8.5 mL SST gel tubes (BD Vacutainer, Franklin Lakes, NJ, USA) and centrifuged at 3400 rpm. Ferritin, insulin, T3, TES and E2 were measured via electrochemiluminescence immunoassay method and IGF-1 was measured via immunochemiluminometric assay. The samples were collected at ~day 3 of altitude exposure. The menstrual phase was not controlled, but was taken into account during data analysis.

Body composition and BMD

Body composition and BMD (whole body, femoral neck, lumbar spine) were measured in an overnight fasted state via DXA (FIN: GE Lunar Prodigy Advance; FLAG: GE Lunar DPX-IQ), according to standardized protocols for body composition as recommended by Nana et al. (2015) and for BMD as recommended by The International Society for Clinical Densitometry (ISCD, 2017).

Food and training logs

Food and training diaries were recorded during the second week at FLAG and immediately after laboratory tests in FIN over a 7-day period. The principal investigator analyzed all dietary records (FIN: Nutri-Flow® software, Flow-Team, Oulu, Finland; FLAG: ESHA Food Processor software, Oregon, US, 2016). Data collection protocols and methods of estimating EI (a combination of weighed and household measure dietary records) have been described in detail in (Heikura et al. in press) while the process of calculating EA following a field-based approach has been explained in Table 5.

Questionnaires

Female participants completed the Low Energy Availability in Females Questionnaire (LEAF-Q, Melin et al., 2014) which was also used to assess self-reported amenorrhea. To

examine the differences between subgroups, cumulative risk scores for Triad (Cumulative Risk Assessment Tool, Joy et al., 2014) and RED-S (Mountjoy et al., 2014) were calculated. The Triad tool was applied for males by replacing amenorrhea with low TES: values within the lowest quartile of clinical range (total testosterone: range of 9-38 nmol/L (Finland)); males with low TES scored 1 point, while males with normal TES scored 0 points. The use of the lowest quartile for TES values was chosen due to clear evidence that although low EA is associated with significant reductions in testosterone values have remained within the normal non-athlete clinical range (Tenforde et al., 2016); therefore, a subclinical threshold of low TES may be more appropriate in identifying LEA in elite endurance athletes. Athletes were grouped according to the Triad scores as low (0-1 points), moderate (2-5 points) or high (≥6 points) Triad risk score based on Joy et al. (2014). RED-S scoring system was applied to include the following conditions: low EA (<30 kcal·kg FFM·day^{-1}), amenorrhea (females) or low TES (males), BMD z-score <-1.0, and a history of one or more stress fractures associated with low EA and/or hormonal dysfunction (see above). The presence of each condition was scored as 1 point, while absence of any condition was scored as 0. The scores of all conditions were added together to form a RED-S score, and athletes were grouped as having low (0-1 points), moderate (2 points) or high (≥3 points) RED-S risk score. All participants answered an informal questionnaire on injury/illness history during the preceding 12 months (questions assessed type of illness/injury and days of absence from running training due to each incident) and all-time history of bone-related injuries (number and type of bone-related injuries during the entire athletic career).

Statistical analysis

Pooled-data statistical were conducted using SPSS Statistics 22 (INM, Armonk, New York, USA). Data were assessed via Shapiro-Wilk goodness-of-fit test and non-parametric

tests were used with non-normally distributed data. Females and males were divided into subgroups based on EA (cut-off: 30 kcal·kg FFM·day^{-1}, Loucks et al., 2011) and reproductive function (amenorrhea: absence of ≥3 consecutive menses, Mountjoy et al., 2014 vs eumenorrhea for females, and low TES vs normal TES for males.

Differences and/or correlation analysis in body composition, dietary intakes, EA, BMD, injury/illness rates and blood measures between sexes, subgroups and cohorts were analyzed with either Student's t-test, one-way ANOVA or Pearson's correlation coefficient (normally distributed data) or Mann-Whitney U-test, Kruskal-Wallis test or Spearman's correlation (non-normally distributed data). BMD was quantified via: Z>-1, normal BMD; Z< -1, a trend for low BMD; Z<-2, clinically low BMD (Torstveit & Sundgot-Borgen, 2005). Analysis of covariance was applied to FLAG females for menstrual cycle phases, with E2 as a covariate, which was non-significant ($p>0.05$). Therefore, we were confident that t-tests were reflective of true differences.

Data are presented as means ± standard deviations (SD), with significance set to $p<0.05$. Differences between-sexes and within-sexes are expressed as Cohen's effect sizes (ES; Hopkins et al., 2009), with ES threshold values of <0.2 (trivial), 0.2-0.5 (small), 0.5-0.8 (moderate) and >0.8 (large).

