Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Neuromedicine and Movement Science

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# The influence of a 2-week training-overload period on performance and indicators of Relative Energy Deficiency in sport in endurance athletes: a pilot study

Master's thesis in Master of science in Physical Activity and Health
Supervisor: Rune Kjøsen Talsnes
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Thank you!

Jeanette Stene Stensheim

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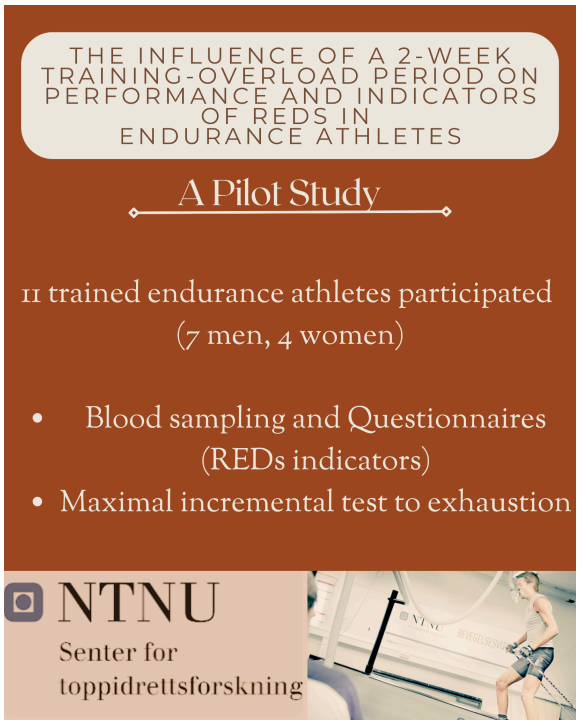
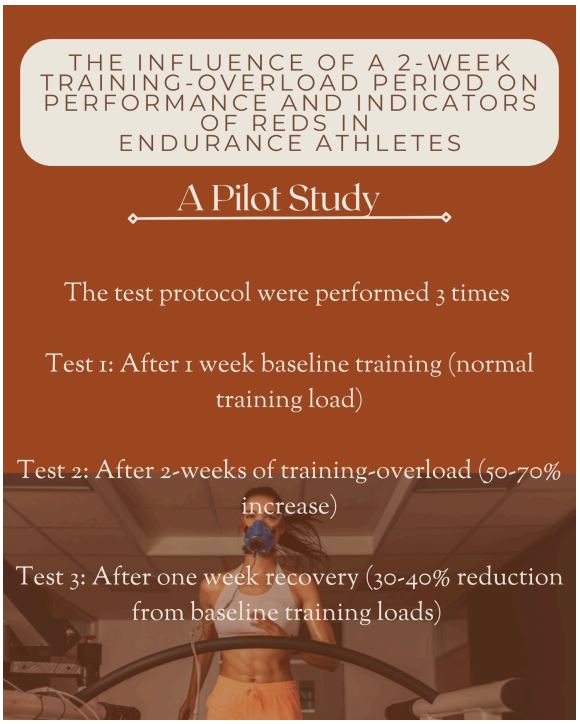
*Infographic.*


**Abstract**

**Introduction:** Competitive endurance athletes regularly go through planned phases of increased training load (training-overload) and tapering (reduction in training load) as part of their training regimes to enhance performance. Nonetheless, increases in training load are not always met by a compensatory increase in energy intake, leading to low energy availability (LEA) and possibly a diagnosis of relative energy deficiency in sport (REDs). Most previous studies in the field have focused on measures of LEA and related biomarkers alone, and only few studies have investigated training-overload and performance together with different contemporary REDs indicators based on the newest IOC consensus statement. Therefore, the overall aim of this study was to investigate the influence of a 2-week training-overload period on performance and REDs indicators in endurance athletes. **Methods:** A total of 11 trained endurance athletes (men=7, women=4, $VO_{2max}$ = 65.5±4.5 and 53.5±3.5 ml/kg/min, respectively) participated in this study. The participants completed a 1-week baseline-training period, a 2-week training-overload period (50-70% progressive increase in training load), and a 1-week recovery period (30-40% reduction in training load from baseline). Immediately after each training period, physiological responses during a maximal incremental time to exhaustion (TTE) test while treadmill-running together with various REDs indicators including the eating disorder questionnaire (EDE-Q), libido questionnaire (LEAMQ), and blood biomarkers (testosterone, cortisol, total and LDL-cholesterol, triiodothyronine, growth hormone, prolactin) were determined. Training data as well as readiness measures were monitored daily. A repeated measures ANOVA was conducted to determine the main effect of time (different weeks of training and between T1, T2, and T3 for main variables). **Results:** TTE did not decline from baseline to after the training overload and increased from baseline to the recovery period (from 339±59 sec to 374±47 sec, $p < .01$, respectively). Physical and mental readiness measures were reduced after the training-overload period (from 6.9 and 7.1 to 5.8 and 5.7 points, $p < .05$, respectively) but returned to baseline levels after the recovery period. No significant changes in any REDs indicators were found between the baseline, training-overload, and recovery period. **Conclusion:** A 2-week training-overload period in endurance athletes has no negative influence on either performance or REDs indicators. This pilot study can be used as a starting point to further investigate the influence of training-overload on performance and REDs indicators in endurance athletes by including a larger sample and subsequently investigating possible sex-differences in responses.

**Key words:** Endurance athletes, training-overload, REDs, biomarkers

## 1.0 Introduction

Endurance athletes aim to improve their performance through finding the optimal balance between training load and recovery, as progressing towards high overall training loads is needed to achieve further training adaptations (Roete et al., 2021). Competitive endurance athletes regularly go through planned phases of increased training load (training overload) as part of their training regimes. These phases provide a larger than usual training stimulus intended to induce the sport-specific adaptations and improved competitive performance (Meeusen et al., 2013). Overreaching is the transient reduction in performance that occurs following training overload due to an imbalance between training stress and recovery (Kuikman et al., 2022). The best competition performances in endurance sports are often achieved following a subsequent taper phase (reduction in training load), which is typically completed after periods of training-overload. Appropriate tapering is considered critical for maximizing performance (Aubry et al., 2014) and a "supercompensation effect" may occur resulting in enhanced performance levels compared to baseline (Kreher, 2016). Based on positive (adaptations) and negative (fatigue) effects of training load on the athlete, the taper aims to reduce negative impact and increase positive training-induced adaptations.

Nonetheless, the high training stress induced by training overload can overwhelm athletes' ability to recover, leading to excessive fatigue and a performance decrement (Roete et al., 2021). Although fatigue is considered a part of the overload stimulus, accumulated levels of fatigue and incomplete recovery can evolve into a continuum ranging from functional overreaching (FOR) to non-functional overreaching (NFOR), and the overtraining syndrome (OTS) (Buyse et al., 2019; Meeusen et al., 2013). FOR is characterized by reduced performance after a phase of training-overload followed by an increased performance after adequate recovery. NFOR/OTS is also characterized by reduced performance but without any "supercompensation effect" following recovery. What typically distinguishes NFOR and OTS is the time needed (weeks to months) to return to normal levels (Kreher & Schwartz, 2012). It is generally thought that states of overreaching might develop into maladaptive states as NFOR and OTS, therefore monitoring athletes' training loads is essential for determining whether they are adapting to their training program, minimizing the risk of injury and illness, and understanding individual responses (Meeusen et al., 2013; Roete et al., 2021). One of the challenges of research on the training-overtraining spectrum is to distinguish between acute fatigue and FOR. Firstly, intensified training which results in acute fatigue leads to increased performance. In FOR athletes, however, the increase in performance after intensified training and the subsequent taper period is smaller or even absent (Aubry et al., 2014). Maximal performance tests and subjective markers of fatigue and readiness are commonly used in training monitoring for evaluation of the athlete's endurance capacity, depicting training-adaptation and performance development (Bentley et al., 2007; Ten haaf et al., 2017). There is evidence that indicates that inducing FOR might not be necessary for improving performance in athletes and might do more harm than good (Bellinger P., 2020; Aubry et al., 2014).

