Low Energy Availability Followed by Optimal Energy Availability Does Not Benefit Performance in Trained Females

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ABSTRACT

OXFELDT, M., D. MARSI, P. M. CHRISTENSEN, O. E. ANDERSEN, F. T. JOHANSEN, M. BANGSHAAB, J. RISIKESAN, J. S. JEPPESEN, Y. HELLSTEN, S. M. PHILLIPS, A. K. MELIN, N. ØRTENBLAD, and M. HANSEN. Low Energy Availability Followed by Optimal Energy Availability Does Not Benefit Performance in Trained Females. *Med. Sci. Sports Exerc.*, Vol. 56, No. 5, pp. 902–916, 2024. **Purpose:** Short periods of reduced energy availability are commonly undertaken by athletes to decrease body mass, possibly improve the power-to-mass ratio, and enhance physical performance. Our primary aim was to investigate the impact of 10 d of low energy availability (LEA) followed by 2 d of optimal energy availability (OEA) on physical performance parameters in trained females. Second, physiological markers at the whole-body and molecular level related to performance were evaluated. **Methods:** Thirty young trained eumenorrheic females were matched in pairs based on training history and randomized to a 10-d intervention period of LEA (25 kcal·fat-free mass (FFM)$^{-1}$·d^{-1}) or OEA (50 kcal·FFM^{-1}·d^{-1}) along with supervised exercise training. Before the intervention, participants underwent a 5-d run-in period with OEA + supervised exercise training. After the LEA intervention, 2 d of recovery with OEA was completed. Participants underwent muscle biopsies, blood sampling, physical performance tests, body composition measurements, and resting metabolic rate measurements. A linear mixed model was used with group and time as fixed effects and subject as random effects. **Results:** Compared with OEA, LEA resulted in reduced body mass, muscle glycogen content, repeated sprint ability, 4-min time-trial performance, and rate of force development of the knee extensors (absolute values; $P < 0.05$). Two days of recovery restored 4-min time-trial performance and partly restored repeated sprint ability, but performance remained inferior to the OEA group. When the performance data were expressed relative to body mass, LEA did not enhance performance. **Conclusions:** Ten days of LEA resulted in impaired performance (absolute values), with concomitant reductions in muscle glycogen. Two days of recovery with OEA partially restored these impairments, although physical performance (absolute values) was still inferior to being in OEA. Our findings do not support the thesis that LEA giving rise to small reductions in body mass improves the power-to-mass ratio and thus increases physical performance. **Key Words:** REDS, FEMALE ATHLETE, MENSTRUAL DYSFUNCTIONS, ENERGY RESTRICTION

APPLIED SCIENCES

In elite sports, shorter periods of reduced energy availability (EA) are commonly undertaken to decrease body mass, possibly to improve the power-to-mass ratio, and enhance physical performance (1,2). However, if not approached cautiously, this may expose athletes to problematic (prolonged, severe, or even chronic) low energy availability (LEA), with potential health risks (3).

EA is defined as the difference in energy intake and exercise energy expenditure relative to fat-free mass (FFM) (4), and LEA represents a state where EA is insufficient to maintain basal physiological body functions. LEA has previously been reported at ≤30 kcal·kg FFM^{-1}·d^{-1} (5,6), although recent studies suggest that this is not a universal threshold for optimal versus impaired body function (7,8). The prevalence of LEA is high among female athletes (9–12). Strikingly, as little as 4 d of LEA has been shown to trigger physiological dysregulation of the hypothalamic–pituitary–thyroid axis (13), the hypothalamic–pituitary–ovarian axis (5), and markers of bone resorption and formation (6) in sedentary females. In addition, we recently

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Submitted for publication July 2023.
Accepted for publication December 2023.
Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.acsm-msse.org).

0195-9131/24/5605-0902/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0000000000003370

reported that 10 d of LEA impaired daily integrated myofibrillar and sarcoplasmic muscle protein synthesis in trained females performing supervised exercise training (14). Although limited longitudinal evidence exists, observational studies in athletes indicate that problematic LEA is associated with a cascade of negative physiological and psychological effects (3).

Given the possible short- (5,6,13,14) and long-term negative effects of LEA on different physiological systems, the relationship between LEA, changes in body composition, and physical performance is complex and requires further investigation. For instance, short periods of LEA may improve performance; however, only if the potential negative physiological effects observed with LEA (5,6,13–15) do not exceed the theoretic benefit of a weight loss. In this perspective, it is critical that the effects of LEA are reversible so that athletes are not left in a condition of impaired performance and health. Until now, the reversal of the physiological and performance-related effects of LEA has been minimally studied (16).

The direct and indirect impact of LEA on sports performance has recently been reviewed (17). It was concluded that more high-quality research was needed to fully understand the effects of LEA on different physiological systems related to sports performance. A well-controlled study (18) in highly trained race walkers (19 males, 3 females) showed that during an intensified training camp, severe LEA (15 kcal·FFM^{-1}·d^{-1}) resulted in a 3% weight loss after 9 d. When followed by a 24-h prerace fueling period, the improvement in race performance was equivalent to controls who received sufficient energy (and carbohydrate) availability (40 kcal·FFM^{-1}·d^{-1}) during training (18). These findings, primarily from male athletes, showed no positive effect of a small body mass reduction on race performance. This challenges theoretical predictions stating that a lighter body will move faster against gravity, and also empirical data suggesting improved performance with reduced body mass (18–21). However, given that females may be more sensitive to LEA than male athletes (22), there is a need for high-quality evidence on the effect of LEA on physical performance in females.

To address the knowledge gap outlined previously, we conducted a randomized controlled trial (RCT) investigating the impact of 10 d with LEA and subsequent 2 d of recovery with optimal energy availability (OEA). We examined physical performance and physiological parameters in trained females during LEA compared OEA. We hypothesized that LEA would impair physical performance compared with OEA; however, 2 d of recovery with OEA would restore physical performance. We aimed to link performance changes with mechanisms, including body composition, muscle glycogen content, and sarcoplasmic reticulum function. We also assessed blood biomarkers, training volume/quality, psychological well-being, and mental readiness.

