Prevalence of surrogate markers of relative energy deficiency in male Norwegian Olympic-level athletes

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ABSTRACT

The syndrome of Relative Energy Deficiency in Sport (RED-S) includes wide-ranging effects on physiological and psychological functioning, performance, and general health. However, RED-S is understudied among male athletes at the highest performance levels. This cross-sectional study aimed to investigate surrogate RED-S markers prevalence in Norwegian male Olympic-level athletes. Athletes (N=44) aged 24.7±3.8 years, body mass 81.3±15.9kg, body fat 13.7±5.8%, and training volume 76.1±22.9 hours/month, were included. Assessed parameters included resting metabolic rate (RMR), body composition, and bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) and venous blood variables (testosterone, free triiodothyronine (fT_3), cortisol and lipids). Seven athletes (16%) grouped by the presence of low RMR (RMR_{ratio} <0.90) (0.81±0.07 vs. 1.04±0.09, p<0.001, effect size 2.6), also showed lower testosterone (12.9±5.3 vs. 19.0±5.3 $nmol \cdot l^{-1}$, p=0.020) than in normal RMR group. In low RMR_{ratio} individuals, prevalence of other RED-S markers (—subclinical— low testosterone, low fT_3, high cortisol and elevated LDL) was (N/number of markers): 2/0, 2/1, 2/2, 1/3. Low BMD (z-score<-1) was found in 16% of the athletes, all with normal RMR. Subclinical low testosterone and fT_3 levels were found in nine (25%) and two (5%) athletes, respectively. Subclinical high cortisol was found in 23% of athletes while 34% had elevated LDL cholesterol levels. Seven of twelve athletes with 2 or more RED-S markers had normal RMR. In conclusion, this study found that multiple RED-S markers also exist in male Olympic-level athletes. This highlights the importance of regular screening of male elite athletes, to ensure early detection and treatment of RED-S.

Keywords: body composition, low energy availability, metabolic rate, hormonal response

Introduction

Relative Energy Deficiency in Sport (RED-S) describes a syndrome with impairment of numerous physiological systems, triggered by low energy availability (LEA) (Mountjoy et al., 2018; Mountjoy et al., 2014). LEA with or without disordered eating (DE) behaviors, impose serious health-risks potentially with clinical manifestations such as endocrine alterations, reproductive function, impaired bone health and cardiovascular risk factors (De Souza et al., 2014; Elliott-Sale et al., 2018; Gibbs et al., 2013; Logue et al., 2020a; McCall & Ackerman, 2019; Melin et al., 2019; Mountjoy et al., 2018; Mountjoy et al., 2014; Nattiv et al., 2007). Until recently, research related to LEA has been predominantly performed in female athletes (De Souza et al., 2014; Logue et al., 2020a; Mountjoy et al., 2018; Mountjoy et al., 2014; Nattiv et al., 2007), competing in sport disciplines where leanness and/or a low body weight is directly (power-to-weight ratio) or indirectly (appearance) related to performance or a specific body weight as a requirement to compete (weight category sports) (Gibbs et al., 2013; Martinsen et al., 2010; Mountjoy et al., 2018; Sundgot-Borgen, 1993; Sundgot-Borgen et al., 2013; Sundgot-Borgen & Torstveit, 2010).

In males, similar negative metabolic and endocrine alterations have been observed, as well as reductions in testosterone levels, which may be associated with reproductive dysfunction, impaired performance, injuries and poor bone health, (De Souza et al., 2019; Elliott-Sale et al., 2018; Friedl et al., 2000; Heikura et al., 2018b; Klomsten Andersen et al., 2018). Elite athletes often have high training loads and energy expenditure, demanding an increase in energy intake which, if not addressed by an accompanying increase in energy intake, may amplify the risk of LEA (Burke et al., 2018). Furthermore, elite athletes in leanness sports may be particularly vulnerable to LEA due to the desire to remain light and lean, with many acknowledging the use of increased training load to facilitate body composition adaptations (Gibbs et al., 2013; Martinsen et al., 2010; Sundgot-Borgen, 1993; Sundgot-Borgen et al., 2013; Sundgot-Borgen & Torstveit, 2010). The incidence of LEA in world-class male elite endurance athletes has been reported to be 25% (Heikura et al., 2018b). Due to potential adverse implications, the development of tools to identify male athletes at risk for RED-S is therefore warranted (Mountjoy et al.,

2018). Preliminary research suggests that males may withstand a lower threshold of LEA compared to females (Koehler et al., 2016; Papageorgiou et al., 2017), however, EA is difficult to assess, making it challenging to use as a practical and reliable measure (Areta et al., 2021; Burke et al., 2018; De Souza et al., 2019; Heikura et al., 2018b). Furthermore, no validated screening tools like the "Low Energy Availability in Females Questionnaire" currently exists for use with males. In total, RED-S in male athletes is understudied with only a few studies investigating RED-S amongst elite male endurance athletes (Heikura et al., 2018a; Heikura et al., 2018b; Logue et al., 2020b), including non-leanness athletes (Logue et al., 2020a; Logue et al., 2020b; Tenforde et al., 2016).

The aim of this study was to investigate RED-S in a Norwegian cohort of male Olympic-level athletes using surrogate markers such as suppressed resting metabolic rate (RMR), impaired bone health, and altered metabolic and endocrine variables.

Material and methods

Study design and recruitment

This study was designed as a cross-sectional study. Athletes were recruited through the Norwegian Olympic and Paralympic Committee and Confederation of Sports. The study was approved by the Norwegian Regional Committees for Medical and Health Research Ethics (2017/2160) and the Norwegian Centre for Research Data (56937/3/STM/LR) and conducted following the 2013 Declaration of Helsinki. Inclusion criteria were senior athlete ≥18 years of age, competing at a national team level in road-cycling, long- and middle-distance running, triathlon, race-walking, rowing, wrestling, biathlon, Nordic combined, cross-country skiing, boxing, powerlifting, soccer, or handball. The exclusion criteria were injuries preventing athletes from participating in their regular training regimen. The recruitment process involved announcements and distributions of invitations via email to both national coaches and athletes, intending to encourage participation. Interested athletes received written information about the study, and those interested in participation signed an informed consent. In total, 44 Olympic-level male athletes accepted participation, competing in the following sports: road-cycling, triathlon, race-walking,

light-weight rowing, wrestling, biathlon, Nordic combined, cross-country skiing, kickboxing, powerlifting competing in high weight-classes (≥93kg), and handball.