Athletic performance and recovery from exercise are enhanced by optimal nutrition (Melin et al., 2024). Athletes competing in endurance sports are at a greater risk of low energy

availability (LEA) as it may be challenging to consume an adequate amount of energy to match the high energy demand from excessive training (Jagim et al., 2022). Relative Energy Deficiency in Sport (REDs) is a clinically diagnosed, multifactorial syndrome characterized by health and performance consequences resulting from exposure to problematic LEA in both male and female athletes (Updated in 2018 and 2023) (Mountjoy et al., 2018; Mountjoy et al., 2023). One of the body's physiological systems that is extremely sensitive to the stress of exercise training is the endocrine system and particularly the components associated with reproductive function. Biomarkers suggested to be linked to LEA and REDs include low leptin and triiodothyronine levels, high levels of cortisol, decreased testosterone and sex-drive (males) (Lundy et al., 2022), as well as altered diurnal patterns of several hormones (Logue et al., 2020; Mountjoy et al., 2023; Dipla et al., 2020). All primary, secondary and other potential emerging indicators of REDs in male and female athletes are presented in the newest clinical assessment tool-version 2 (CAT-2) to help diagnose and assess risk for developing REDs. The cause of LEA is complex and may include disordered eating behaviors, depressive symptoms, unhealthy body image, inadequate nutritional knowledge or suppressed appetite to compensate for high training loads (Melin et al., 2024; Wasserfurth et al., 2020). Eating disorders (ED) or disordered eating (DE) are serious mental diseases and behaviors that frequently appear in female athletes (Torstveit et al., 2007; Fahrenholtz et al., 2022). Eating Disorder Examination-Questionnaire (EDE-Q) is a well-established measurement tool used in both clinical and research purposes for assessing disordered eating. An elevated EDE-Q score is one of the primary REDs indicators (CAT 2).

There are symptom similarities between those often associated with training-overload (with or without OTS diagnosis) and REDs, with both initiated from a hypothalamic-pituitary origin (Stellingwerff et al., 2021). The predominant REDs diagnostic criteria centered around performance reduction and endocrinology (sex and metabolic hormones), gastrointestinal and cardiovascular dysfunction (Logue et al., 2020; Mountjoy et al., 2023). On the other hand, training-overload studies have centered primarily on associated hormone and blood markers which are suggested to be associated with reduced endurance performance and a decline in training adaptation. Stellingwerff et al., (2021) have presented evidence to suggest that LEA and carbohydrate availability may be a confounding factor in a significant number of training-overload studies, resulting in misdiagnoses of training-overload/OTS, instead of LEA leading to REDs . Underperformance due to LEA may not always be noticeable as it can be masked by the positive influence of lower body weight in endurance sports. If not recognized, LEA can lead to severe health issues that can affect the ability to train and compete (Mountjoy et al., 2023; Wasserfurth et al., 2020). Not all athletes who undergo training overload experience LEA or reduced carbohydrate availability concurrent with REDs symptoms (Armstrong et al., 2022). As LEA does not always lead to reduced performance, it is also possible that athletes may be affected by both overreaching and LEA independently (Kuikman et al., 2022).

Stenquist et al., (2020) demonstrated that 4 weeks of high-intensity training in trained males resulted in positive performance responses such as an increased aerobic peak power output and functional threshold power. However negative consequences related to health, potentially caused by LEA were also observed (Stenqvist et al., 2020). In contrast, a study investigated the effects of a 9-week intensified aerobic training period and 3 weeks of

recovery on signs of overload in 9 healthy active males (Bresciani et al., 2014). No significant changes were found in testosterone and cortisol or other biomarkers (hemoglobin, markers of oxidative stress). In a recent study, 10 days of LEA resulted in impaired performance in trained females, with subsequent reductions in muscle glycogen. Two days of recovery with optimal energy availability partially restored these impairments, although physical performance was still inferior to being in optimal energy availability (Oxfeldt et al., 2023). There is currently a gap in the literature on how training-overload affects performance and possible changes in REDs indicator in endurance athletes, and to our knowledge, there are no recent studies conducted with the updated REDs indicators. Most studies regarding LEA/REDs have focused on measures of LEA and related biomarkers alone, and few studies have investigated training-overload and performance together with a wider array of REDs indicators. Despite the relatively high prevalence of both overreaching and REDs among endurance athletes, detection at early stages is challenging, and no single, validated, and available method for monitoring and early detection exists (Heikura et al., 2022; Stellingwerff et al., 2021).

Therefore, the overall aim of this study was to investigate the influence of a 2-week training-overload on performance and REDs indicators in endurance athletes.

## 2.0 Methods

### *2.1 Participants*

A total of 11 endurance athletes (men=7, women=4) including runners (n=5), triathletes (n=4), and orienteers (n=3) at a recreational level (Tier 2-3 (McKay et al., 2022)) participated in this study. Inclusion criteria for participation included a minimum of 2 years' experience with the endurance sport, 5 or more endurance sessions per week, and aged between 18 and 30 (see Table 1 for characteristics of the participants). Participants were ineligible to participate if they had current injuries or illnesses that prevented participation in regular or alternative exercise training.

### *2.2 Ethical considerations*

The principles of the Helsinki Declaration were followed. The participants were provided with information including informed consent, protocols, risks and benefits, data handling, and how they could access their results, and were informed of their rights to at any given time withdraw from the study. The study protocol was approved by the Research Ethics Committee in Mid-Norway (Reference number: 656529). Signed informed consent was obtained before the start of the study.

*Table 1. Characteristics of the participants before the start of the study.*

| | Males | Females |
|---|---|---|
| Age (years) | 24.8±2.8 | 26.4±2.5 |
| Body mass (kg) | 72.9±5.4 | 69.6±7.4 |
| Height (cm) | 180.9±3.9 | 171.2±5.6 |
| BMI | 22.3±1.6 | 23.8±1.4 |
| $VO_{2max}$ (ml/kg/min) | 65.5±4.5 | 53.5±3.5 |
| Sports experience (years) | 3.6±3.9 | 13.2±5.2 |
| Training hours per week (h) | 8±3.2 | 6.1±1.8 |
| Training sessions per week (n) | 6.8±3 | 5±1.2 |
| 5-km personal best (min:sec) | 16:30±1:30 | 19:45±1:30 |
| 10-km personal best (min:sec) | 36:30±1:50 | 39:30±2:45 |

*Presented with means and standard deviations. Abbreviations: BMI = Body mass index, $VO_{2max}$ = maximal oxygen uptake.*

### *2.3 Study design*

The participants completed a 1-week baseline training, a 2-week training overload (50-70% progressive increase in training load), and a 1-week recovery period (30-40% reduction in training load from baseline). After recruitment and before the start of the project eligible participants were invited to a familiarization test (T0) and information meeting. There was one test after baseline training (T1), one test after training-overload (T2) and one test after recovery (T3). The procedure for each test was as follows: blood samples in a fasted state, a standardized pre-exercise meal and filling out a questionnaire, and a maximal incremental test in the laboratory. There was no blood sampling at T0. The training load for the different periods were based on the average training load of the athlete over 2-3 weeks of training monitoring before the start of the study.