METHODS

Experimental Design and Participants

This study was part of a larger RCT examining the effects of LEA compared with OEA on several physiological parameters in trained females (14). We have previously reported the effect of 10 d of LEA on cumulative myofibrillar and sarcoplasmic muscle protein synthesis (14). Here we present outcomes on the effect of the LEA period and subsequent short-term recovery with OEA-induced refueling on physical performance and physiological parameters compared with being in OEA the whole period.

This study included 30 healthy, trained eumenorrheic females who completed three dietary phases in a parallel group design; 1) a run-in period of 5 d with OEA + supervised exercise training, 2) an intervention period of 10 d with LEA or OEA + supervised exercise training in both groups, 3) a 2-d recovery period with OEA (Fig. 1). LEA was provided 25 kcal·FFM^{-1}·d^{-1} and OEA 50 kcal·FFM^{-1}·d^{-1}. A series of laboratory tests were conducted after each dietary phase (day 6 (Pre), 16 (Post) and 18 (Recovery); Fig. 1), including dual-energy x-ray absorptiometry (DXA), resting metabolic rate (RMR), blood and muscle biopsy sampling, and physical performance; Maximal isometric and isokinetic strength, countermovement jump (CMJ) height,

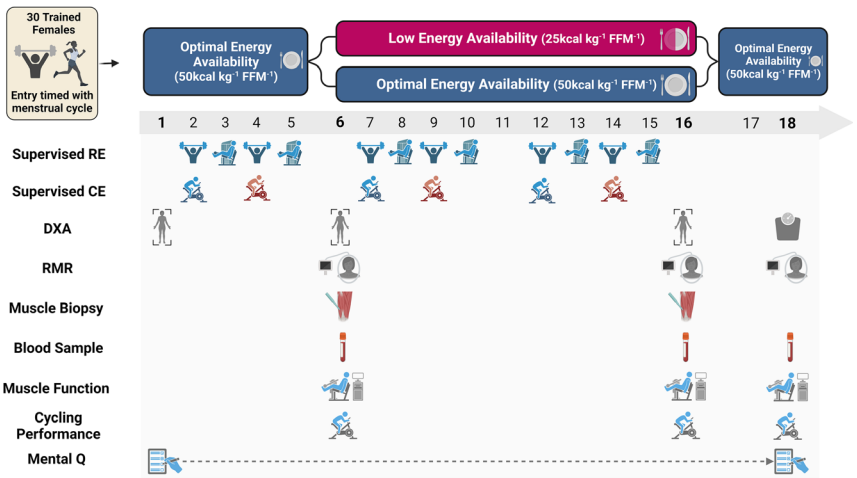

FIGURE 1—Schematic overview of the experimental period. Blue icon indicates moderate-intensity training; red icon indicates high-intensity interval training), CE, cardiovascular exercise; Mental Q; Mental Questionnaire; RE, resistance exercise. Created with Biorender.com

repeated sprint ability, and 4-min time-trial performance. However, the DXA and biopsy procedures were not performed on day 18 after recovery. Individualized meal plans ensuring LEA or OEA were provided as prepackaged meals and snacks during the entire study period, and a standardized breakfast was consumed before the performance testing. Mental questionnaires were collected daily.

Trained eumenorrheic females with similar age, body composition, and training status (see Ref. (14) for details) were recruited through social media and posters distributed at the local university and fitness centers. All participants fulfilled the following inclusion criteria: 1) healthy females aged 18–30 yr with a body mass index between 18.5 and 30 $kg \cdot m^{-2}$; 2) exercising between 4 and 10 times per week, with at least one resistance and at least one aerobic-based or interval-based training session per week; and 3) not used any form of hormonal contraceptive for the past 6 months. Females were excluded from participation if they 1) had experienced irregular menstruation (oligomenorrhea or amenorrhea) within the last 6 months (absence of bleeding); 2) showed markers of LEA (low energy availability in females questionaire, ≥8; eating disorder examination questionnaire, <2.3; and RMR ratio, <0.90; see Ref. (14)); 3) had donated blood within the last month; 4) had not been weight stable for the last 6 months; 5) had suffered from illness, diseases, injuries, or pain that would compromise the training protocol or otherwise affect performance; or 6) were smokers, vegans, or pregnant.

The present study was conducted at the Department of Public Health, Aarhus University, Denmark. It was conducted per the Declaration of Helsinki, approved by the Central Denmark Region Committees on Health Research Ethics (1-10-72-319-20) and registered at Clinical.Trials.gov (ID: NCT04821076). All participants signed an informed consent form before their enrollment into the study.

Screening and familiarization. Before the experiments, all participants visited the laboratory for a screening session and three familiarization sessions, as described elsewhere (14). Briefly, the screening session was performed to identify potential markers of LEA and verify that the participants fulfilled the inclusion criteria (14). If deemed eligible to participate, females completed three familiarization sessions, including assessment of maximal oxygen uptake ($\dot{V}O_{2max}$) on an ergometer bike (familiarization 1), habituation to the exercise training program (familiarization 1 + 2), and 8–12 repetition maximum testing (familiarization 3) (14). Furthermore, habituation to the CMJ test, repeated sprint test, and 4-min time trial was performed on familiarization 2, and isokinetic testing on familiarization 3.

Experimental period. The experimental period started in accordance with each participant's menstrual cycle (1–5 d after the start of menstrual bleeding) and approximately 1 to 2 wk after familiarization. Days 0–16 of the experimental period have previously been described in detail (14). Briefly, participants arrived at the laboratory, overnight fasted in the morning to complete a series of laboratory tests on day 1 (baseline), day 6 after the OEA run-in period, day 16 after the 10 d of either LEA or OEA, and day 18 after 2 d of recovery with refueling (Fig. 1).

Dietary Intervention

The dietary intervention has previously been described in detail (14). In short, participants were provided individualized meal plans throughout the experiment to ensure EA of either 25 or 50 $kcal \cdot FFM^{-1} \cdot d^{-1}$ after subtracting exercise energy expenditure depending on the time period or group. The level of OEA was chosen based on an earlier study reporting a mean EA of 50 $kcal \cdot FFM^{-1} \cdot d^{-1}$ in eumenorrheic female athletes (10). The level of LEA was based on previous literature showing disruption of the hypothalamic–pituitary–thyroid and ovarian axis at EA of <30 $kcal \cdot FFM^{-1} \cdot d^{-1}$ (5,13). The diets in the two groups were matched for protein content (2.2 g·kg lean $mass^{-1} \cdot d^{-1}$). All foods were provided as a mix of prepackaged breakfast and snacks and packed premade meals for lunch and dinner (Getfitfood.dk, Skovlunde, Denmark). Compliance with the diet was monitored through individual food logs in which the participants reported if they did not consume the prescribed food or consumed any food outside the prescribed diet (14).