Assessment protocol

All tests were performed from January to March 2018, between 5-9 a.m. starting with RMR measurements, followed by body composition and BMD assessment before blood sampling.

Resting metabolic rate

Athletes either slept overnight at the laboratory in Oslo, Norway or arrived in a 12-hour fasted and rested state using motorized transport with minimal bodily movement. On arrival, subjects were placed in a quiet and dimly lit room maintained at a constant temperature (21°C). For a detailed description of measurement, see Table 1.

Body composition and bone health

Following RMR assessment, stretch stature, body weight, body composition and BMD were measured as described in Table 1.

Biochemical markers

A venous blood sample was drawn as described in Table 1, and serum was analysed at commercial clinical laboratory (Fürst, Oslo, Norway).

Insert Table 1 here

RED-S criteria

Following the procedure of Heikura et al. (2018b), athletes were given a score based on a positive- (1 point) or a negative (0 points) prevalence related to the following symptoms of RED-S; low body fat defined as <5% (Sundgot-Borgen et al., 2013), underweight defined as BMI <18.5 $kg \cdot m^2$ (Sundgot-Borgen et al., 2013), low BMD, defined as a Z-score <-1 in lumbar spine or femur neck (Nattiv et al., 2007), low RMR, defined as an RMR_{ratio}<0.90 using the Cunningham (1980) equation (Strock et al., 2020b), subclinical low testosterone, defined as within the lowest quartile of clinical range defined by

the laboratory (<14.8 nmol·l^{-1}) or fT_3 (<4.3 pmol·l^{-1}), subclinical high cortisol (defined as within the highest quartile of clinical range) (>537 nmol·l^{-1}) or elevated LDL levels (>3.0 mmol·l^{-1}).

Statistics

Data were analyzed using STATA for Windows (v. 16; Stata Corp LCC, College Station, TX, USA). The dataset was controlled for signs of non-normality using histograms, QQ-plot, and the Shapiro-Wilk test. Athletes (n=44) were included and divided into two groups based on energetic status (low vs normal RMR) (Strock et al., 2020b). Differences between energetic status were assessed using the welch test for unequal variances. Contingency data were analyzed using the Fisher exact test. Between group differences are expressed with Cohen's D effect size (ES) with the following threshold; trivial (<0.2), small (0.2-0.5), moderate (0.5-0.8), and large (>0.8). Relationships between RED-S variables were investigated using linear regression. Statistical significance level was defined as $p<0.05$, and data are presented as mean ± standard deviation.

Results

Descriptive data are presented in Table 2.

Insert Table 2 here

RED-S criteria

Of the 44 athletes, 32 (73%) had either 0 or 1 RED-S criteria present seven athletes (16%) presented with 2 criteria, four athletes (9%) with 3 criteria, and one athlete (2%) with 4 criteria (Figure 1). Detailed criteria points, including absolute values for all athletes with low RMR are presented in Table 3, including all athletes with low BMD independent of the presence of other markers, as well as two athletes with 3 simultaneously present criteria other than low RMR.

Insert Figure 1 and Table 3 here

RED-S surrogate markers

Table 4 summarizes the RED-S surrogate markers investigated according to energetic status, while Figure 2 summarizes RED-S markers. Overall, the low RMR group had lower testosterone compared to the normal RMR group (Table 4). No significant linear relationship was found between RMR_{ratio} and surrogate biochemical markers of RED-S ($p>0.05$).

Insert Table 4 here

None of the athletes had low body fat or were underweight. Of the 44 athletes included, seven (16%) had low RMR_{ratio}. Of these seven athletes, two athletes had no other RED-S marker present, two athletes had one marker present, two athletes had two markers present, while one athlete had three other RED-S markers present simultaneously. Seven athletes (16%) had low BMD in the lumbar spine, with four of them having no other RED-S marker present. A total of eleven athletes (25%) had subclinical low testosterone levels, including one athlete with clinically low levels (<8 $nmol \cdot l^{-1}$). Two athletes (5%) had subclinical low fT_3. Ten athletes (23%) had subclinical high cortisol, and 15 athletes (34%) had elevated LDL levels.

Leanness vs. non-leanness athletes

Thirty-four of the athletes participated in leanness sports, while the remaining 10 were involved in non-leanness sports. No significant differences in prevalence were observed between leanness and non-leanness athletes (Figure 2).

Insert Figure 2 here

Discussion

This is one of few studies investigating surrogate markers of RED-S in a larger group of Olympic-level male athletes, including both leanness- and non-leanness sports athletes (Drew et al., 2018; Drew et al., 2017). The primary findings of this investigation were that most athletes displayed none or few single markers related to RED-S independent of current low RMR. However, seven athletes (16%) were identified with low RMR with the majority of these displaying additional RED-S markers. LEA can be present with or without DE behaviors and is more prevalent among female athletes, especially in sports

where leanness is associated with performance (Gibbs et al., 2013; Mountjoy et al., 2014; Sundgot-Borgen, 1993; Sundgot-Borgen et al., 2013; Sundgot-Borgen & Torstveit, 2010; Tenforde et al., 2016). Hence, screening and identifying athletes at risk of DE behaviors is therefore important, however, is time consuming and requires the expertise of a multi-disciplinary team (Wells et al., 2020).