RESULTS

Tables 1 and 2 summarize body composition, training/performance status and dietary intake from the pooled dataset of 59 athletes, comparing sub-groups of males and females based on EA and reproductive status, while these sub-groups are examined in Tables 3 and 4 in terms of metabolic and health parameters.

Reproductive/metabolic function, bone health and injury/illness rates

Overall, 37% of females were amenorrheic (FIN: 50%; FLAG: 30%). Forty percent of males (FIN: 55%; FLAG: 33%) were low TES, while none were clinically low (<9 nmol/L). FLAG females had higher spine, left and right femur Z-scores (p=0.024, *d*=0.084; p=0.022, *d*=0.85; p=0.041, *d*=0.75, respectively), higher IAAF scores (p=0.002, *d*=1.15) and higher T3 (p<0.001, *d*=2.59) and insulin (p=0.036, *d*=0.81) concentrations than FIN females. Number of days missed training due to bone injuries was 2-fold greater in FIN vs FLAG females (25±41 vs 12±23 days, p=0.33, *d*=0.39 respectively) and 5-fold greater in FIN vs FLAG males (10±20 vs 2±8 days, p=0.28, *d*=0.53 respectively). Similarly, FLAG males had higher values for T3 concentrations (p<0.001, *d*=2.33), EA (p=0.047, *d*=0.85), reported intakes of energy (p=0.032, *d*=0.93) and CHO (p<0.001, *d*=1.55) and IAAF scores (p<0.001, *d*=0.91) than FIN males. Conversely, FLAG males had lower whole-body BMD than FIN males (p<0.001, *d*=2.13). Compared with eumenorrheic females, amenorrheic females had lower E2, T3 and BMD and higher LEAF-Q scores, while low TES in males was associated with low T3 (Table 3). On the contrary, apart from TES values in males, there were no differences in hormone concentrations or BMD (Table 4) between low and moderate EA groups of either sex.

Figure 1 shows the prevalence of 0, 1, and ≥2 career stress fractures amenorrheic and eumenorrheic females (1.46±1.27 vs 1.00±1.38 SF's, respectively, p=0.26, d=0.41), and in low vs normal TES males (2.10±2.10 vs 0.50±0.86 SF's, respectively, p=0.042, d=1.01). Effect size calculations showed that amenorrheic and low TES athletes had 4.5 times more training absences due to bone injuries compared to others (Table 3).

Correlations

In females, whole-body BMD correlated negatively with LEAF-Q score (*r*=-.410, p=0.015). In males, correlations were found between all-time fracture history and TES (*r*=-

.485, p=0.016). In all athletes pooled, both Triad score (r=-.338, p=0.009) and RED-S criteria (r=-.396, p=0.002) correlated negatively with T3 and positively with all-time fracture history (r=.566, p<0.001 and r=.328, p=0.011, respectively) and negatively with spine Z score (r=-.263, p=0.044 and r=-.323, p=0.013, respectively).

Triad and RED-S risk assessment

Athletes were scored based on available Triad criteria. Triad symptoms were absent in 34% of females, while 34%, 26% and 6% presented with one, two and three Triad conditions, respectively; this was even higher in amenorrheic females. Amenorrheic females had a significantly higher cumulative risk score for the Triad compared to eumenorrheic females (5.2±1.9 vs 1.7±1.4 points; p<0.001, *d*=2.10). The same was true for low vs moderate EA females (4.4±2.3 vs 2.4±2.1 points; p=0.027, *d*=0.91). Of all females pooled, 31%, 51% and 17% were classified into low-, moderate- and high-risk categories, respectively. Low TES males had higher adapted Triad scores compared to normal TES males (2.0±1.1 vs 0.1±0.4 points; p=0.009, *d*=1.43). Similarly, males with low EA had higher scores than males with moderate EA (2.8±0.4 vs 0.6±0.8 points; p<0.001, *d*=3.00). Of males, 63% were classified into low and 37% into moderate risk categories. Females had higher scores than males (3.0±2.3 vs 1.2±1.2; p<0.001, *d*=1.03). Based on Triad risk categories, T3 levels in females (3.0±0.5 vs 3.5±0.7 vs 2.7±0.4 pmol·L^{-1}, low, moderate and high risk groups, respectively; p=0.021 between moderate and high) and in males (4.0±0.6 vs 3.4±0.7 pmol·L^{-1}, low and moderate risk groups, respectively; p=0.032) were significantly lower and the number of all-time fractures in females (0.3±0.5 vs 1.4±1.4 vs 2.2±1.3 fractures, low, moderate and high risk groups, respectively; p=0.013 between low and high) and in males (0.3±0.5 vs 2.7±1.9 fractures, low and moderate risk groups, respectively; p<0.001) significantly higher in higher score groups. In addition, although not statistically significant, the number of training days lost due to bone

injuries in the preceding year was markedly higher in both females (3±11 vs 18±34 vs 34±38 days, low, moderate and high risk groups, respectively; p=0.13) and males (2±8 vs 10±20 days, low and moderate risk groups, respectively; p=0.23) with higher risk scores.