### *2.4 Training monitoring and prescriptions*

All athletes recorded their training and "daily parameters'' using an online training diary developed by the Norwegian Top Sport Centre (Olympiatoppen) which the research team had access to and used to follow-up participants during the entire project period. Training variables monitored included training time, intensity, and frequency, whereas the "daily parameters" included self-reported resting heart rate, perceived sleep quality, mental and physically perceived readiness to train, rating of perceived exertion (RPE), and daily shape. Training impulse score (TRIMP) and session RPE load (sREPE load) were calculated as a measure of training intensity and training duration (Mujika, 2017). All participants received an individualized training plan with how much training volume they should preferably spend in low-intensity training (LIT), moderate-intensity training (MIT), and

high-intensity training (HIT). The intensity distribution was held similar as they usually trained, only the volume in each zone was increased. Participants were told to keep strength training similar as usual/ make no changes. Instructions about zones/how to register correctly according to heart rate and perceived exertion were given to participants before the start of the study. Throughout the study, members of the research team would remind participants to register training and nutrition 48-h before each test and to wear the same shoes/equipment.

After blood sampling in a fasted state, the participants were provided a standardized pre-exercise meal consisting of approximately 1.5 grams of carbohydrate per kg of body weight 75 minutes before the laboratory protocol following pre-event sport nutrition guidelines (Thomas et al., 2016). The participants were also asked to monitor their nutritional intake 48-h before arriving at the lab using a food diary and to replicate this before the subsequent tests. All food diaries were checked for completeness and were processed using the Norwegian food database (Kostholdsplanleggeren) by the research team. Descriptives of nutrition before each test are presented in Table 2.

*Table 2. Average nutritional intake over the 48-h period before the tests (n=11).*

| | T1 | T2 | T3 |
|---|---|---|---|
| Fluid (ml) | 3548.3±1564.8 | 3650.9±1278.5 | 3660±1330.2 |
| Energy (Kcal) | 2909.3±989.7 | 3310.4±1614.4 | 3068.4±1659 |
| Carbohydrates (g) | 379.3±141 | 421.7±241 | 406±296 |
| Carbohydrates (g/kg) | 5.1±1.5 | 5.7±2.9 | 5.42±3.5 |
| Fat (g) | 85.7±31 | 116.5±45.2 | 91.4±36.7 |
| Protein (g) | 115.3±46 | 136.4±66.5 | 123.8±57.5 |
| Protein (g/kg) | 1.6±0.6 | 1.8±0.8 | 1.7±0.7 |

*Presented with means and standard deviations. Abbreviations: T1 = Test after 1 week of normal training loads, T2 = Test after 2 weeks of training-overload, T3 = Test after 1 week of recovery training.*

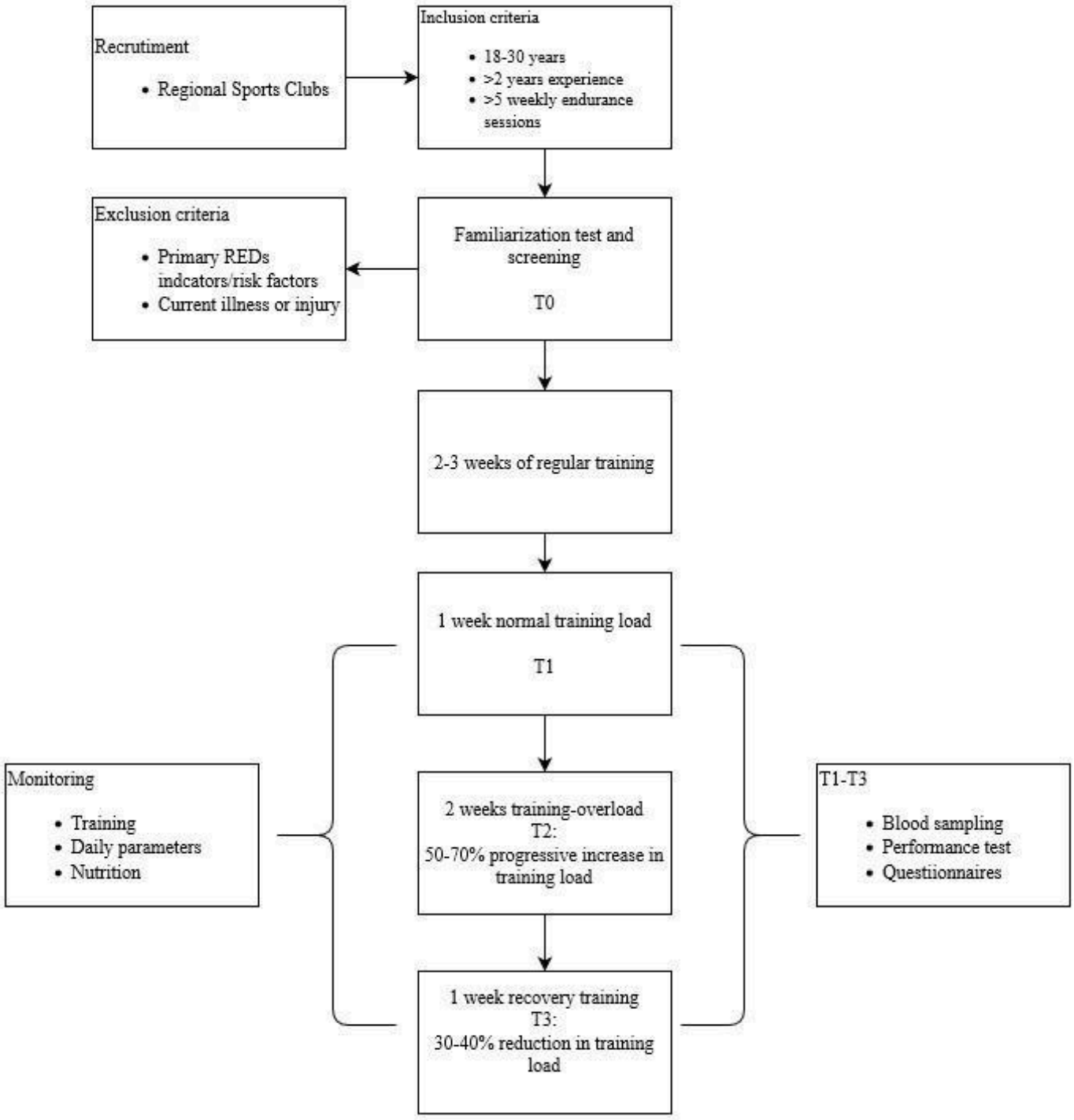



*Figure 1. Flowchart of the recruitment process and study design.*

### *2.5 Performance and test protocol*

The protocol consisted of a 10-min standardized warm-up and maximal incremental test to exhaustion. Before warm-up, the participant's height (first time) and body mass were assessed. The warm-up was performed as a treadmill running at an 1.5% incline (8 km/h for women and 10 km/h for men, respectively). A maximal incremental test to exhaustion (TTE) was performed at 5.5% incline with a starting speed of 10 km/h and 12 km/h for women and men, respectively. Subsequently, the speed was increased by (1 km/h) every minute until exhaustion. Treadmill running was performed on a 2.5 x 0.7-m motor-driven treadmill (RL 2500, Rodby, Vange, Sweden). Respiratory variables were measured using open-circuit indirect calorimetry with mixing chamber (Vyntus CPX, Vyaire Medical, Mettawa, IL, United States) and HR by a Garmin Forerunner 935 (Garmin Ltd., Olathe, KS, USA). RPE using the 6-20-point Borg scale and [La] were taken immediately after completing the test. Lactate was measured using the stationary Biosen C-Line lactate analyzer (Biosen, EKF Industrial Electronics, Magdeburg, Germany). Respiratory variables and HR were measured continuously, and $VO_{2max}$ was defined as the highest 1-min average. HRmax was defined as the highest 5-s HR measurement, whereas RPE was determined directly after, and lactate approximately 2 minutes after. Peak speed (VPeak) was defined as the average speed during the last minute of the TTE. The participants were blinded to time and were given information about time left before the next increase in speed (at 30 s and 15 s). TTE was used as the main performance measure in the study.