Prevalence of clustered RED-S markers

The prevalence of RED-S in athletes has been reported to range between 22-58% (Logue et al., 2020a). However, few of these studies have investigated male athletes at elite- or Olympic levels. In a study by Heikura et al. (2018b), 25% of their world-class male middle- and long-distance runners and racewalkers were identified with LEA, with significant lower testosterone levels in the LEA-group. Assessing RED-S related markers, 40% of their population also had lower testosterone and T_3 levels, including a 4.5 times greater incidence of bone injury in these athletes, despite BMD being unimpaired, but did not assess RMR (Heikura et al., 2018b). Woods et al. (2017) reported reduction of ~2 $kcal \cdot kgFFM^{-1} \cdot day^{-1}$ in RMR and body weight in elite rowers undertaking a 4-week intensified training period, without an apparent increase in EI. We recently reported a case study on a male combat athlete cutting weight for competition during 7 weeks of EA ~20 $kcal \cdot kgFFM^{-1} \cdot day^{-1}$ and 1 week ~3 $kcal \cdot kgFFM^{-1} \cdot day^{-1}$, showing a clear concomitant decrease of RMR_{ratio} under 0.9 (Cunningham, 1980) and RED-S markers falling outside clinical reference ranges (Langan-Evans et al., 2020). Similarly, in the current study, we identified seven athletes (16%) with low RMR, five of whom had multiple other RED-S markers, such as subclinical low testosterone and fT_3, subclinical high cortisol and elevated LDL. Interestingly, we also identified two athletes without low RMR, yet with 3 other RED-S markers present, such as subclinical low testosterone, low BMD, subclinical high cortisol, and elevated LDL, warranting further scrutiny (Table 4). However, our findings provide preliminary data suggesting that RMR_{ratio} may be a practical tool to identify athletes at risk of RED-S, representing a novel approach attempting to overcome the difficulties of assessing EA (Areta et al., 2021; Burke et al., 2018; De Souza et al., 2019).

Metabolic alterations

FFM is one of the most significant determinants of RMR, and reductions in RMR have been reported in male athletes with LEA(Torstveit et al., 2018; Woods et al., 2017; Woods et al., 2018). When energy availability is insufficient for basal physiological processes, the body prioritizes processes essential for survival, reducing RMR to conserve energy, including suppression of reproduction, growth, metabolism, and bone formation (De Souza et al., 2019; Mountjoy et al., 2014; Nattiv et al., 2007). An RMR_{ratio} of <0.90 has been recognized as a surrogate marker of LEA in exercising females (McCall & Ackerman, 2019; Strock et al., 2020a; Strock et al., 2020b). Furthermore, research by Strock and coworkers has identified that RMR_{ratio} accurately reflects total T_3 status in females, making it a useful marker of prolonged energy deficiency (Strock et al., 2020a; Strock et al., 2020b). In our study, seven athletes had low RMR, with five of them also having subclinically low testosterone. Interestingly, five out of seven of these athletes had very low RMR, ranging from 0.68-0.83 (Table 3), with similar deficits to that observed in females with anorexia nervosa (Marra et al., 2002). Most athletes with low RMR also presented subclinically low testosterone levels, strengthening a link to LEA, similar to the findings of Heikura et al. (2018b). However, rather than using RMR_{ratio} as a sole diagnostic tool, a combination with other markers such as hypotension, underweight and subclinically low testosterone levels in males are recommended (Staal et al., 2018). Furthermore, the 0.90 cut-off point was initially established in exercising females (De Souza et al., 2008), making it to some extent challenging to apply to athletes, who generally have a higher fat-free mass compared to non-athletes. Finally, though we acknowledge that selecting the Cunningham (1980) equation among different predictive formulas for the RMR_{ratio} cut-off of 0.90 may appear arbitrary, a strong rationale for the use of this predictive equation exists: 1) The few studies in males in this area also utilized the Cunningham (1980) equation, making our results comparable to others in the current literature (Langan-Evans et al., 2020; Torstveit et al., 2018; Torstveit et al., 2019; Wilson et al., 2018), and 2) we observed a very large effect size (Cohen's *d* 2.6 (95% CI 1.6-3.6), Table 4) in the low RMR_{ratio} group compared to the normal RMR which would yield similar results using other formulas giving slightly different RMR_{ratio} values. We are aware of the importance of comparison of different equations and the need for further exploration of cut-off values in males to define presence of adaptive thermogenesis. However, such exploration is beyond the scope of the current work and we hope that the findings of this study provide evidence to substantiate further research to

establish whether the proposed cut-offs are transferrable to males (Strock et al., 2020a; Strock et al., 2020b) as well as how RED-S markers are related to low RMR in males.

T_3 is essential for growth, metabolism, and reproduction with ties to LEA (Elliott-Sale et al., 2018). As a result of reduced energy intake, the hypothalamic-pituitary-thyroid axis adapts and alters levels of both T_3 and thyroxine to conserve energy for vital functions (Logue et al., 2020a; McCall & Ackerman, 2019). T_3 might also be a more useful marker of LEA than other thyroid function tests in males (McCall & Ackerman, 2019). Furthermore, low T_3 levels have frequently been linked to low testosterone levels (De Souza et al., 2019; Friedl et al., 2000; Heikura et al., 2018b; McCall & Ackerman, 2019). In our study, two athletes displayed subclinical low fT_3. Only one of these athletes belonged to the energy deficit group, and also displayed very low RMR_{ratio} (0.77) and clinical low testosterone levels (4.3 $nmol.l^{-1}$). In a study on male special forces soldiers experiencing prolonged starvation, researchers observed substantial reductions of both T_3 and testosterone during the eight-week course (Friedl et al., 2000). In the Heikura et al. (2018b) study, athletes with low testosterone had lower T_3 levels compared to athletes with normal testosterone levels, while no difference were observed between the groups of LEA and moderate EA. Similar T_3 findings are observed in studies were recreational trained males are exposed to short periods of LEA (~15 $kcal \cdot kg^{-1}FFM \cdot day^{-1}$) compared to optimal EA (40-45 $kcal \cdot kg^{-1}FFM \cdot day^{-1}$), possibly due to males being less sensitive to short periods of LEA compared to females (Koehler et al., 2016; Papageorgiou et al., 2017). In our study, athletes with severe low RMR did not show signs of subclinical low fT_3, warranting more research to explore the relation between LEA, RMR and fT_3 in males.