In addition, RED-S scoring system was used to score athletes based on symptoms of low EA. Differences in RED-S scores were significant between amenorrheic and eumenorrheic females (2.4±0.8 vs 0.5±0.7; p<0.001, *d*=2.53) and between low vs moderate EA females (2.2±0.9 vs 0.7±1.0; p=0.001, *d*=1.58). Likewise, differences between low and moderate TES males (2.0±1.2 vs 0.6±0.8; p<0.001, *d*=2.62) and between low and moderate EA males (2.7±0.8 vs 0.3±0.5; p<0.001, *d*=3.45) were significant. The division resulted in 43%, 43% and 14% of females and 50%, 29% and 21% of males classified into low, moderate and high RED-S risk categories, respectively. Assessment of RED-S risk categories did not find significant differences in T3 levels or injury indices in females, whereas in males T3 concentrations (4.0±0.5 vs 4.0±0.8 vs 3.1±0.4 $pmol \cdot L^{-1}$, low, moderate and high risk groups, respectively; p=0.018 between low and high) were significantly lower and the number of all-time fractures (0.6±0.9 vs 0.7±1.3 vs 3.2±2.2 fractures, low, moderate and high risk groups, respectively; p=0.021 low vs high, p=0.039 moderate vs high) significantly higher in higher score groups. In addition, although not statistically significant, the number of training days lost due to bone injuries in the preceding year was markedly higher in both females (7±20 vs 15±27 vs 48±50 days, low, moderate and high risk groups, respectively; p=0.08) and males (3±9 vs 0 vs 18±25 days, low and moderate risk groups, respectively; p=0.09) with higher risk scores.

DISCUSSION

This is the first study to examine EA, metabolic and reproductive hormonal function, BMD, injury and illness rates and dietary characteristics in a large cohort (n=59) of national to world-class (19% were Olympians) female and male distance athletes during a high load pre-

competition training block. We divided athletes into subgroups based on reproductive function (menstrual status and TES levels) as well as a single time-point assessment of EA status (a cutoff point of 30 kcal·kg FFM·day^{-1}). Around 40% of females and males were amenorrheic or had low TES, respectively. These athletes had significantly lower sex hormone and T3 concentrations and 4.5 times greater incidence of bone injuries compared to eumenorrheic females and males with normal testosterone levels. We found that the Triad and RED-S tools were able to detect symptoms of impaired endocrine-metabolic function and bone health (all-time fractures) in both sexes with better accuracy than the assessment of EA based on food and training logs.

Seminal work by Loucks and colleagues, within the standardized conditions of the laboratory, showed that low EA is the primary reason for the suppression of the metabolic and reproductive axis in females. These sophisticated studies established that below EA levels of ~30 kcal·kg FFM·day^{-1} the likelihood of suppression of female reproductive and metabolic hormones increases (Loucks & Callister, 1993; Loucks & Heath, 1994). Emerging data suggest that males, too, incur reciprocal effects (Tenforde et al., 2016), albeit at a lower EA threshold (between 20-25 kcal·kg FFM·day-1: Fagerberg, 2017; Koehler et al., 2016). Such perturbations can have serious effects on bone (Mountjoy et al., 2014; Tenforde et al., 2015) and other aspects of physiological function and performance (Mountjoy et al., 2014).

Although the negative effects of low EA on hormonal function are robustly detected in controlled laboratory settings, our study clearly demonstrates that the real-world measurement of EA, via dietary and exercise recording, is challenging and lacking in sensitivity as diagnostic tool for the presence of low EA. Indeed, EA is assessed indirectly, based on self-reported records of food intake and estimates of energy expenditure. These techniques have inherent problems related to reliability and validity, and may significantly skew EA calculations to both