Exercise Training Program

A supervised exercise training program was performed from days 1 to 16 consisting of three 4-d training blocks, including heavy resistance exercise for the upper and lower body and moderate and high-intensity cardiovascular exercise on a bicycle ergometer (Fig. 1) (14). Upper body resistance exercise sessions were performed on the same days as cardiovascular exercise, whereas lower body resistance exercise was performed on separate days.

As described previously in detail (14), the moderate-intensity exercise consisted of 45–60 min of cycling with a constant cadence at an intensity corresponding to 80% of the ventilatory threshold. The high-intensity exercise consisted of 6–10 intervals of 2 min at 85%–90% W max, intercepted by 1 min of rest. Heart rate, cadence, and rate of perceived exertion (RPE) on the Borg scale were monitored throughout sessions (14). Capillary blood samples were collected by finger-prick with a disposable lancet Pre and 30 s after exercise for immediate lactate analysis (Lactate Scout+; EKF Diagnostics, Barleben, Germany) on the first and last sessions.

The upper- and lower-body resistance exercise sessions consisted of four upper- and lower-body exercises, respectively (14). The exercises were performed as 3 sets of 8–10 repetitions starting at an intensity of 12-repetition maximum (RM) with 2 min of rest between sets. The load was progressively increased throughout the experimental period when participants could complete 3 × 10 repetitions in a given exercise. Training volume, resistance training–specific RPE, and session RPE were monitored throughout the sessions (14).

Exercise energy expenditure was calculated from the predicted training volume each participant was assigned to complete, subtracted by measured resting energy expenditure (REE; kilocalories per hour) during the same time period (14). The energy expenditure during the cardiovascular exercise was calculated from the predicted total work based on the prescribed training program in watts (assuming the mechanical

efficiency of cycling is 25% (23)). The energy expenditure during resistance exercise was calculated from the predicted total work prescribed in the training program in kilos based on the 8–12 RM test with adjustments for age, height, fat mass, and lean mass (24). Calculated exercise energy expenditure subtracted from REE was in both groups 434 ± 97 kcal on days performing moderate-intensity exercise, 314 ± 57 kcal on days performing high-intensity interval training, and 117 ± 17 kcal on days performing lower-body resistance exercise.

Questionnaires

Self-perceived fatigue was assessed daily using a visual analog scale. Participants were instructed to rate their "feeling of fatigue" each night before going to bed using a visual analog scale of 100 mm, where 0 mm corresponded to feeling extremely energetic and 100 mm represented feeling extremely fatigued.

Mental readiness was assessed before each training session and on days 6, 16, and 18 before the performance tests. Mental readiness was evaluated using the questionnaire presented by McLean et al. (25), comprising five questions to asses fatigue, sleep quality, general muscle soreness, stress levels, and mood on a five-point scale. Mental readiness was determined by summing the scores from the five questions.

Dual-Energy X-ray Absorptiometry

Body mass was measured using a Tanita SC 330 (Tanita Europe B.V., 2132 NG Hoffddorp, the Netherlands) before each DXA and on day 18. Changes in body composition and the measurement of FFM for the EA equation were determined in a GE Lunar iDXA series scanner (GE Healthcare, Madison, WI) equipped with the enCORE software v16.0 (GE Healthcare), following standardized procedures as previously described (14). The precision of measurement (percent coefficient of variation) based on weekly quality assurance reports from our DXA showed 0.4% for bone mineral density, 0.4% for lean mass, and 0.4% for fat mass.

Resting Metabolic Rate

REE was determined by indirect calorimetry using a ventilated hood system (Q-NRG; Cosmed, Rome, Italy). All measurements were corrected using alcohol (ethanol) burning (26) to account for potential inaccuracies associated with indirect calorimetry as previously described (14).

Muscle Biopsies

Resting muscle biopsies, with local anesthesia, were obtained from the vastus lateralis using a Bergstrom needle with suction. All biopsies were obtained from the nondominant leg using standardized procedures described elsewhere (14).

Blood Samples

Blood samples were obtained from the antecubital vein at rest and centrifuged 1200g × 10 min before plasma was collected and stored at −80°C. Samples were analyzed in batches for thyrotropin (thyroid-stimulating hormone (TSH)), total triiodothyronine (T_3), glucose, insulin, cortisol, testosterone, and sex hormone–binding globulin (SHBG) at the Department of Clinical Biochemistry, Aarhus University Hospital, Skejby, Denmark, using standard procedures accredited according to ISO/IEC 15189. The free Androgen index was calculated as the ratio between total testosterone and SHBG multiplied by 100.

Maximal Isometric and Isokinetic Muscle Strength

Maximal isometric and isokinetic peak torque and rate of force development (RFD) were assessed for the knee extensors of the dominant leg using an isokinetic dynamometer (Humac Norm; CSMI, Stoughton, MA), previously described in detail (27). After a standardized warm-up, isokinetic peak torque was measured by performing one submaximal followed by five maximal isokinetic contractions at $300°\cdot s^{-1}$ with 30 s of rest between trials. Maximal isometric torque, RFD, and impulse were measured for the knee extensors by performing one submaximal and three 4-s maximal isometric contractions at 70° knee joint angle (0° = full knee extension) with 60 s of rest between successive trials. If the peak torque in the last recording was higher than the previous, another attempt was given. Torque recordings were sampled at 1000 Hz (16-bit A/D converter; National Instruments Corporation, Austin, TX) and were analyzed using customized code written in MATLAB software (The MathWorks, Natick, MA). Maximal voluntary contraction strength was determined as the highest isometric peak torque. RFD and contractile impulse (28) from 0 to 30, 0 to 50, 0 to 100, and 0 to 200 ms relative to the onset of force were derived from the trial with the highest impulse (torque-time integral) from 0 to 200 ms. Force onset was defined as the instant where force increased above baseline force by 2% of the respective peak torque. All trials were visually inspected for any countermovements at the onset of contraction, in which case trials were omitted from the RFD analysis.