Reproductive function

Indisputable evidence shows that shorter periods of LEA causes suppression of reproductive- and metabolic functions in females (Loucks & Thuma, 2003), however, this is not fully understood in male athletes, and evaluation is difficult and may require sperm analysis (De Souza et al., 2019; Elliott-Sale et al., 2018; Tenforde et al., 2016). It has been stated that testosterone in males plays a critical role in both sexual, bodily development, and cognitive aspects as well as physiological advantage in sports performance (Hackney, 2020). Research on male soldiers undergoing prolonged starvation has shown

dramatic reductions in testosterone levels (Friedl et al., 2000). In male long-distance runners, race walkers and cyclists, LEA has been reported to strongly correlate with reduced testosterone levels (Heikura et al., 2018b; Keay et al., 2018; Melin et al., 2019). Experimental data has shown a causal effect between LEA and reduced testosterone only in one (Kojima et al., 2020) out of two studies (Koehler et al., 2016). In our study, a total of 11 athletes (25%) had sub-clinically low testosterone. However, our subclinical low testosterone levels findings may arise from hypogonadotropic hypogonadism (Arce et al., 1993; De Souza et al., 1994; De Souza et al., 2019; Tenforde et al., 2016) or the Exercise Hypogonadal Male Condition (EHMC), a maladaptation within the reproductive system due to athletes persistent and chronic exposure to large volumes of exercise training (Hackney, 2020). The first findings of lower total and free testosterone levels in male athletes compared with sedentary controls were reported by Arce et al. (1993) and De Souza et al. (1994). The subclinical testosterone levels were associated with low normal sperm count, decreased motility and morphological changes that may compromise fertility (Arce et al., 1993). These findings were confirmed in a large, randomised training study (n=286) where subjects were assigned to five 120-minute sessions/week of moderate-intensity exercise (60% of VO_{2max}) or high-intensity exercise (80% VO_{2max}) (Safarinejad et al., 2009). The results demonstrated that strenuous long-term exercise with significant weight loss resulted in a significant decrease in plasma sex hormone concentrations and impaired reproductive capacity. Re-analyzing previous data, Hackney and Lane (2018) found a ~30-35% reduction in testosterone levels in endurance-trained distance runners with ≥5 years- compared to those with <5 years of endurance training experience, although the reduction was unlikely to be caused by LEA, since no other health problems were reported (Hackney, 2020). In the Heikura et al. (2018b) study, low testosterone was found in 40% of participants. However, no differences in EA between the groups were reported, although athletes with LEA had significant lower testosterone levels compared to the moderate EA group. In the Koehler et al. (2016) study, no reductions in testosterone levels between groups were found. It is unclear whether the four-day period of LEA was long enough to observe changes in subclinical markers in the latter study (Koehler et al., 2016). Establishing baseline values for endocrine markers may be warranted, where sudden and unexpected drops in values should trigger further investigations into the cause to distinguish between the potential onset of RED-S or EHMC. However, broad scientific evidence supports the fact

that LEA causes reduction in testosterone, and that low testosterone levels are detrimental for performance (Hackney, 2020; Hackney et al., 2017). Thus, it is interesting to observe that subclinically low testosterone is present among almost all athletes with low RMR (Table 4), strengthening the association to LEA among these athletes (Arce et al., 1993; De Souza et al., 1994; De Souza et al., 2019; Hackney, 2020; Tenforde et al., 2016).

Impaired bone health

Several parameters influence bone health, mostly endocrine and nutritional aspects, as well as mechanical loading. Long term LEA is strongly linked to impaired bone health in female athletes (De Souza et al., 2014; Mountjoy et al., 2018; Mountjoy et al., 2014; Nattiv et al., 2007) and data on male athletes are now emerging (Barrack et al., 2017; Heikura et al., 2018b; Klomsten Andersen et al., 2018; Kraus et al., 2019; Papageorgiou et al., 2017; Tenforde et al., 2018; Viner et al., 2015). This includes increased risk of bone stress injuries among runners (Barrack et al., 2017; Kraus et al., 2019; Tenforde et al., 2018), as well as high prevalence of low BMD among cyclists (Klomsten Andersen et al., 2018; Viner et al., 2015). Viner et al. (2015) found a high prevalence of both LEA (70%) and low BMD (40%) across a professional cycling season, and Klomsten Andersen et al. (2018) found that 58% of elite cyclists had low BMD. In a randomized controlled trial, Papageorgiou et al. (2017) found decreased bone formation and increased bone resorption in females exposed to LEA, but not in males. Researchers speculate whether the 5-day LEA restriction among males was insufficient to see such changes, emphasizing the need for more research in this field (Papageorgiou et al., 2017). In a study by Heikura et al. (2018a), they reported no associations between the incidence of LEA and low BMD among world-class male endurance athletes. In our study, seven athletes (16%) were identified with low BMD, with or without other RED-S markers, highlighting the importance of screening athletes for low BMD independent of the presence of other signs of RED-S. BMD is, affected by an array of variables such as a chronic energy deficiency in the past, family history of osteoporosis, physical activity level, sedentary lifestyle, and dietary intake (Nattiv et al., 2007), variables which we did not assess. Finally, screening, and early detection of declining BMD are especially important in athletes at risk of LEA due to the detrimental effects and the lengthy process of regaining lost BMD (De Souza et al., 2014).