under/overestimate the true value during the short time period of assessment (Schoeller, 1995; Hills et al., 2014). For example, self-reporting resulted in an underestimation of EI by 5-21% (Stubbs et al., 2014), whereas the use of MET's overestimated REE by 20% (Byrne et al., 2005), and wearable devices underestimated EE by ~100-600kcal/day (Murakami et al., 2016), albeit in non-athlete populations. Furthermore, there is no reference time-frame over which a dietary/exercise assessment should be conducted, nor any standardized protocols by which any of the components should be measured. Although an EA assessment has appeal as apparently low-cost, low-technology and non-invasive test, in reality, it carries a large burden in terms of athlete compliance and practitioner time. Cross-sectional estimates of low EA in athletes vary from 51 to 63% (Koehler et al., 2013; Melin et al., 2014; Melin et al., 2015). In our study, low EA (based on a cut-off of 30 $kcal \cdot kg^{-1}$ $FFM \cdot day^{-1}$) was found in 25% of male and 31% of female athletes but was poorly correlated with factors known to be affected by low EA, including reproductive, metabolic and bone health (Table 4). Indeed, interestingly, the only difference between EA groups was a finding of lower TES levels in males with low EA. Despite the challenges associated with EA assessment, we are confident that the rigorous control applied to obtain high quality dietary/training data allowed us to estimate EA to a sufficient detail. Our findings are in line with previous studies (Koehler et al., 2013; Melin et al., 2014) who failed to find an association between dietary EA and physiological markers of low EA in females. However we also included males in our analysis, and indeed showed a significant difference between males who had low vs moderate EA. While interesting, given no differences were found in other markers of EA between male EA groups, whether this finding is of relevance remains to be seen. Furthermore, as in our study, the EA assessment may occur at a different time point (and, in the case of FLAG, a different environment), to the scenarios in which poor match between energy intake and exercise energy expenditure may have caused health issues. Finally, other dietary factors often found in concert with low EA may have an

independent or additive contribution to the outcomes (Reed et al., 2011). Indeed, in line with previous studies Melin et al. (2016) who found significantly higher fibre intakes in oligo-amenorrheic vs eumenorrheic females, we noted a higher fiber intake reported by our amenorrheic females than their eumenorrheic conterparts.

Instead, qualitative screening tools (LEAF-Q, RED-S and Triad risk assessment tools) and/or quantitative measurements of reproductive function (blood reproductive and metabolic hormones, BMD, resting metabolic rate), may provide a better representation of an individual's longer-term EA, rather than just a snap-shot, and may be a more sensitive way to diagnose low EA. Indeed, based on the current cross-sectional analysis, it appears that current reproductive function provides an equally good, but more objective marker of appropriate energy support for overall health: we found lower levels of T3, sex hormones and BMD (females) in amenorrheic females and low TES males compared to others. Furthermore, when we divided athletes into low, moderate and high risk groups based on available Triad Cumulative Risk Assessment Tool (Joy et al., 2014) and a modified version of the RED-S consensus paper (Mountjoy et al., 2014), we found significantly lower T3 concentrations and up to 9-fold higher number of all-time fractures in higher risk groups of both female and male athletes. For females, the Triad tool seemed to work better, while in males, both tools produced similar outcomes. In addition, although not significantly different, both tools showed up to 10-times higher frequency of lost training days due to bone injuries in the preceding year in the higher risk groups.

The prevalence of amenorrhea (37%) in the current study was in line with some (Barrack et al., 2014; Gibbs et al., 2014) but not all (Melin et al., 2014; Pollock et al., 2010) previous studies, whereas, low BMD (17% in females, 0% in males) was significantly less than previously reported in females (Melin et al., 2014; Pollock et al., 2010). Interestingly, our cohort of elite athletes were of higher caliber than previously reported in the literature (27%

Olympians in FLAG), thus various differences in cohort rates of amenorrhea or BMD may not be surprising. While the use of menstrual status is probably one of the best markers of long-term EA, the use of oral contraceptives may mask the equation. In case of the use of oral contraceptives, perhaps other signs of chronic low EA, including low BMD, increased incidence of bone related injuries, and low metabolic hormone concentrations, could be used to detect low EA in these athletes. Interestingly, low testosterone males had poorer health, despite being within the reference range. Indeed, the clinical cut-offs may not be applicable for elite athletes in assessing RED-S. The available literature lacks a more systematic analysis of the prevalence of these issues in male athletes.

Our findings of a significantly higher LEAF-Q score (12.8 vs 8.3 points) in amenorrheic vs eumenorrheic females support the use of the LEAF-Q to assess increased risk of the Triad (Melin et al., 2014). We detected significant differences in the Triad and RED-S scores between the reproductive subgroups by categorizing athletes based on the Triad (Joy et al., 2014) and RED-S (Mountjoy et al., 2014) tools. This supports the work of Tenforde and colleagues (2017) who classified 239 amenorrheic/oligomenorrheic collegiate females into low- (71%), moderate- (25%) and high-risk (4%) categories based on the Triad tool, and found that moderate and high-risk category athletes were twice and nearly four times more likely to sustain a bone injury than low-risk athletes. We noted a higher prevalence of EA-associated problems in the lower caliber Finns. Whether this reflects cultural/genetic differences, the additional resources afforded to higher caliber athletes that might reduce the risk of problems, or the natural selection process by which the healthier athletes progress to higher levels, can't be judged from our data but merits additional investigation. Future research should focus on finding a practical yet valid tool(set) for the assessment of low EA in female and male athletes to address this and other themes.