### *2.6 REDs indicators*

Participants completed a questionnaire in connection with a familiarization test and information meeting for screening of potential REDs/health risks. Both men and women with severe REDs risk would be excluded from participation. REDs symptoms and classification of severe risk were based on the latest IOC clinical guidelines for the assessment and risk stratification of REDs (Mountjoy et al., 2023). For female participants, menstrual/hormonal contraceptive status was recorded (i.e., having a natural menstrual cycle or the type of hormonal contraceptive (combined or progesterone only), delivery method, names and concentrations of exogenous hormones) (Elliott Sale et al., 2021).

All blood sampling and handling were conducted by certified personnel at NTNU Centre for Elite Sports Research. Blood samples were taken after lying down for 10 minutes and according to standard procedures for venous blood samples. Blood samples of testosterone (total, free and bioavailable), cortisol, human growth hormone (HGH), sex hormone binding globulin (SHBG), prolactin, and cholesterol (total and low-density lipoprotein) were taken after 12 h of fasting and individually at the same time of the day (7:10-9:20). Serum-gel-tubes 3x5ml (3x1ml kryo-tubes) were used. Samples were centrifuged after 30 minutes at 2200*g for 10 minutes, and separated serum was immediately removed. Blood samples were stored at -20°C in a freezer at the lab before they were sent to St. Olav Hospital for analyses (St. Olavs, Medical biochemistry). Triiodothyronine serum and SHBG concentrations were analyzed with Siemens Atellica CH390 and Immunoassay Chemiluminescence as the assay method. Cortisol serum concentration was analyzed with Siemens Atellica IM1600 and assay method Immunoassay Chemiluminescence. HGH serum was analyzed with Roche Cobas Pro e801 and assay method Immunoassay

Electrochemiluminescence. Testosterone serum concentrations were analyzed with Agilent 1290, Agilent 6465, Triple Quad LC/MS-MS, and assay method Liquid-liquid extraction, High-pressure Liquid Chromatography, Mass spectrometry. Total cholesterol and LDL-cholesterol serum concentrations were analyzed with Siemens Atellica CH390 and assay method Enzymatic assay with 596 nm light. Analytical variations and responses in REDs indicators are presented in table 3 below. Exact times for each blood sample, when it was centrifuged and when it was stored were registered, and all tubes were marked with ID-numbers for each participant.

*Table 3. Biomarkers and analytical variations.*

| | CV% | CVi% | CVt% | REDs indicator response |
|---|---|---|---|---|
| Total cholesterol | 1.1 | 5.4 | 5.5 | Secondary (above reference) |
| LDL cholesterol | 1.2 | 7.8 | 7.9 | Secondary (above reference) |
| Triiodothyronine | 2.2 | 7.9 | 8.2 | Primary (reduced) |
| Total testosterone | 6.0 | 9.3 | 11.1 | Primary (reduced) |
| Free testosterone | 6.0 | 9.3 | 11.1 | Primary (reduced) |
| Bioavailable testosterone | 6.0 | 9.3 | 11.1 | Primary (reduced) |
| Cortisol | 8.2 | 20.9 | 22.5 | Potential (increased or changes) |
| SHBG | 3.7 | 12.1 | 12.7 | Potential* |
| HGH | 0.6 | na | na | Potential* |
| Prolactin | 2.7 | 6.9 | 7.4 | Potential* |

*Abbreviations: CV% = analytical variation, CVi% = intra biological variation, CVt% = total variation, LDL = low-density lipoprotein, SHBG = sex hormone binding globulin, HGH = human growth hormone, *changes must be interpreted together with other markers*

In the time-period between the standardized pre-exercise meal and the exercise test, the participants performed a digital questionnaire developed using www.nettskjema.no. Two versions of the questionnaire were used. One extended version was used before and after the project period (in connection with the familiarization test and test 3) to primarily screen for REDs indicators and collect information on training history. The other shorter version was used in connection with tests 1-3 and did not include the above-mentioned additional questions. The developed questionnaire covered the following dimensions: Eating Disorder

Examination Questionnaire (EDE-Q) was used to screen participants for disordered eating as part of the REDs "risk umbrella" according to the latest IOC statement (Mountjoy et al., 2023). EDE-Q was included both prior to the overload-protocol (for exclusion) and after (T3) to detect any possible changes because of the period with increased training load. EDE-Q global score of >2.30 for females and 1.68 for males is considered a primary indicator of disordered eating as part of the REDs risk assessment tool (Mountjoy et al., 2023). The EDE-Q covered the following aspects: Dietary restraint, eating concerns, weight concerns and shape concerns. Participants were asked to rate their experiences and behaviors over a specific time period (the past 28 days) on a Likert scale.

For male participants, 4 questions (A1-2 and B1-2) related to libido and morning erections were included based on the developed and validated low-energy availability questionnaire in males (LEAM-Q) (Lundy et al., 2022), in which these subjective markers have been shown to be most sensitive in detecting low energy availability in male athletes and considered an emerging indicator of REDs (Mountjoy et al., 2023). In subsequent analysis, low sex drive (libido) was identified when A1 and A2 received a score ≥2 and B2 a score of ≥ 1 (Lundy et al., 2022).

### *2.7 Statistical analysis*

Data were tested for normality using Shapiro-Wilk's test of normality. The data were checked for outliers by inspecting Q-Q plots and no meaningful outliers were identified. For normally distributed continuous data, descriptive statistics are presented as mean ± standard deviation (SD). For ordinal data, descriptive statistics are presented as median and interquartile range (IQR). A repeated measures ANOVA was conducted to determine the main effect of time (different weeks of training and between T1, T2, and T3 for main variables). The F-value and associated p-value for the main effect, the degrees of freedom for each effect, and the effect sizes (Eta squared) were reported. Post hoc tests with unadjusted p-values were assessed when applicable with a paired samples t-test to test for significant differences. Cohen's d effect sizes were also calculated. For ordinal data, the non-parametric Friedman test was used (between T1, T2 and T3). A Wilcoxon signed rank test was conducted for EDE-Q global and subscale scores (between T0 and T3). The statistical significance level was set at <.05. All analyses were performed with Microsoft Excel 2010 (Microsoft Corporation, WA) and IBM SPSS Statistics version 29.0 (SPSS Inc, Chicago, IL).

## 3.0 Results

There were eleven participants (men=7, women=4) who completed the study (training, performance, and questionnaires), while data from blood biomarkers were obtained from only ten participants (men=7, women=3) due to technical measurement error.

### *3.1 Training data*

The participants increased their training load (TRIMP-score) by 49%±68% (p <.001) in the first week of T2 and 73%±75% (p <.001) in week 2 of T2 compared to T1. From T2 to T3, there was a 38%±43% (p <.001) reduction in training load (see Figure 2 and Table 4 for detailed training data).