Cortisol

Cortisol, a steroid hormone related to stress, is likely to contribute to increased adiposity during energy abundance and is an essential catabolic hormone secreted to ensure glucose homeostasis during prolonged exercise and starvation (Elliott-Sale et al., 2018). Increases in cortisol during severe caloric restriction and fasting has been observed in humans, and hypercortisolemia might directly affect reproductive function or serve as a biomarker of stress and reproductive dysfunction in amenorrheic athletes (Elliott-Sale et al., 2018). The role of cortisol in relation to LEA in male athletes is not fully understood. Studying American soldiers, Friedl et al. (2000) suggested that augmented cortisol levels were associated with reduced body fat after four weeks of semistarvation during military training. In support of this, the soldier with the highest observed levels of cortisol began the course with minimal fat-reserves and lost most bodyweight (Friedl et al., 2000). In a recent study by Torstveit et al. (2018), a larger single-hour energy deficit was associated with higher cortisol values among well-trained male endurance athletes. Another study found that higher exercise dependency scores were associated with a more negative energy balance and higher cortisol levels among well-trained male cyclists and runners (Torstveit et al., 2019). In contrast, cortisol did not differ between a group of nine male long-distance runners with LEA compared to eight non-athletes with optimal EA (Hooper et al., 2017). We observed a 23% prevalence of athletes with subclinically high cortisol levels in our study. Furthermore, high cortisol levels (one clinical and two subclinical) were present among three athletes in combination with other RED-S markers (Table 3). However, cortisol as a marker itself of LEA should be interpreted with care, especially since stress and exercise *per se* is known to acutely increase cortisol levels (Hackney, 2020) with elite athletes exhibit large training volumes (Woods et al., 2017). More in-depth research is needed to better understand the effects of LEA on cortisol especially in the male population (Elliott-Sale et al., 2018).

Cardiovascular health

Cardiovascular risk factors in both male and female athletes related to LEA is understudied. In females, unfavorable lipid profiles in amenorrhoeic athletes, with elevated TC and LDL levels, have been reported (Melin et al., 2019; Rickenlund et al., 2005). The mechanism for an impaired lipid profile in

amenorrhoeic athletes is suggested to be related to estrogen deficiency, since increased levels of LDL have been associated with hypogonadotropic hypogonadism in anorexia nervosa patients (Meczekalski et al., 2013), and athletes with amenorrhea (Rickenlund et al., 2005). However, elevated TC and LDL levels has also been reported in female eumenorrheic athletes with current low or reduced EA and/or disordered eating behavior, suggesting that alterations in cholesterol synthesis might be triggered by energy deficiency, despite normal weight and normal estrogen levels (Melin et al., 2015). Therefore, more research is needed to establish whether cardiovascular outcomes in female athletes can occur independent of estrogen deficiency. Research on male athletes is even more limited. Friedl et al. (2000), observed a progressive increase in both total-, LDL- and high-density lipoprotein (HDL) during the 8-week military course, potentially related to changes in thyroid hormones and insulin-like growth factor 1 (IGF-1). Male judo players (n=11) undergoing a self-selected 7-day energy restriction prior to competition showed no changes in TC, LDL, or HDL (Filaire et al., 2001). In our study, one-third of the total sample displayed elevated LDL levels. We were, however, not able to investigate the athletes' family history or diet to explore for potential dietary causes of elevated blood lipids. More research on risk factors for cardiovascular health among male athletes is needed to improve the understanding of the complexity and possible link to RED-S.

Leanness vs. non-leanness athletes

Most of the investigated RED-S signs were also present among the investigated non-leanness athletes. These signs included low RMR, low BMD, subclinical low testosterones, subclinical high cortisol, and elevated LDL (Figure 2). A priory, we expected that athletes belonging to leanness sports would be more prone to exhibit a higher prevalence of surrogate markers of RED-S. Therefore, we hypothesized that male leanness athletes would exhibit both higher incidence and more severe cases of RED-S. Unfortunately, the small sample size in this study makes the sample highly biased and should be taken into consideration when interpreting the results. Despite this, it is still interesting to observe that some non-leanness athletes displayed signs of energetic deficit in combination with other RED-S markers, warranting further investigations among this group of athletes.

In conclusion, symptoms of chronic energy conservation related to RED-S were found in this group of Norwegian male Olympic-level athletes. Seven athletes (16%) had low RMR among this group of athletes, with the majority clustering with several additional RED-S markers, emphasizing the needs to further scrutinize these athletes. Furthermore, several RED-S markers were identified independent of current low RMR, including low BMD, subclinical testosterone, subclinical low fT_3 and subclinical high cortisol, emphasizing the need to further investigate the use of clustering of such RED-S risk factors among other groups of athletes.

Strengths and limitations

Although LEA underpins RED-S, it is well recognized that EA is notoriously difficult to assess and evaluate on free living athletes (Areta et al., 2021; Burke et al., 2018; De Souza et al., 2019; Heikura et al., 2018b). As an alternative approach to identify athletes at risk of RED-S, we chose to accurately quantify variables, known to reflect adaptations to chronic energetic stress, such as RMR using a canopy hood, BMD and body composition using DXA, as well as blood sampling, as described in recent studies (Elliott-Sale et al., 2018; Heikura et al., 2018b; Koehler et al., 2016; Lee et al., 2020; Logue et al., 2020a; Staal et al., 2018; Woods et al., 2017; Woods et al., 2018). The limitations of this study are: 1) a cross-sectional design does not enable establishing any cause-effect relationships, 2) lack of data on EA and no assessment of whether athletes prior to testing had attempted to moderate body mass, thus actively facilitating a state of LEA, 3) being weight stable at the time of testing were not part of the inclusion criteria, 4) the prevalence of athletes with clustering of RED-S markers may be influenced by athletes current training phase at time of testing (Heikura et al., 2018b; Woods et al., 2017), which was not controlled for, 5) the two groups representing low- and normal RMR, as well as leanness and non-leanness differ in size, hence comparison should be interpreted with care, 6) not all athletes were tested during pre-season, due to practical reasons and 7) excluding injured athletes from participation may have induced a survivorship bias, and future research may include injured athletes in their analysis to get a better understanding of the RED-S syndrome. Finally, the use of upper and lower quartiles for normative ranges, compared to using clinical cut-offs, when interpreting hormones must be taken into consideration, as research applying this method is very limited (Heikura et al., 2018b).