Countermovement Jump

Maximal vertical jump height was determined in a CMJ performed on a force platform (AMTI Force and Motion, Watertown, MA). In brief, participants were instructed to place their hands on their hips and jump as high as possible. One submaximal jump was performed as a specific warm-up. Four maximal jumps were recorded with 60 s of rest in between. The highest jump was chosen for further analysis. Vertical ground reaction force was sampled at 1000 Hz and analyzed using customized software (MathWorks).

Repeated Sprint Test

Anaerobic peak power and repeated sprint ability were evaluated with a repeated sprint test consisting of 5 × 6-s maximal sprints separated by a 24-s recovery period, performed on a mechanically braked bicycle ergometer (Monark ergomedic 894E Peak Bike; Monark Exercise AB, Vansbro, Sweden) equipped with the Monark Anaerobic test software (Monark

Exercise AB). Seat height was standardized relative to individual leg length, and the brake loads were set to 10% of the participant's body mass. The repeated sprint test was initiated by a sprint-specific warm-up consisting of two 2- to 3-s practice sprint starts followed by 90-s passive recovery. Hereafter, participants were instructed to reach a pedaling frequency of 80 rpm and, after a 3-s countdown, to pedal as fast and forcefully as possible and stay seated throughout the 6 sec sprint. When a pedal frequency of 100 rpm was reached, the dedicated software automatically applied the braking force and started the timer. Twenty-four seconds of active recovery (pedaling at 40 W, ~80 rpm) separated the five repeated sprints of 6 s. Five seconds before each sprint, participants were instructed to reach 80 rpm and prepare for the next countdown. The test personnel provided strong verbal encouragement throughout each sprint. Power output in watts were recorded and saved for further analysis using the Monark Anaerobic Test Software (Monark Exercise AB).

Four-Minute Time-Trial Performance

Four-minute time-trial performance was evaluated on the same Monark peak bike after 10 min of rest from the repeated sprint test. The load was based on iPPO and preferred cadence during intense work from the incremental test using the formula by Christensen and Bangsbo (29):

$$\text{Load PT (N)} = (\text{iPPO}/11) + (\text{average cadence of last 3 increments in the incremental test}/-10) + 10$$

The test was initiated by a 1-min lead-in at 40 W (~80 rpm), followed by a 3-s countdown, before the braking force was applied. Participants were instructed to complete as much work (i.e., watt) as possible throughout the 4 min. The only verbal feedback was information on elapsed time in 30-s increments and 10 s the last 30 s. Power output was recorded and saved for further analysis using the Monark Anaerobic Test Software (Monark Exercise AB).

Muscle Glycogen Content

Whole-muscle glycogen content was determined using spectrometry with methods described by Passonneau and Lowry (30) and modified by Ørtenblad et al. (31). Freeze-dried and dissected muscle tissue (~1.5 mg) was boiled in 0.5 mL 1 M HCl for 150 min before being cooled and centrifuged at 3500g × 10 min at 4°C. The supernatant was then collected and mixed with 1 mL of reagent solution (Tris-buffer (1 M), distilled water, ATP (100 mM), $MgCl_2$ (1 M), $NADP^+$ (100 mM), and G-6-PDH) before hexokinase was added to initiate the reaction. Absorbance was recorded for 60 min before the glycogen content was calculated.

SR Vesicle Ca^{2+} Uptake and Release

The fluorescent dye technique was used to determine Ca^{2+} uptake and release rates in SR vesicles as described in detail elsewhere (32). Free [Ca^{2+}] was determined by the fluorescent Ca^{2+} indicator Indo-1 (1 mM; 20 Hz, Ratiomaster RCM; Photon Technology International, Brunswick, NJ). The assay buffer consisted of 165 mM KCl, 22 mM Hepes, 7.5 mM oxalate, 11 mM NaN_3, 5.5 μM TPEN, 20 μM $CaCl_2$, and 2 mM $MgCl_2$ (pH 7.0 at 37°C). Muscle homogenate (30 μL) was mixed with 750 mL of assay buffer, SR vesicle Ca^{2+} uptake was initiated by adding ATP (5 mM), and Ca^{2+} uptake was recorded for 3 min before [Ca^{2+}] reached a plateau. Upon measurements of Ca^{2+} uptake, the SR Ca^{2+} ATPase was blocked with cyclopiazonic acid (40 μM) before SR vesicle Ca^{2+} release was initiated by the addition of 4-chloro-M-Cresol (4-CmC) (5 mM). Raw data for [Ca^{2+}] were mathematically fitted using monoexponential equations as previously described (Curve Fitting Toolbox version 1.1.1; The MathWorks) (33). Time for free [Ca^{2+}] to decrease by 63% of the initial free [Ca^{2+}] (τ) was calculated as $1/b$ from the equation; $y = ae^{-bt} + c$, where y is the free [Ca^{2+}], t is time, and a, b, and c are constants assigned from MATLAB. There were no differences in constant c (Nadir Ca^{2+}) between trials, time, or within same subject at various time points. SR Ca^{2+} release rate was obtained by mathematically fitting the data points during the first 30 s of release to the equation; $y = a[1 - e^{-b\,(t\,-\,c)}]$. This was back-extrapolated to Nadir [Ca^{2+}], and the rate of Ca^{2+} release was determined as the derivate of the initial release. Assays of Ca^{2+} uptake and release were performed in duplicates and values and expressed as arbitrary units; Ca^{2+}·g protein^{-1}·min^{-1}. Protein content in the muscle homogenate was measured in triplicates using a standard kit (Pierce BCA protein reagent no. 23225).

Statistics

The statistical analyses were carried out in STATA (STATA version 17; StataCorp, College Station, TX), and graphical illustrations were made in GraphPad Prism (GraphPad Software, Version 8, San Diego, CA). The sample size calculation was based on the primary outcome of the RCT: changes in myofibrillar fractional synthetic rate in response to LEA, which has previously been published (14). All data were visually inspected for normal distribution using QQ plots, and models were validated by inspecting standardized residuals against the fitted values. Test for differences between groups at baseline was performed using a Student's unpaired t-test. A linear mixed model was performed to evaluate the main effects and interactions between groups (LEA and OEA) and time (Pre vs Post, Pre vs Recovery), with group and time as fixed effects and participant as a random effect. *Post hoc* analysis of significant interactions was performed by multiple comparisons. The level of statistical significance was chosen as $P \leq 0.05$ (two-tailed). Change scores were calculated by subtracting post scores from pre scores. All values are presented as means ± SD unless otherwise specified.