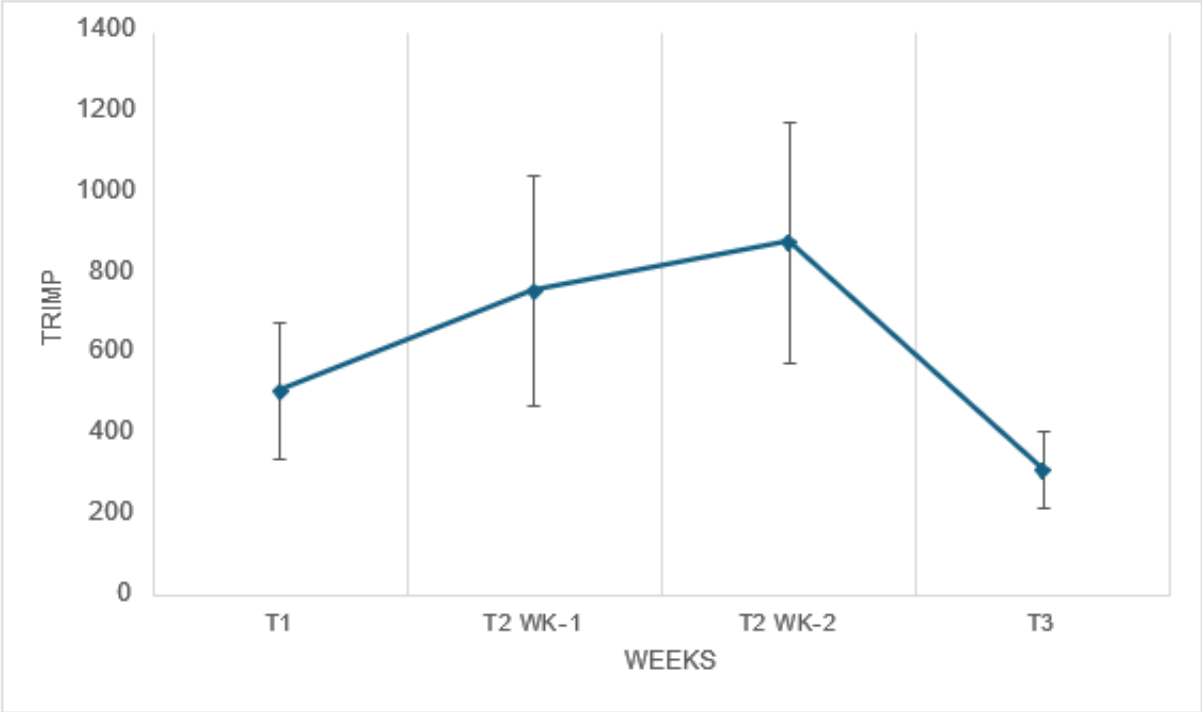



*Figure 2. Changes in training load (TRIMP-score) for the 4-week study duration (mean ± standard deviation). Abbreviations: T1 = week 1 of baseline training, T2 wk-1 = week 1 of the overload period, T2 wk-2 = week 2 of the overload period, T3 = 1 week of recovery training. *Changes in training load (p <.001).*

*Table 4. Training data of all participants (n=11)*

| | T1 | T2 wk-1 | T2 wk-2 | T3 |
|---|---|---|---|---|
| Endurance volume (h) | 6.8± 2.0 | 10.2±3.7 | 11.8±4.0 | 4.4±1.8 |
| Total volume (h) | 7.5±2.1 | 11.2±3.3 | 12.5±3.9 | 5.0±1.6 |
| LIT (h) | 5.4±1.7 | 8.1±3.4 | 9.5±4.1 | 3.7±1.8 |
| MIT (h) | 0.9±0.6 | 1.3±0.9 | 1.5±1.0 | 0.4±0.4 |
| HIT (h) | 0.5±0.4 | 0.7±0.6 | 0.7±0.5 | 0.3±0.5 |
| sRPE | 5.0±0.7 | 5.5±1.0 | 5.6±0.9 | 4.8±0.7 |
| sRPE load | 2219±720 | 3540±1572 | 4129±1809 | 1496±1809 |
| HR rest (bpm) | 47.6±8.8 | 47.6±7.9 | 43.8±14.54 | 46.3±7.6 |

*Presented with means and standard deviations. Abbreviations: LIT = Low Intensity Training, MIT= Moderate Intensity Training, HIT= High Intensity Training, sRPE= Session Rating of Perceived Exertion, sRPE load= Session Rating of Perceived Exertion load, T1 = week 1 of baseline training, T2 wk-1 = week 1 of the overload period, T2 wk-2 = week 2 of the overload period, T3 = 1 week of recovery training.*

Physical readiness was significantly different between T1, T2 wk-1, T2 wk-2 and T3 $F(3.0, 30.0) = 2.93$, partial eta squared: 0.22 indicating a large effect between tests, $p<.05$. Post hoc analysis revealed that physical readiness had a 0.9-point reduction from T2 wk-1 to T2 wk-2 ($p<.05$, Cohen's $d= 0.7$), and a 0.8-point increase between T2 wk-2 and T3 ($p<.05$,

Cohen's d= 0.7), see Table 5. There were no significant changes between other tests (T1 and T2-wk 1 or 2, or T1 and T3), see Table 5.

Mental readiness was significantly different between T1, T2 wk-1, T2 wk-2 and T3 F(2.3, 23.92) = 3.32, partial eta squared: 0.25 indicating a large effect between tests, p<.05. Post hoc analysis revealed that mental readiness had a 1.4-point reduction from T1 to T2 wk-2 (p<.05, Cohen's d= 0.6), and a 1.1-point reduction from T2 wk-1 to T2 wk-2 (p<.05, Cohen's d= 0.8), see Table 5.

Daily shape and sleep quality were not statistically significant between time points. Mental and physical readiness, daily shape, and sleep quality data are presented in Table 5.

*Table 5. Daily variables for all participants (Median (IQR))*

| | T1 | T2 wk-1 | T2 wk-2 | T3 |
|---|---|---|---|---|
| Readiness physical (1-10) | 6.9 (7.5-6.3) | 6.7 (6.9-6.3) | 5.8 (6.5-4.7) | 6.6 (7.3-5.9) |
| Readiness mental (1-10) | 7.1 (7.4-6.2) | 6.8 (7.4-6.3) | 5.7 (6.6-4.8) | 6.5 (7.0-5.9) |
| Sleep quality (1-10) | 7.3 (7.8-6.5) | 7.3 (7.5-6.4) | 7.1 (8.0-6.6) | 7.3 (8.0-6.0) |
| Daily shape (1-10) | 6.8 (7.2-5.4) | 7.0 (7.3-6.2) | 6.3 (6.9-5.3) | 6.7 (7.0-6.3) |

*Abbreviations: T1 = week 1 of baseline training, T2 wk-1 = week 1 of the overload period, T2 wk-2 = week 2 of the overload period, T3 = 1 week of recovery training.*

### *3.2 Performance*

The main performance outcome (TTE) was significantly different between T1, T2 and T3 (F(1.4, 14.5) = 12.3, partial eta squared: 0.55). Post hoc analysis revealed that TTE increased by 7.8±14% from T1 to T3 (p <.01, Cohen's d = 1.3) and by 10.3±17% from T2 to T3 (p <.01, Cohen's d = 1.8). However, there was no significant change between T1 and T2 (see Figure 3).