CONCLUSIONS

A relatively high proportion of national class-elite female and male distance athletes showed signs of chronic energy conservation, including amenorrhea (37% of females) and low testosterone (40% of males). Amenorrheic females were characterized by lower BMD, sex hormone and T3 concentrations, and 4.5-fold higher rates of injury, compared to eumenorrheic females. Low TES males showed lower T3 concentrations, lower EA and 4.5-fold higher rates of injury compared to males with normal TES. The assessment of risk scores based on Triad and RED-S criteria revealed lower T3 concentrations and higher number of all-time fractures in both female and male athletes with higher risk scores. Our findings suggest that assessing physiological symptoms of low EA (hormone concentrations, menstrual function, bone density) or using LEAF-Q, Triad and RED-S screening tools provides a better assessment of the overall health status of an individual than a snapshot of current EA.

ACKNOWLEDGEMENTS

The study was designed by IH, TS, DB, AM, AU and LB; data were collected and analyzed by IH, TS, AU, DB and LB; data interpretation and manuscript preparation were undertaken by IH, TS, AU, DB and LB. All authors approved the final version of the paper. This study was financially supported by grants from the Canadian Sport Institute, Australian Catholic University, Australian Institute of Sport, Finnish Sport Institute Foundation, and Finnish Sport Research Foundation. The authors wish to thank Risto Puurtinen (University of Jyvaskyla), Salli Tommola (University of Jyvaskyla), Hypo2 High Performance Sport Center, and Northern Arizona Orthopaedics for their role in the study, and Prof. Jennifer Peat for statistical support during data analysis. Finally, the authors wish to thank all the participants for volunteering for the study.

CONFLICTS OF INTEREST

The authors and funding agents do not have any conflicts of interests.

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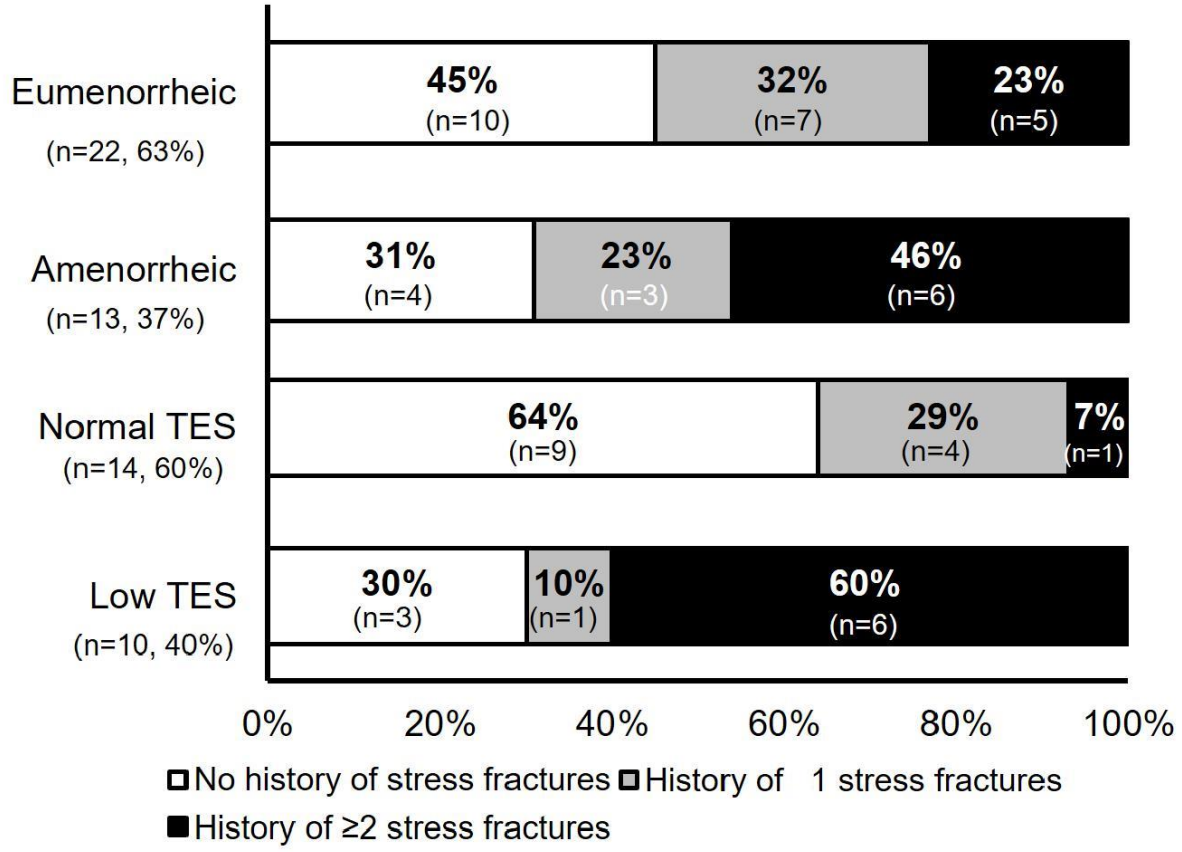