RESULTS

All participants completed the planned training protocol without injuries (14), but two participants (OEA: n = 1, LEA: n = 1) did not complete the final day of testing (day 18).

Body composition. Changes from Pre to Post in body composition derived from DXA have previously been presented in Ref. (14). Scale-based body mass was reduced in LEA from Pre to Post by 1.8 kg (95% confidence interval (CI), 1.5–2.0 kg; $P < 0.001$) in comparison to OEA (group–time interaction, $P < 0.001$) who was weight stable (0.1 kg; 95% CI, −0.3 to 0.1 kg; $P = 0.475$). From Pre to Recovery, LEA decreased scale-based body mass by 1.4 kg (95% CI, 1.2–1.7 kg; $P < 0.001$), which was greater (group–time interaction, $P < 0.001$) than the 0.3-kg reduction in OEA (95% CI, 0.1–0.6 kg; $P = 0.015$). Body mass changed −0.3 kg; 95% CI, −0.1 to −0.6 kg from Baseline to Pre in both groups.

Isometric and dynamic muscle strength and rate of force development. No difference was observed between groups at Pre for maximal isometric knee extensor strength ($P = 0.365$). Maximal isometric knee extensor strength and isometric peak torque·kg^{-1} did not change from Pre to Post (both time: $P > 0.7$ and group–time interaction: $P > 0.2$). However, maximal isometric knee extensor strength and isometric peak torque·kg^{-1} increased from Pre to Recovery in both groups (time: $P = 0.008$, group–time interaction: $P = 0.649$; time: $P = 0.005$, group–time interaction: $P = 0.924$, respectively; Figs. 2A, B).

No difference was observed between groups at Pre for maximal isokinetic knee extensor strength ($P = 0.202$). Maximal isokinetic knee extensor strength demonstrated between-group differences from Pre to Post (time: $P = 0.114$, group–time interaction: $P = 0.031$), however, no differences were detected from Pre to Post in LEA (−3.1 N·m; 95% CI, −7.3 to 1.0 N·m; $P = 0.139$) and OEA (3.4 N·m; 95% CI, −0.8 to 7.5 N·m; $P = 0.114$; Fig. 2C). From Pre to Recovery, a trend for between group differences was observed (group–time interaction: $P = 0.053$), indicating that OEA increased maximal isokinetic strength (6.2 N·m; 95% CI, 1.5–10.9 N·m; $P = 0.010$), whereas LEA did not change (0.3 N·m; 95% CI, −4.5 to 5.0 N·m; $P = 0.913$; Fig. 2C). No change was observed when expressing isokinetic peak torque relative to body mass from Pre to Post (time: $P = 0.118$, group–time interaction: 0.213). However, from Pre to Recovery, isokinetic peak torque·kg^{-1} increased in both groups (time: $P = 0.005$, group–time interaction: $P = 0.173$; Fig. 2D).

RFD derived from maximal isometric contraction at 0–100 ms from Pre to Post showed no difference between groups (time: $P = 0.780$, group–time interaction: $P = 0.061$). However, from Pre to Recovery (group–time interaction: $P = 0.030$) RFD from 0 to 100 ms was reduced by 113 (N·m)·s^{-1} in LEA (95% CI, 29 to 197 (N·m)·s^{-1}; $P = 0.008$), in contrast to OEA who did not change (22 (N·m)·s^{-1}; 95% CI, −67 to 112 (N·m)·s^{-1}; $P = 0.621$; Fig. 2E). No change was observed when expressing RFD from 0 to 100 ms relative to body mass from Pre to Post (time: $P = 0.707$, group–time interaction: $P = 0.124$). However, from Pre to Recovery, LEA decreased RFD relative to body mass from 0 to 100 ms by 1.46 (N·m)·s^{-1}·kg^{-1} (95% CI, 0.13–2.79 (N·m)·s^{-1}·kg^{-1}; $P = 0.031$), in contrast to OEA who did not change (0.49 (N·m)·s^{-1}·kg^{-1}; 95% CI, −0.92 to 1.91 (N·m)·s^{-1}·kg^{-1}; $P = 0.494$; group–time interaction: $P = 0.048$; Fig. 2F). For RFD at 0–30, 0–50, and 0–200 ms, no differences between groups were found at any time point (see Supplemental Table 1, Supplemental Digital Content, Rate of force development (RFD) of the knee extentors, http://links.lww.com/MSS/C992).

Countermovement jump. CMJ height was not different between groups at Pre ($P = 0.536$). Jump height did not change from Pre to Post (time: $P = 0.236$, group–time interaction: $P = 0.522$) or Pre to Recovery (time: $P = 0.076$, group–time interaction: $P = 0.834$; Fig. 2G).

Repeated sprint ability. No difference between groups was observed at Pre in average power output (OEA: 551 W (95% CI, 503–599 W) vs LEA: 565 W (95% CI, 508–623 W), $P = 0.691$), mean peak power output (OEA: 611 W (95% CI, 561–660 W) vs LEA: 627 W (95% CI, 568–686 W), $P = 0.651$), and highest peak power output measured in the repeated sprint test (OEA: 634 W (95% CI, 581–687 W) vs LEA: 654 W (95% CI, 597–711 W), $P = 0.588$).

Average power and mean peak power output over the five sprints decreased in LEA from Pre to Post (29.2 W (95% CI, 13.1–45.3 W; $P < 0.001$) and 29.5 W (95% CI, 14.5–44.5 W; $P < 0.001$), respectively), in comparison to OEA (group–time interaction: $P = 0.002$, $P < 0.001$) who did not change: 7.2 W (95% CI, −9.4 to 23.9 W; $P = 0.395$) and 12.1 W (95% CI, −3.4 to 27.6 W; $P = 0.127$), respectively; Figs. 3A, B. Between-group differences were observed in peak power (group–time interaction, $P = 0.026$); however, no differences were detected from Pre to Post in LEA (−18.7 W; 95% CI, −38.7 to 1.2 W; $P = 0.066$) and OEA (13.9 W; 95% CI, −6.8 to 34.5 W; $P = 0.188$; Fig. 3C).