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Author contribution

MKT, TBS, AKM, GS, JLA, JI GP, and IG designed the study. JLA, JI, GS, GP, and IG participated in the conception of the study. GS and JI performed data collection. TBS analyzed data. TBS, AKM, and MKT interpreted the results of the experiments and drafted the manuscript. All authors edited and approved the final version of the manuscript.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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612 **Tables:**

Table 1. Overview of methods used to assess and evaluate RED-S surrogate markers

Component	Summary of method	Comments/references
Resting metabolic rate (RMR)		
mRMR	Indirect calorimetry using an automated system with a ventilated canopy hood (Vyntus CPX, CareFusion, Hoechberg, Germany, SentrySuite v. 2.21.4). The system was calibrated before each test following manufacturer directions. Participants laid in supine position for 5 minutes, before canopy was positioned. All were instructed to remain still and not fall asleep. VO_2 and VCO_2 were assessed over a 25-min period, and the last 20 min of measurements used to assess RMR. Typical error of measurement (CV%) for this methodology in the laboratory was 4.5% (95% confidence limits 3.5-6.2%)	Compher et al. (2006) Areta et al. (2019) Weir (1990)
pRMR	500 + 22 × LBM (kg)	Cunningham **(**1980**)** based on Thompson and Manore (1996)
RMRratio	Calculated as mRMR/pRMR	De Souza et al. (2008)
Physique		
Body composition + BMD (femur neck and lumbar spine L1-L4)	Via Dual-energy X-ray absorptiometry (DXA), strictly adhering to protocol. Urinary specific gravity was measured using a digital refractometer (Atago UG-α cat.no. 3464, Atago U.S.A Inc., Bellevue, WA). Scans were performed in the total body mode on a narrow fan-beam DXA scanner (Lunar iDXA, EnCore v. 16.20 software, GE Healthcare, Madison, WI with the combined NHANES/Lunar reference database). The coefficient of variation for the laboratory was 0.0%, 1.0%, 0.3%, 0.3% for body mass, fat mass, lean mass, and bone mass, respectively. All scans were conducted by the same technician using the standard thickness mode as determined by the auto-scan feature in the software and analysed automatically by the DXA software.	Kerr et al. (2016) Nattiv et al. (2007) Mountjoy et al. (2014)
Height	Measured without shoes to the nearest 0.1 cm using a wall-mounted centimeter scale (Seca Optima, Seca, Birmingham, UK)	
Body weight	Measured in underwear to the nearest 0.01 kg with an electronic scale (Seca, model 861, Birmingham, UK)	
BMI	Total body weight (kg) / body height squared in meter ($kg \times m^2$)	
FFMI	FFM (kg) / body weight squared in meter ($kg \times m^2$)	
FMI	Fat mass (kg) / body weight squared in meter ($kg \times m^2$)	
Biochemical markers		
Blood sampling	A venous blood sample was drawn from an antecubital forearm vein by a qualified nurse. 5 mL Vacuette Z Serum Sep clot activators were filled and subsequently centrifuged at 3000RPM for 10 minutes (Statspin Express 4, Beckman Coulter, CA, USA) within a limit of ≥ 20 minutes but ≤ 40 minutes. 2mL Cryotube vials were filled with serum and cooled to 2 degrees Celsius before being transported for analysis	Blood was centrifuged at 1500×g for 12 minutes and serum was analysed for total- and free testosterone (analytic CV 7.6%), free T_3 (fT_3; 3.0%), cortisol (8.2%), low-density lipoprotein (LDL; 2.0%) and total cholesterol (TC; 1.9%)

BMD: bone mineral density, BMI: body mass index, FFM: fat-free mass, FFMI: fat-free mass index, FMI: fat mass index, LBM; lean body mass, mRMR: measured RMR, pRMR: predicted RMR, T_3: Triiodothyronine

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Table 2. Descriptive characteristics of athletes in total and categorized according to energetic status

Measurement	**Total** **n = 44**	**Low RMR** **n = 7**	**Normal RMR** **n = 37**	**P-value**	**Effect size** **(95% CI)**
Age (years)	24.8 ± 3.8	26.0 ± 3.3	24.6 ± 3.9	0.338	0.2 (-1.1-0.4)
Stature (cm)	181.3 ± 8.4	177.9 ± 8.8	181.8 ± 8.2	0.297	0.5 (-0.3-1.3)
Weight (kg)	81.3 ± 15.9	80.5 ± 19.1	81.5 ± 15.5	0.907	0.1 (-0.8-0.9)
BMI (kg·m^2)	24.7 ± 4.4	25.3 ± 4.4	24.6 ± 4.4	0.724	0.1 (-0.9-0.7)
FFM† (kg)	69.4 ± 11.2	71.2 ± 12.9	69.0 ± 10.9	0.682	0.2 (-1.0-0.6)
FFMI (kg·m^2)	21.6 ± 2.7	22.4 ± 2.9	21.4 ± 2.7	0.425	0.2 (-1.2-0.4)
FMI (kg·m^2)	3.6 ± 2.4	3.2 ± 1.8	3.7 ± 2.5	0.567	0.2 (-0.6-1.0)
Body fat† (%)	14.7 ± 6.9	12.2 ± 4.5	15.2 ± 7.2	0.167	0.4 (-0.6-1.0)
Training volume (hours/month)	76.1 ± 22.9	72.9 ± 18.6	76.7 ± 22.7	0.638	0.2 (-0.6-1.0)
L1-L4 Z-score†	0.59 ± 1.62	1.86 ± 1.92	0.35 ± 1.46	0.083	1.0 (0.2-1.8)
Femur Z-score†	0.96 ± 1.14	1.33 ± 0.82	0.89 ± 1.19	0.280	0.3 (-0.3-0.5)

Data are presented as mean ± standard deviation. P-value and ES indicates difference between low and normal RMR group. Effect size expressed as Cohens D with 95% confidence interval (CI), † measured by DXA. Abbreviations: BMI: body mass index, DXA: dual-energy X-ray absorptiometry, FFM: fat-free mass, FFMI: fat-free mass index, FMI: fat-mass index

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Table 3. A detailed description of athletes with one, two and ≥3 RED-S points