Figure 1. The prevalence of amenorrheic, eumenorrheic, low testosterone (TES) and normal TES athletes with 0, 1, or ≥2 career stress fractures (SF). White bars, no history of SF; grey bars, history of 1 SF; black bars, history of ≥2 SF. Values in each bar represent the percentage (%) and number (n) of athletes in each category.

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Table 1. Body composition and self-reported dietary and training data in female and male athletes categorized into (amenorrhea, AME; low testosterone, low TES) and normal (eumenorrhea, EUM; normal TES) reproductive function subgroups. Values are means ± standard deviations.

	Females			Males		
	AME (n=13)	EUM (n=22)	ES[a]	Low TES (n=10)	Normal TES (n=14)	ES[a]
Age (yr)	23.8 ± 4.4	26.7 ± 2.9 **	0.81	27.4 ± 3.4	26.9 ± 4.5	0.12
Height (m)	1.69 ± 0.04	1.68 ± 0.05	0.04	1.83 ± 0.06	1.81 ± 0.06	0.31
Weight (kg)	52.9 ± 5.8	54.7 ± 5.0	0.33	70.5 ± 6.7	67.8 ± 6.2	0.42
Body fat (%)	11.3 ± 4.1	12.1 ± 2.9	0.22	6.6 ± 1.1	7.2 ± 1.9	0.39
Energy ($kJ \cdot kg^{-1} \cdot d^{-1}$)	208 ± 40	201 ± 36	0.17	212 ± 39	212 ± 22	0.01
CHO ($g \cdot kg^{-1} \cdot d^{-1}$)	6.0 ± 1.3	6.0 ± 1.2	0.03	6.5 ± 2.0	6.6 ± 0.9	0.05
PRO ($g \cdot kg^{-1} \cdot d^{-1}$)	2.4 ± 0.6	2.0 ± 0.4 *	0.71	2.2 ± 0.3	2.1 ± 0.3	0.39
Fat ($g \cdot kg^{-1} \cdot d^{-1}$)	1.7 ± 0.5	1.8 ± 0.4	0.13	1.7 ± 0.5	1.8 ± 0.4	0.16
Fibre ($g \cdot d^{-1}$)	54.1 ± 35.4	37.7 ± 9.7 *	0.73	47.2 ± 14.5	48.3 ± 17.9	0.07
IAAF score	1077 ± 45	1102 ± 52	0.50	1075 ± 83	1071 ± 84	0.05
LEAF-Q score	12.8 ± 4.8	8.3 ± 3.7 **	1.08		N/A	
Running ($km \cdot wk^{-1}$)	101 ± 28	90 ± 30	0.40	117 ± 41	110 ± 24	0.22

* $p<0.05$, ** $p<0.01$ significant within-sex difference; [a] denotes magnitude of within-sex difference.

CHO, carbohydrate; PRO, protein; IAAF score, International Association of Athletics Federation scoring tables 2011; LEAF-Q score, Low Energy Availability in Females Questionnaire (Melin et al. 2014)

Table 2. Dietary and training data in female and male athletes categorized into low energy availability (EA) and moderate EA. Values are means ± standard deviations.