From Pre to Recovery, average power and peak power did not change (group–time interaction: $P = 0.176$, $P = 0.071$, respectively). In contrast, between-group differences were observed in mean peak power from Pre to Recovery (group–time interaction: $P = 0.045$); however, no differences were detected from Pre to Recovery in LEA (−10.9 W; 95% CI, −27.8 to 6.0 W; $P = 0.206$) and in OEA (12.7 W; 95% CI, −3.0 to 28.4 W; $P = 0.206$; Figs. 3A–C).

When expressing average power, mean peak power output, and peak power over the five sprints relative to body mass, trends were observed from Pre to Post in LEA for reductions in average power·kg^{-1} (group–time interaction: $P = 0.052$) and mean peak power·kg^{-1} (group–time interaction: $P = 0.018$), but no change in peak power·kg^{-1} (time: $P = 0.234$, group–time interaction: $P = 0.298$; Figs. 4A–C). From Pre to Recovery, no change was observed in average power·kg^{-1} (time: $P = 0.498$, group–time interaction: $P = 0.176$) and peak power·kg^{-1} (time: $P = 0.053$, group–time interaction: $P = 0.281$), whereas mean peak power·kg^{-1} increased in both groups (time: $P = 0.048$, group–time interaction: $P = 0.220$; Figs. 4A–C).

Four-minute time-trial performance. Average power output derived from the 4-min time trial was not different between groups at Pre (OEA: 209 W (95% CI, 193–224 W) vs LEA: 218 W (95% CI, 199–236 W), $P = 0.432$). Four-minute time-trail average power decreased in LEA from Pre to Post by 5.4 W (95% CI, 1.3–9.6 W; $P = 0.010$), in comparison to OEA who increased average power by 4.9 W (95% CI,

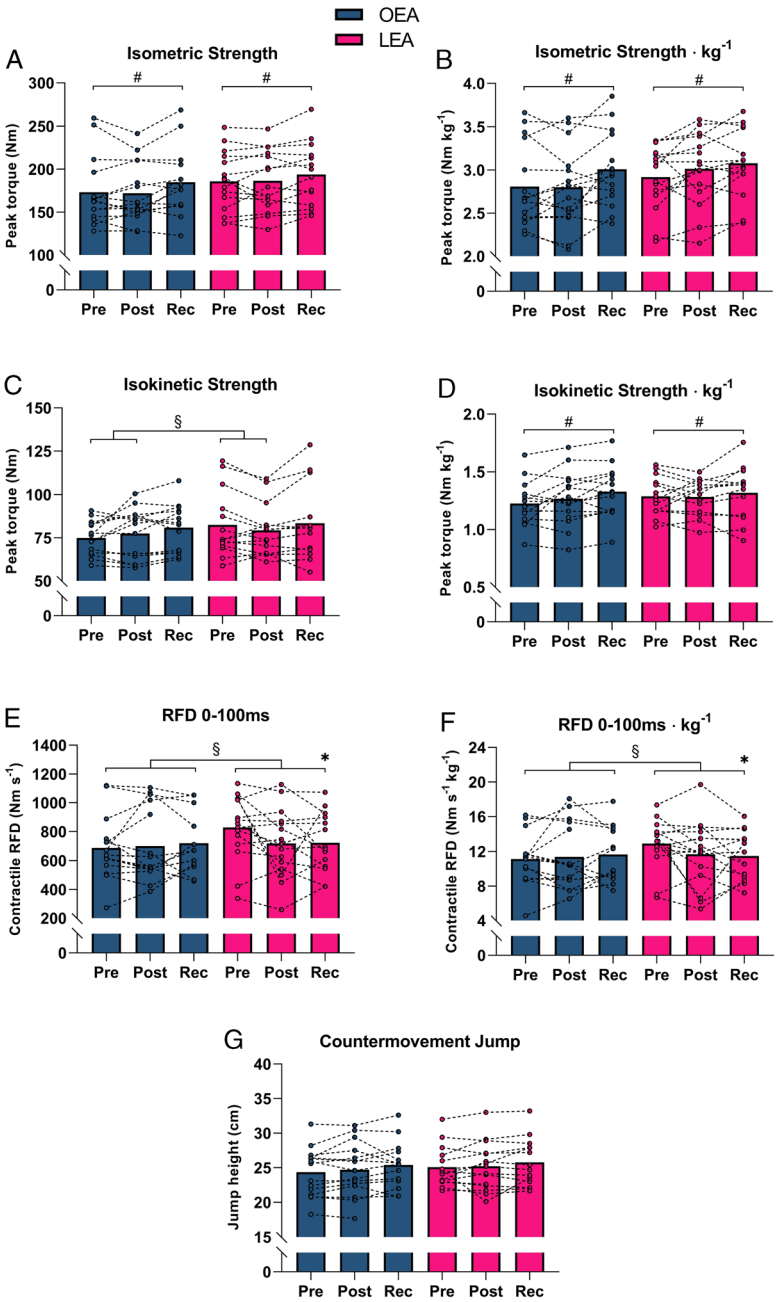

FIGURE 2—Changes in isometric strength (A), isometric strength relative to body mass (B), isokinetic strength (C), isokinetic strength relative to body mass (D), RFD from 0 to 100 ms (E), RFD from 0 to 100 ms relative to body mass (F), and CMJ (D) from before (Pre), after a 10-d period with either optimal (OEA) or LEA in combination with training (Post), and after 2 d of recovery with OEA (Rec). Data are presented as means with individual data points. #Effect of time $P < 0.05$; §$P < 0.05$ group–time interaction; *$P < 0.05$ from Pre.