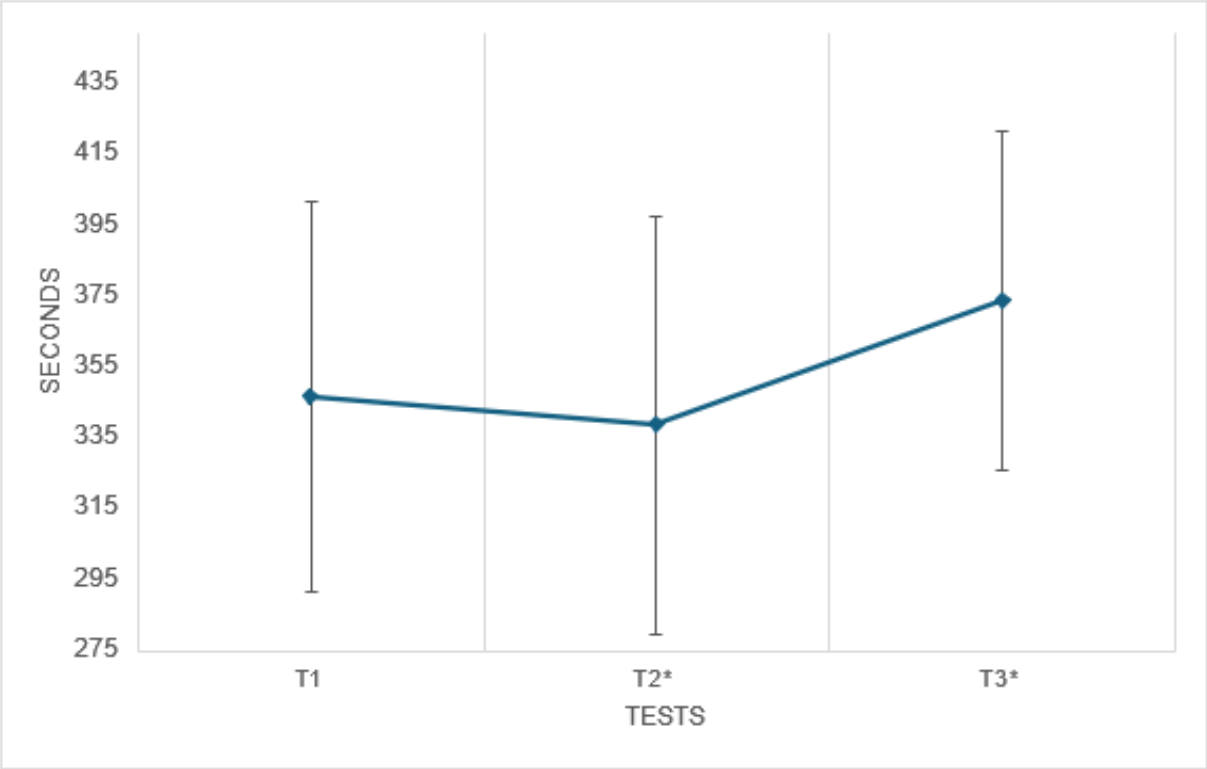



*Figure 3. Changes in performance (TTE) for the 4-week study duration for all participants (mean and standard deviation) *Changes between T1 and T3, and changes between T2 and T3 were significant (p<.01).*

Descriptives of the other physiological variables are presented in Table 6.

*Table 6. Performance variables (n=11)*

| | T1 | T2 | T3 |
|---|---|---|---|
| Body mass (kg) | 72.5±6.6 | 72.4±6.4 | 72.4±6.5 |
| Lactate peak (mmol) | 9.9±1.5 | 8.3±1.9* | 11.0±2.0* |
| Speed-peak (km/t) | 16.1±1.5 | 16.0±1.4* | 16.6±1.4* |
| HR peak (bpm) | 190.8±9.5 | 186.9±9.5 | 190.5±9.0 |
| $VO_{2max}$ (ml/kg/min) | 61.8±6.8 | 61.2±6.7 | 62.9±6.8 |
| RER peak | 1.10±0.03 | 1.08±0.05 | 1.11±0.04 |
| RPE (6-20) | 19.1±0.7 | 18.8±0.6 | 19.3±0.4 |

*Presented with mean and standard deviations. Abbreviations: T1 = Test after 1 week of normal training loads, T2 = Test after 2 weeks of training-overload, T3 = Test after 1 week of recovery training. *Changes between T1 and T3, and between T2 and T3 were significant (P<.01).*

### *3.3 REDs indicators*

There were no significant changes between tests for any of the blood biomarkers. Descriptives are presented in Table 7.

*Table 7. Blood biomarkers for all participants (n=10)*

| | T1 | T2 | T3 | Reference |
|---|---|---|---|---|
| Total cholesterol (nmol/l) | 4.2±0.8 | 4.0±0.6 | 4.4±0.5 | [2.9-6.1] |
| Cholesterol LDL (nmol/l) | 2.4±0.7 | 2.2±0.5 | 2.6±0.4 | [1.5-5.1] |
| Trio Thyroid Free (pmol/l) | 5.9±1.0 | 5.8±0.8 | 5.9±0.7 | [3.5-6.5] |
| Testosterone (nmol/l) | 18.8±13.6 | 18.5±13.8 | 16.9±13.4 | [6.73-31.9] |
| Testosterone free (nmol/l) | 0.4±0.3 | 0.4±0.3 | 0.3±0.3 | |
| Testosterone bioavailable (nmol/l) | 9.2±6.6 | 8.6±6.4 | 7.9±6.4 | |
| Cortisol (nmol/l) | 632.1±187 | 645.5±209 | 667±231 | [133-537 (994)*] |
| SHBG (nmol/l) | 59.1±60.2 | 61.5±60.1 | 66.5±77.9 | [13-72, 20-151] |
| HGH (ug/l) | 2.2±4.7 | 1.6±2 | 1.6±2.9 | [0.13-9.88] |
| Prolactin (mlU/l) | 259±49 | 292±120 | 284±93 | [61-314, 63-533] |

*Presented with mean and standard deviations. Abbreviations: LDL = low-density lipoprotein. T1 = Test after 1 week of normal training loads, T2 = Test after 2 weeks of training-overload, T3 = Test after 1 week of recovery training. *reference females*

There were no significant changes in EDE-Q global or subscales of EDE-Q from T0 to T3, see Table 8 for descriptives.

*Table 8. EDE-Q global questionnaire (n=11)*

| | T0 | T3 | Norm. females | Norm. males |
|---|---|---|---|---|
| Eating | 0.00 (0.00-0.00) | 0.00 (0.00-0.00) | [0.5±1.0] | [0.2±0.4] |
| Weight | 0.40 (00.00-0.40) | 0.00 (0.00-0.40) | [1.6±1.5] | [0.5±0.7] |
| Figure | 0.12 (0.00-0.30) | 0.00 (0.00-0.25) | [1.8±1.6] | [0.7±0.8] |
| Restriction | 0.00 (0.00-0.20) | 0.00 (0.00-0-20) | [1.2±1.3] | [0.5±0.7] |
| Global | 0.13 (0.00-0.31) | 0.06 (0.00-0.20) | [1.3±1.1] | [0.4±0.5] |

*Presented with median and interquartile range. Abbreviations: EDE-Q weight, restriction, eating, figure = subscale scores, EDE-Q global = mean of all subscale scores. T0 = test before the start of the study, T3 = after one-week recovery. Norm. = normative values for healthy individuals (without an eating disorder).*

Descriptive statistics for libido questions for males are presented in Table 11. There were no significant differences between the tests in the different subscales of the questionnaire.