	Females			Males		
	Low EA (n=11)	ModEA (n=24)	ES[a]	Low EA (n=6)	ModEA (n=18)	ES[a]
Age (yr)	25.0 ± 4.5	25.9 ± 3.5	0.22	26.9 ± 3.8	27.2 ± 4.2	0.07
Height (m)	1.69 ± 0.06	1.68 ± 0.04	0.04	1.83 ± 0.08	1.81 ± 0.05	0.32
Weight (kg)	54.0 ± 6.8	54.1 ± 4.6	0.02	69.8 ± 6.9	68.6 ± 6.4	0.19
Body fat (%)	11.8 ± 4.6	11.8 ± 2.8	0.01	6.5 ± 0.9	7.1 ± 1.7	0.44
Energy ($kJ \cdot kg^{-1} \cdot d^{-1}$)	181 ± 27	214 ± 37 *	1.02	185 ± 35	222 ± 21 **	1.34
CHO ($g \cdot kg^{-1} \cdot d^{-1}$)	5.4 ± 1.1	6.3 ± 1.2	0.75	5.6 ± 1.9	6.9 ± 1.1 *	0.86
PRO ($g \cdot kg^{-1} \cdot d^{-1}$)	2.1 ± 0.6	2.2 ± 0.5	0.18	2.1 ± 0.2	2.1 ± 0.3	0.06
Fat ($g \cdot kg^{-1} \cdot d^{-1}$)	1.4 ± 0.3	1.9 ± 0.4 **	1.14	1.4 ± 0.5	1.9 ± 0.4 **	1.28
Fibre ($g \cdot d^{-1}$)	40.7 ± 11.1	45.2 ± 27.8	0.23	45.3 ± 12.4	48.7 ± 17.6	0.23
IAAF score	1113 ± 41	1083 ± 52	0.65	1057 ± 100	1077 ± 77	0.23
LEAF-Q score	11.1 ± 4.8	9.4 ± 4.6	0.35		N/A	
Running ($km \cdot wk^{-1}$)	115 ± 23	84 ± 28 **	1.19	130 ± 43	107 ± 25	0.68

* $p<0.05$, ** $p<0.01$ significant within-sex difference; [a] denotes magnitude of within-sex difference.

CHO, carbohydrate; PRO, protein; IAAF score, International Association of Athletics Federation scoring tables 2011; LEAF-Q score, Low Energy Availability in Females Questionnaire (Melin et al. 2014)

Table 3. Metabolic and reproductive hormone concentrations, bone density and injury and illness history in female and male athletes categorized into low (amenorrhea, AME; low testosterone, low TES) and normal (eumenorrhea, EUM; normal TES) reproductive function subgroups. Values are means ± standard deviations.

	Females			Males		
	Amenorrhea (n=13)	Eumenorrhea (n=22)	ES[a]	Low TES (n=10)	Normal TES (n=14)	ES[a]
Reproductive hormones						
E2 (pmol·l^{-1})	27 ± 19	111 ± 79 ***	1.71		N/A	
Total testosterone (nmol·l^{-1})		N/A		15.1 ± 3.0	25.0 ± 7.1 ***	1.86
Metabolic hormones						
IGF-1 (nmol·l^{-1})	225 ± 75	237 ± 67	0.17	197 ± 50	197 ± 52	0.00
T3 (pmol·l^{-1})	2.9 ± 0.7	3.4 ± 0.6 *	0.77	3.5 ± 0.7	4.0 ± 0.6 *	0.77
Insulin (pmol·l^{-1})	3.9 ± 4.1	4.2 ± 1.5	0.11	2.7 ± 1.7	3.6 ± 1.5	0.56
Bone characteristics						
BMD (g·cm^2)	1.164 ± 0.041	1.222 ± 0.076 *	0.98	1.297 ± 0.083	1.282 ± 0.073	0.19
Spine Z score	-0.3 ± 0.9	0.3 ± 0.8 *	0.73	0.3 ± 0.7	0.3 ± 0.9	0.04
Left femur Z score	1.0 ± 0.8	1.6 ± 1.1	0.68	1.2 ± 0.7	1.5 ± 1.0	0.39
Right femur Z score	1.0 ± 0.8	1.5 ± 1.1	0.59	1.2 ± 0.7	1.6 ± 1.0	0.52
Injuries						
Injuries and illnesses (d)	58 ± 48	32 ± 35	0.63	16 ± 17	14 ± 10	0.15

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	Females			Males		
	Amenorrhea (n=13)	Eumenorrhea (n=22)	ES[a]	Low TES (n=10)	Normal TES (n=14)	ES[a]
Bone injuries (d)	32 ± 41	7 ± 18	0.85	9 ± 19	2 ± 8	0.52
EA (kcal·kg FFM^{-1}·d^{-1})	32 ± 12	35 ± 9	0.29	31 ± 12	35 ± 5	0.47

* $p<0.05$, **$p<0.01$ significant within-sex difference; [a] denotes magnitude of within-sex difference.

E2, estradiol; IGF-1, insulin-like growth-factor-1; T3, triiodothyronine; BMD, bone mineral density; Z score, age-matched reference value for BMD; Injuries and illnesses, the number of training days lost to injury and illness over the preceding 12 months prior to study; Bone injuries, the number of training days lost to bone injuries during the preceding 12 months; EA, energy availability.