0.7–9.0 W; $P = 0.021$; group–time interaction: $P = 0.001$). Furthermore, between-group differences were observed from Pre to Recovery (group–time interaction: $P = 0.028$), demonstrating that OEA increased average power by 8.2 W (95% CI, 4.3–12.1 W; $P < 0.001$), in comparison to LEA that did not change (2.0 W (95% CI, −1.83 to 5.9 W); $P = 0.302$; Fig. 3D). When expressing average power output relative to body mass, both groups increased from Pre to Post (time: $P = 0.018$, group–time interaction: $P = 0.104$), and from Pre to Recovery (time: $P < 0.001$, group–time interaction: $P = 0.290$; Fig. 4D).

RMR and thyroid hormones. Changes from Pre to Post in REE, TSH, and T_3 are presented in Ref. (14). From Pre to Recovery, REE was reduced in LEA by 75 kcal·d^{-1} (95% CI, 40.7–110.8 kcal·d^{-1}; $P < 0.001$), in comparison to OEA (group–time interaction: $P < 0.001$) that did not change (27.6 kcal; 95% CI, −7.5 to 62.6 kcal·d^{-1}; $P = 0.123$; Table 1). In contrast, no between-group differences were observed in TSH (time: $P = 0.915$, group–time interaction: $P = 0.718$) or T_3 (time: $P = 0.154$, group–time interaction: $P = 0.234$) from Pre to Recovery (Table 1).

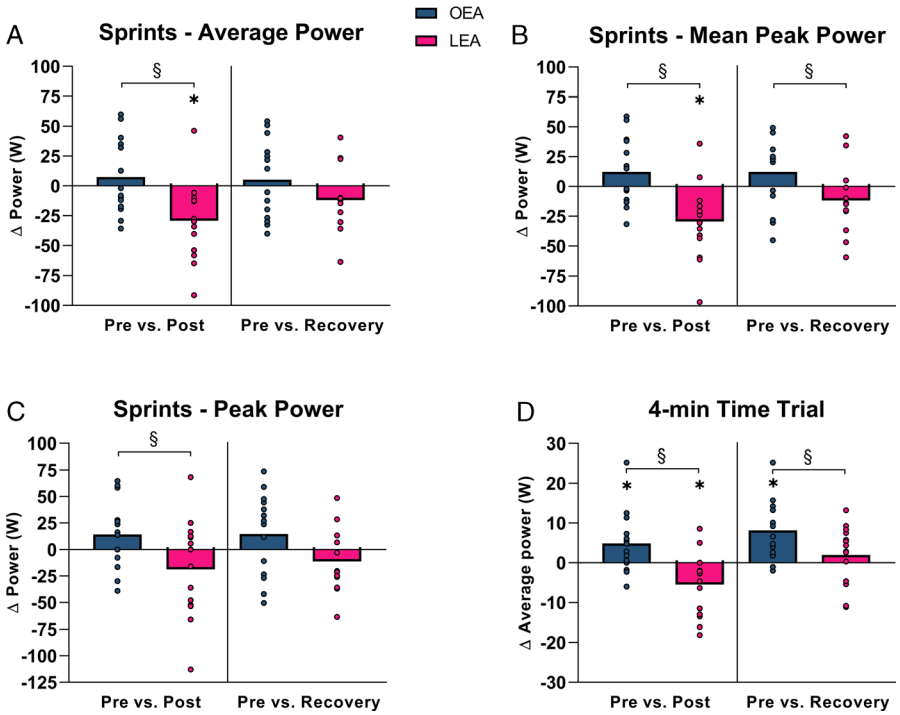

FIGURE 3—Changes in repeated sprint ability (Sprints) measured as average power (A), mean peak power (B), peak power (C), and 4-min time trail performance (D) from before (Pre), after a 10-d period with either optimal (OEA) or LEA in combination with training (Post), and after 2 d of recovery with OEA (Rec). Data are presented as means with individual data points. §$P < 0.05$ group–time interaction; *$P < 0.05$ from Pre.

Blood parameters related to the anabolic and catabolic status. Changes from Pre to Post in glucose, insulin, cortisol, cortisol/insulin ratio, testosterone, SHBG, and androgen index are presented in (14). Briefly, LEA resulted in reduced glucose, SHBG, and androgen index Pre to Post, whereas the cortisol/insulin ratio increased (14). From Pre to Recovery, no between-group differences were observed in glucose (time: $P = 0.659$, group–time interaction: $P = 0.316$), insulin (time:

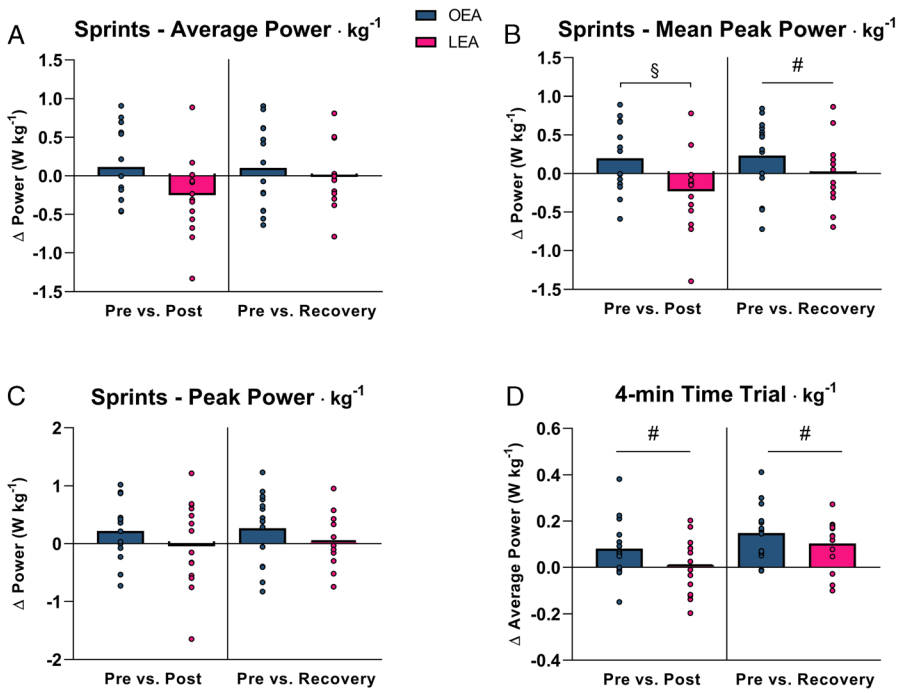

FIGURE 4—Changes relative to body mass in repeated sprint ability (Sprints) measured as average power (A), mean peak power (B), peak power (C), and 4-min time-trial performance (D) from before (Pre), after a 10-d period with either optimal (OEA) or LEA in combination with training (Post), and after 2 d of recovery with OEA (Rec). Data are presented as means with individual data points. #Effect of time $P < 0.05$; §$P < 0.05$ group–time interaction.