*Table 9. Libido questionnaire for males (n=7)*

| | T1 | T2 | T3 | Low libido identification |
|---|---|---|---|---|
| Sex drive (A1) | 0(1) | 1(1) | 0(1) | >2 |
| Sex drive last month (A2) | 0(0) | 0(0) | 0(0) | >2 |
| Morning erections (B1) | 0(0) | 0(1) | 0(1) | >2 |
| Compared to normal (B2) | 0(0) | 0(0) | 0(0) | >1 |

*Presented with median and interquartile range. Abbreviations: A1: 0 = high, 1 = moderate, A2: 0 = stronger than usual or about the same, B1: 0 = 5-7 per week, 3-4 per week, 1 = 1-2 per week, B2: 0 = more often or about the same. T1 = Test after 1 week of normal training loads, T2 = Test after 2 weeks of training-overload, T3 = Test after 1 week of recovery training.*

## 4.0 Discussion

The overall aim of this study was to investigate the influence of a 2-week training-overload period on performance and REDs indicators in endurance athletes. The main findings were that although all participants increased their training load substantially, there was no significant influence on either performance measures of REDs indicators following the 2-week training overload period. An 8% increase in TTE was found following the recovery period, implying a positive training effect of the 4-week study protocol.

### *4.1 Training data*

The training protocol was planned so that the training load would progressively increase from week 1 to week 2 in the overload period. The intensity distribution was kept the same as during the baseline period, while the volume of endurance training in each intensity zone (LIT, MIT or HIT) increased 40-70% from week 1 to week 2 of the overload period. Subsequently one week of recovery with a 30% reduction in training load compared with

baseline training. Physical and mental readiness to train decreased after the training-overload protocol (at T2) but returned to baseline levels after recovery (at T3). That subjective well-being and readiness to train are sensitive to changes in training load has been revealed in previous studies (Symons et al., 2023; Ten Haaf et al., 2017), and are frequently used to detect overreaching development in periods of training-overload (Bresciani et al., 2011). In accordance with these findings the present study indicates that physical and mental readiness are able to significantly reflect changes in training loads.

### *4.2 Performance*

The 2-week training-overload followed by 1-week recovery increased performance indicating a positive adaptation from the training load prescribed in this study. It is fair to assume that the training load was appropriate for the participants based on the increase of performance after recovery and that subjective fatigue markers (physical readiness, mental readiness, daily shape, and sleep quality) were restored or unaffected by the training protocol. All individuals increased their maximal performance or running speed, and none of the participants could be defined as overreached at the time of recovery (T3). A study by Aubry et al., proposes that greater gains in performance can be achieved when a higher training load is prescribed before the taper but not in the presence of FOR, and suggests that FOR provides a higher risk of fatigue and maladaptation, including increased infection risk (Aubry et al., 2014; Bellinger P., 2020). In the present study, [La]max was significantly reduced after the training-overload period which corresponds to previous work of Le Meur et al., where lactate levels were able to detect overreached athletes. Although, in the current study, since performance (TTE, Vpeak) and [La]max were significantly improved after only one week of recovery this might strengthen the possibility that FOR is not necessary for increases in performance.

Even though underperformance is the main symptom of FOR (Meeusen et al., 2013), it is possible that there are several contextual factors that may influence the metabolic consequences associated with FOR and classifying this training-induced state of fatigue based purely on a decrement in performance may be an oversimplification (Bellinger P., 2020). Subjective measures may reflect an athlete's psychological readiness to perform, with psychological state known to influence performance. Readiness to train/other subjective markers of fatigue have successfully detected overreaching and FOR in previous studies (Symons et al., 2023; Ten Haaf et al., 2017). So, the decline in subjective readiness measures in the current study might be an indication of FOR, even though no significant decline in performance was observed between T1 and T2.

### *4.3 REDs indicators*

There were no significant changes observed in any of the REDs indicators (blood biomarkers and questionnaires) suggesting that training-overload had no influence on REDs indicators in the present study. However, lowered testosterone values and decreased libido/sex drive in male athletes have been found in previous studies (Hackney, 2018), and in a recent validation of the LEAM-Q those classified as having low sex drive by the questionnaire demonstrated multiple disturbances in clinical markers of LEA. For instance, sex drive was associated with total testosterone, triiodothyronine, and free testosterone:cortisol ratio (Lundy et al., 2022). Since there were no significant changes in these biomarkers in the

present study, it's unsurprising that there were also no changes in libido scores. There is evidence that lower libido is an effect of changes in testosterone, and this has been linked to both overreaching and LEA in male athletes (Cupka, 2023). Moreover, testosterone can be suppressed as a result of increases in cortisol or inadequate energy intake, or both (Kuikman et al., 2022). Besides, loss of interest in sex and decreased sex-drive can be affected by medication or other individual differences that are not connected to REDs. Accordingly, assessment of changes in libido should probably be interpreted in connection with other REDs indicators and exclusion of other conditions. In addition, it can be challenging to obtain accurate information on this topic given the possible stigma and/or embarrassment around revealing low sex drive or reduced morning erections (Lundy et al., 2022). For EDE-Q scores it might be more complex, since EDs/DEs are equally a risk and a possible consequence of LEA and REDs (Mountjoy et al., 2023). Thus, EDE-Q scores could be elevated with no other signs of REDs and in that case, it could indicate a risk for later developing REDs if measures or not taken. Alternatively, an athlete could show signs of REDs without elevated EDE-Q scores since insufficient energy intake can happen inadvertently through lack of knowledge, loss of appetite or time to plan and prepare nourishing meals. Some athletes may experience an acute performance increase due to initial body weight loss at the onset of LEA (e.g., increased relative maximal oxygen uptake). Although the elevated level of performance is ultimately unsustainable, some athletes attribute the acute increase in performance to excessive exercise and DE behaviors, while a desire to pursue more praise and success creates an evil cycle of rigid behaviors ultimately leading to REDs. In the present study, there were only four females, and since the risk for EDs/DEs have shown a higher risk for females than males (Melin et al., 2015), this might contribute to the lack of findings or significant change in the EDE-Q scores.

A systematic review (Kuikman et al., 2022) found that most training periods (around 60%) with maintained or increased training loads did not result in impaired athletic performance or evidence of LEA, which corresponds with the present findings. Nevertheless, increases in training load do not always lead to a compensatory increase in energy intake and this may occur inadvertently due to appetite-suppressing hormone alterations that occur with exercise (Koehler et al., 2016; Schaal et al., 2021). Several studies have indicated associations with performance and responses in biomarkers when energy intake was not matched with energy expenditure (Woods et al., 2018; Schaal et al., 2021). In the current study, participants were encouraged to increase their energy intake, which may have contributed to the positive influence from the training-overload. Training-overload and focus on the required energy intake/ diet for the specific phases should go hand in hand in the athletes training-plans/regimes (Stellingwerff et al., 2019). Athletes experiencing LEA either increase, stagnate, or decrease performance, depending on the intensity of LEA adaptation and importance of body weight on their performance. Body composition appears to be one of an array of variables impacting performance, and its influence should not be overstated (Melin et al., 2024; Wasserfurth et al., 2020). Sports practitioners and coaches should encourage athletes to focus on increasing their caloric intake "by discipline" rather than according to their appetite during critical periods of training-overload in their training regimes (Schaal et al., 2021). A sudden increase in performance may also be an early sign of LEA, especially if this is followed by a decline and or stagnation of performance in spite of training load being maintained or increased. Although in the present study there

were no changes in body mass/BMI, or too low BMI at the start of the study which may strengthen the fact that there were no changes in the other REDs indicators either. Lowered triiodothyronine values have been consistently seen in athletes in LEA states from both sexes (Mountjoy et al., 2023; Melin et al., 2024), and for females it has appeared more frequently in those with LEA together with menstrual dysfunction or suppressed ovarian function (Mountjoy et al., 2023; Schaal et al., 2021). The females that participated in this study did not report any signs of menstrual dysfunction at the start of the study.