Table 4. Metabolic and reproductive hormone concentrations, bone density and injury and illness history in female and male athletes categorized into low energy availability (EA) and moderate EA. Values are means ± standard deviations.

	Females			Males		
	Low EA (n=11)	ModEA (n=24)	ES[a]	Low EA (n=6)	ModEA (n=18)	ES[a]
Reproductive hormones						
E2 (pmol·l^{-1})	70 ± 82	85 ± 73	0.19	N/A		
Total testosterone (nmol·l^{-1})	N/A			14.8 ± 3.6	22.9 ± 8.0 *	1.40
Metabolic hormones						
IGF-1 (nmol·l^{-1})	205 ± 51	245 ± 74	0.64	201 ± 55	196 ± 50	0.10
T3 (pmol·l^{-1})	3.1 ± 0.6	3.3 ± 0.7	0.31	3.4 ± 0.8	3.9 ± 0.6	0.71
Insulin (pmol·l^{-1})	3.1 ± 2.0	4.6 ± 2.9	0.61	2.2 ± 1.8	3.6 ± 1.4	0.88
Bone characteristics						
BMD (g·cm^2)	1.196 ± 0.082	1.202 ± 0.067	0.09	1.316 ± 0.088	1.279 ± 0.072	0.46
Spine Z score	0.1 ± 0.8	0.0 ± 0.9	0.13	0.1 ± 0.8	0.4 ± 0.8	0.42
Left femur Z score	1.5 ± 1.0	1.3 ± 1.0	0.17	1.5 ± 0.9	1.3 ± 0.9	0.22
Right femur Z score	1.4 ± 1.0	1.3 ± 1.0	0.17	1.6 ± 0.9	1.3 ± 0.9	0.38
Injuries						
Injuries and illnesses (d)	35 ± 46	45 ± 40	0.23	18 ± 22	14 ± 10	0.25

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	Females			Males		
	Low EA (n=11)	ModEA (n=24)	ES[a]	Low EA (n=6)	ModEA (n=18)	ES[a]
Bone injuries (d)	9 ± 20	19 ± 34	0.37	15 ± 24	2 ± 7	0.84
EA (kcal·kg FFM^{-1}·d^{-1})	24 ± 6	38 ± 8 ***	2.00	21 ± 6	37 ± 4 ***	3.20

* p<0.05, **p<0.01 significant within-sex difference; [a] denotes magnitude of within-sex difference.

E2, estradiol; IGF-1, insulin-like growth-factor-1; T3, triiodothyronine; BMD, bone mineral density; Z score, age-matched reference value for BMD; Injuries and illnesses, the number of training days lost to injury and illness over the preceding 12 months prior to study; Bone injuries, the number of training days lost to bone injuries during the preceding 12 months; EA, energy availability.

Table 5. Method for estimating energy availability (EA) and each of its components based on food and training records (field-based approach).

Energy availability (EA) equation	$\frac{\text{EI – EEE}}{\text{FFM}}$
Energy intake (EI)	• Estimate total daily EI (kcal/d) via food records • Usually 4 to 7-day food records provide the best result (in terms of compliance and reflection of real-life dietary habits) • Prefer weighed food records over more vague estimations of portion sizes • Educate the athlete to minimize errors in recording • Analyze food records with a dietary analysis software to get an estimate of daily EI
Exercise energy expenditure (EEE) and Resting energy expenditure (REE)	*REE* • Estimate REE by using the Cunningham equation (Cunningham, 1991) • Divide total daily REE by 24h to yield hourly REE *EEE* • Estimate EEE via training diaries, where information on exercise mode, duration, intensity (heart rate or ratings of perceived exertion) is included • Assign each training session or its parts a metabolic equivalent (METs; Ainsworth et al., 2000) value that best corresponds to the type and intensity of that activity • Multiply the MET for each training session/part of the by the duration of the session or part of that session to yield MET hours • Multiply MET hours by the hourly REE to yield total EEE (tEEE) • Subtract REE that would have occurred regardless of exercise from tEEE so that only the additional energy cost of exercise is included in the EEE • Use this EEE value as part of the equation provided above
Fat-free mass (FFM)	FFM can be obtained via Dual-energy X-ray Absorptiometry or air displacement plethysmography (BodPod) provided 'best practice' conditions for body composition analysis are utilised. Alternatively well standardised skinfold meausures or bioelectrical impedence analysis may be used.
Interpretation of EA values	The following cutoffs have been established (Loucks et al., 2011): *Low EA*: EA <30 kcal·kg FFM·day^{-1} *Moderate EA*: EA 30-45 kcal·kg FFM·day^{-1} *Optimal EA*: EA >45 kcal·kg FFM·day^{-1}