Athlete	**Sport**	**Low RMR** Ratio < 0.90	**Low BMD** Z-score < -1.0	**Subclinical low TES** < 14.8 nmol·l^{-1}	**Subclinical low fT3** < 4.3 pmol·l^{-1}	**Subclinical high COR** > 537 nmol·l^{-1}	**Elevated LDL** > 3.0 nmol·l^{-1}	**Fat%**
1 RED-S points								
1	Wrestling	**YES** (0.89)	NO (+4.3)	NO (21.0)	NO (5.3)	NO (479)	NO (2.7)	8.1
2	Kickboxing	**YES** (0.68)	NO (-0.4)	NO (17.0)	NO (5.2)	NO (483)	NO (2.6)	11.6
3	Triathlon	NO (1.06)	**YES** (-1.2)	NO (16.0)	NO (6.3)	NO (459)	NO (2.6)	9.7
4	Triathlon	NO (1.06)	**YES** (-1.1)	NO (22.0)	NO (6.7)	NO (283)	NO (2.7)	13.4
5	Rowing	NO (0.98)	**YES** (-1.1)	NO (30.0)	NO (5.9)	NO (390)	NO (1.6)	17.9
6	Rowing	NO (1.08)	**YES** (-1.9)	NO (18.0)	NO (6.0)	NO (406)	NO (2.2)	15.5
2 RED-S points								
7	Kickboxing	**YES** (0.77)	NO (+3.2)	**YES** (13.0)	NO (4.8)	NO (404)	NO (2.0)	9.7
8	Powerlift	**YES** (0.89)	NO (+0.3)	**YES** (9.0)	NO (6.5)	NO (386)	NO (2.6)	21.5
9	Cycling	NO (1.03)	**YES** (-1.2)	NO (20.0)	NO (5.8)	NO (478)	**YES** (3.9)	13.3
10	Cycling	NO (1.06)	**YES** (-1.8)	NO (27.0)	**YES** (3.6)	NO (518)	NO (1.9)	9.0
11	Rowing	NO (0.96)	NO (+0.8)	**YES** (13.0)	NO (5.3)	NO (451)	**YES** (3.1)	8.1
12	Nordic combined	NO (0.92)	NO (-0.8)	NO (25.0)	NO (5.1)	**YES** (544)	**YES** (3.1)	9.0
13	Powerlift	NO (1.04)	NO (+1.5)	**YES** (13.0)	NO (5.4)	NO (236)	**YES** (4.9)	26.1
≥3 RED-S points								
14	Cycling	**YES** (0.77)	NO (-0.1)	**YES** (4.3)	**YES** (3.9)	**YES** (573)	NO (2.8)	8.6
15	Kickboxing	**YES** (0.83)	NO (+2.4)	**YES** (13.0)	NO (4.9)	**YES** (711)	NO (2.8)	14.1
16	Powerlift	**YES** (0.83)	NO (+3.4)	**YES** (13.0)	NO (5.4)	NO (222)	**YES** (4.5)	26.1
17	Powerlift	NO (1.05)	**YES** (-1.9)	**YES** (12.0)	NO (5.7)	NO (417)	**YES** (3.1)	35.4
18	Rowing	NO (1.08)	NO (-0.5)	**YES** (8.0)	NO (5.4)	**YES** (633)	**YES** (3.1)	10.7

Abbreviations: BMD: bone mineral density, RMR: resting metabolic rate, TES: total testosterone, fT_3: free triiodothyronine, COR: cortisol, LDL: low-density lipoprotein, ( ) represent absolute values of measurements

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Table 4. Reproductive and metabolic hormones of athletes in total and categorized according to energetic status

Measurement	**Total** **n = 44**	**Low RMR** **n = 7**	**Normal RMR** **n = 37**	**P-value**	**Effect size** **(95% CI)**
RMR_{ratio}	1.00 ± 0.13	0.81 ± 0.07	1.04 ± 0.09	<0.001	2.6 (1.6-3.6)
Relative RMR ($kcal \cdot kgFFM^{-1} \cdot day^{-1}$)	29.4 ± 4.1	23.6 ± 1.8	30.4 ± 3.3	<0.001	2.2 (1.2-3.1)
Total testosterone ($nmol \cdot l^{-1}$)	18.1 ± 5.9	12.9 ± 5.3	19.0 ± 5.3	0.020	1.2 (0.3-2.0)
Free testosterone ($nmol \cdot l^{-1}$)	0.37 ± 0.11	0.28 ± 0.13	0.39 ± 0.10	0.061	1.1 (0.3-1.9)
Free T_3 ($pmol \cdot l^{-1}$)	5.6 ± 0.7	5.1 ± 0.8	5.7 ± 0.7	0.127	0.8 (0.0-1.6)
Cortisol ($nmol \cdot l^{-1}$)	451 ± 115	465 ± 154	449 ± 106	0.789	0.2 (-1.0-0.7)
TC ($mmol \cdot l^{-1}$)	4.7 ± 0.8	5.0 ± 0.8	4.6 ± 0.8	0.270	0.5 (-0.3-1.3)
LDL ($nmol \cdot l^{-1}$)	2.7 ± 0.8	2.9 ± 0.8	2.6 ± 0.8	0.606	0.2 (-1.0-0.6)