Furthermore, reduced hormonal responses have been shown in studies in which subjects underperformed (Woods et al., 2018) as well as in studies in which subjects maintained their performance level after intensified training (Stenquist et al., 2020). One particularly prevalent sign of REDs is that of decreased performance (Logue et al., 2020). The fact that the participants in this study increased their performance and readiness to train after the recovery period might indicate that the endocrine and metabolic system were balanced/unaffected. This is in accordance with Saw et al. (2016), who showed that subjective markers come ahead of disruptions of biomarkers after intensified training. Two recent studies (Oxfeldt et al., 2023; Wasserfurth et al., 2020) indicate that energy restriction in combination with heavy training loads might not be worth the risk of maladaptation, and the possible consequences on health might outweigh any potential performance benefit. Nonetheless, more studies are needed to get a better understanding of how biomarkers and performance manifest in connection with LEA/REDs. These preliminary findings should be studied further with a larger sample size and address potential sex-differences on training-overload and REDs indicators in endurance athletes. Especially since there is evidence indicating that males are more robust than females when inducing LEA for short periods (Melin et al., 2024), and it might be appropriate to test the same individuals over a longer time period.

### *4.4 Methodological considerations*

The present study included REDs indicators from the newly updated CAT2 assessment tool used for the evaluation of athletes or active individuals who are suspected of having LEA and acts as a guide for clinicians and coaches for return-to-play decisions (Mountjoy et al., 2023). Significant scientific progress in REDs severity and risk assessment has been made since the original IOC REDs Clinical Assessment Tool (CAT) was published in 2015 (Melin et al., 2015). Because problematic LEA is the underlying etiology for the health and performance outcomes of REDs, various LEA indicators (signs and symptoms) have emerged as the current best practice for clinical assessment and research purposes. High-performance environments in endurance sports seem to be at bigger risk (Wasserfurth et al., 2020), and systematic reviews on LEA and/or REDs have mainly studied elite- or non–athletes (Gibbs et al., 2013; Logue et al., 2020). Therefore, it is important to include more recreationally active and highly trained individuals as well. Prevalence rates for EDs are high among elite athletes, particularly female athletes, and those competing in endurance sports (leanness sports). EDs also occur more frequently among male athletes than in non-athletic male controls. Further studies should emphasize including enough female participants and to study the possible sex differences in the development of REDs in highly trained individuals. Male athletes seem to be less sensitive to LEA/can sustain hormonal and metabolic balance and performance. Still, it's important to consider that REDs

occur in male athletes as well and that symptoms of training stress and/or REDs can manifest differently. Since this was a pilot study with a low sample size, the power to detect differences was likely too low, and further studies are needed including larger samples from both sexes. Nonetheless, there's no denying the facts/risk of REDs and the importance of detection and prevention with around 60 % prevalence in endurance sports. Overall, coaches must internalize these basic considerations: proper training loads combined with adequate nutrition are two critical inseparable factors for achieving peak athletic performance (Stellingwerff et al., 2019). In the end, all biomarkers should be contemplated in association with other markers and symptoms, and being aware that other health issues or medications can affect the results of biomarkers. Besides, the inter-individual differences are one of the reasons it might be challenging to obtain meaningful results at group-level.

**5.0 Conclusion**

A 2-week training-overload period in endurance athletes had no negative influence on either performance or REDs indicators. This pilot study can be used as a starting point to further investigate the influence of training-overload on performance and REDs indicators in endurance athletes by including larger samples and analyses of potential differences between men and women in the response to training overload.

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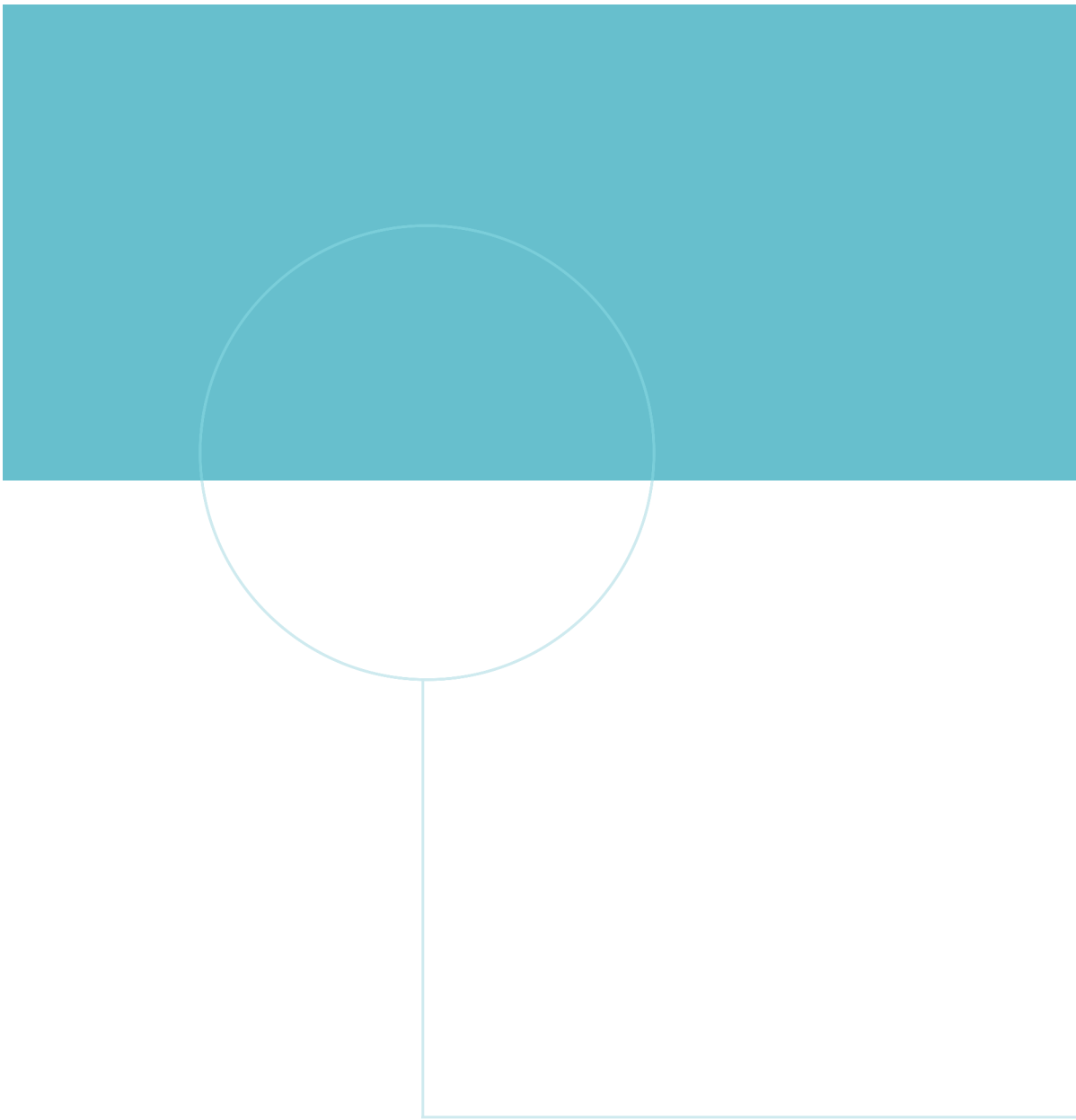

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