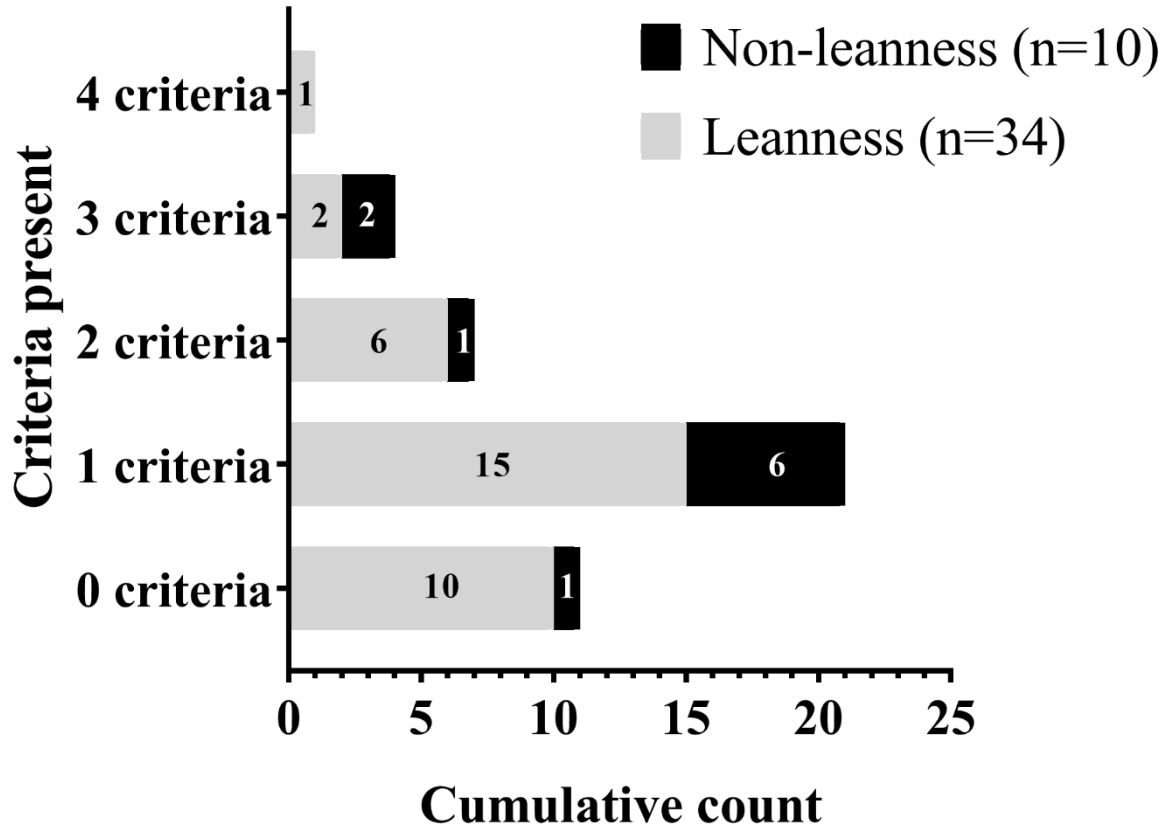
Data are presented as mean ± standard deviation. P-value and ES indicates difference between low and normal RMR group. Effect size expressed as Cohens D with 95% confidence interval (CI). Abbreviations: FFM: fat-free mass, T_3: Triiodothyronine RMR: Resting metabolic rate, TC: total cholesterol, LDL: low-density lipoprotein.

Figure legends:

Figure 1. Between-group and cumulative count (x-axis) of the numbers of RED-S criteria present among the athletes divided into leanness and non-leanness groups. Abbreviations: RED-S: relative energy deficiency in sport

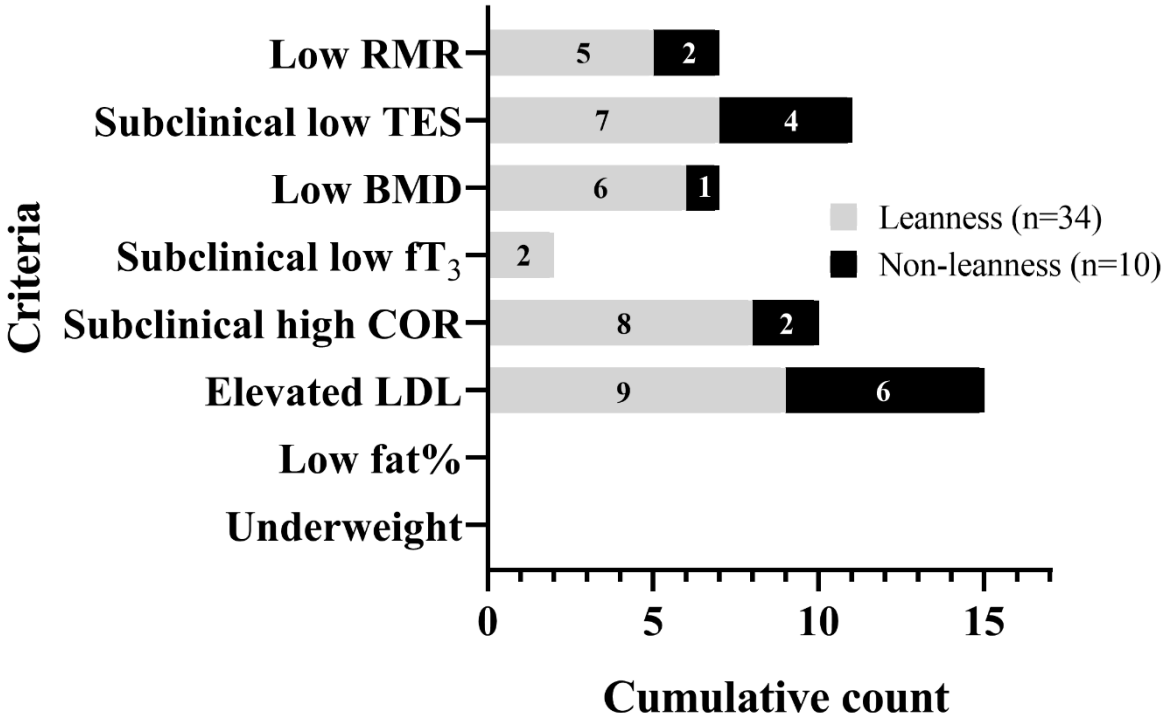
Figure 2. Individual RED-S criteria cumulative represented (x-axis) and numbers present within each athlete group (numbers displayed in each bar). Abbreviations: BMD: bone mineral density, COR: cortisol, fat%: fat percentage, LDL: low-density lipoprotein, RED-S: relative energy deficiency in sport, RMR: Resting metabolic rate, fT_3: Free triiodothyronine, TES: testosterone.

631 **Figures**:



632

633


Criteria
Low RMR
Subclinical low TES
Low BMD
Subclinical low fT3
Subclinical high COR
Elevated LDL
Low fat%
Underweight
5
2
7
4
6
1
2
8
2
9
6
Leanness (n=34)
Non-leanness (n=10)
0
5
10
15
Cumulative count

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