TABLE 1. Changes in RMR and blood parameters.

	Pre		Recovery	
	OEA	LEA	OEA	LEA
REE (kcal·d^{-1})	1423 ± 157	1536 ± 164	1463* ± 143	1482*,** ± 130
TSH (nMmol·L^{-1})	2.14 ± 0.8	2.00 ± 0.7	2.11 ± 0.9	1.85 ± 0.4
T_3 (nMmol·L^{-1})	1.58 ± 0.2	1.62 ± 0.3	1.50 ± 0.2	1.44 ± 0.3
Glucose (mMol·L^{-1})	4.82 ± 0.4	4.97 ± 0.3	4.84 ± 0.3	4.87 ± 0.3
Insulin (pMmol·L^{-1})	36.6 ± 12.0	31.3 ± 9.6	37.5 ± 20.0	30.8 ± 10.4
Cortisol (nMmol·L^{-1})	412 ± 72	387 ± 118	385 ± 48	377 ± 87
Cortisol/insulin ratio	12.6 ± 4.8	13.9 ± 7.2	12.0 ± 4.0	15.6 ± 12.0
Testosterone (nMmol·L^{-1})	1.10 ± 0.4	1.06 ± 0.3	1.06 ± 0.2	1.22 ± 0.4
SHBG (nMmol·L^{-1})	69.4 ± 25.8	69.1 ± 23.7	79.3*,** ± 33.4	97.4*,** ± 36.9
Androgen index	1.64 ± 0.4	1.64 ± 0.5	1.48 ± 0.5	1.37 ± 0.5

Values are presented as mean ± SD. Pre and Post comparison are presented in Ref. (11).
*P < 0.05 group–time interaction.
**P < 0.05 vs Pre.

P = 0.674, group–time interaction: P = 0.726), cortisol (time: P = 0.224, group–time interaction: P = 0.445), the cortisol/insulin ratio (time: P = 0.796, group–time interaction: P = 0.454), testosterone (time: P = 0.756, group–time interaction: P = 0.073), and androgen index (time: P = 0.126, group–time interaction: P = 0.546; Table 1). However, from Pre to Recovery, SHBG increased in both groups (LEA: 26.5 nmol·L^{-1} (95% CI, 17.2–35.8 nmol·L^{-1}), P < 0.001; OEA: 9.9 nmol·L^{-1} (95% CI, 0.9–18.8 nmol·L^{-1}), P = 0.031), but the increase was more pronounced in LEA (group–time interaction: P = 0.012; Table 1).

Muscle glycogen content. Muscle glycogen content was similar between groups at Pre (P = 0.204, Fig. 5A). Muscle glycogen content decreased in LEA from Pre to Post by 82 mmol·kg dry weight^{-1} (95% CI, −40 to −123 mmol·kg dry weight^{-1}; P < 0.001), in contrast to OEA who increased muscle glycogen content by 64 mmol·kg dry weight $^{-1}$ (95% CI, 24–105 mmol·kg dry weight^{-1}; P = 0.002; group–time interaction: P < 0.001).

Sarcoplasmic reticulum function. Ca^{2+} uptake and release rates were similar between groups at baseline (P = 0.673 and P = 0.247, respectively). Ca^{2+} uptake did not change from Pre to Post in both groups (time: P = 0.864, group–time interaction: P = 0.787; Fig. 5B). However, in OEA, Ca^{2+} release rate tended to increase from Pre to Post (P = 0.053), in comparison to the Ca^{2+} release rate in LEA that did not change (P = 0.424; group–time interaction: P = 0.048; Fig. 5C).

For the Ca^{2+} uptake and release rate analyses, two samples (OEA: n = 1, LEA: n = 1) were more than 3 SDs outside the mean. When these samples were excluded from the analyses, Ca^{2+} uptake remained unchanged from Pre to Post (time: P = 0.804, group–time interaction: P = 0.968). However, Ca^{2+} release rate did no longer reach statistical significance from Pre to Post (time: P = 0.179, group–time interaction: P = 0.119).

Training volume and training variables. Upper-body training volume did not change from the run-in period to the early intervention period (time: P = 0.085, group–time interaction: P = 0.953), yet lower-body training volume was increased in both groups (time: P < 0.001, group–time interaction: P = 0.144). From the run-in period to the late intervention period, both upper-body training volume and lower-body training volume increased (time: P < 0.001, group–time interaction: P = 0.549, time: P < 0.001, group–time interaction: P = 0.109, respectively; Table 2).

Average power output in the HIIT-session did not change from the run-in period to the early intervention period (time: P = 0.679, group–time interaction: P = 0.596). However, OEA increased average power output from the run-in period to the late intervention period (4.5 W; 95% CI, 1.9–7.2 W; P = 0.001), in comparison to the average power in LEA, which did not change (−0.3 W; 95% CI, −3.0 to 2.2 W; P = 0.802; group–time interaction: P = 0.010; Table 2). No between-group differences were observed for HIIT-RPE, HIIT-HR, and HIIT-Lactate (all, group–time interaction: P > 0.2; Table 2). Similarly, no between-group differences were observed for MIT-RPE and MIT-HR (both, group–time interaction: P > 0.3; Table 2).

Questionnaires. When comparing mean fatigue of the run-in period with the intervention period, no between-group differences were observed (time: P = 0.436, group–time interaction: P = 0.112; Fig. 6A).

When comparing the mean mental readiness of the run-in period with the intervention period, no between-group differences were observed (time: P = 0.397, group–time interaction: P = 0.303; Fig. 6B). When mental readiness before the performance testing on days 6 and 16 was evaluated, no between-group differences were observed from days 6 to 16 (time: P = 0.514, group–time interaction: P = 0.774). However, mental readiness increased in both groups from day 16 to day 18 (1.9; 95% CI, 0.9, 2.8; time: P < 0.001, group–time interaction: P = 0.224; Fig. 6C).

No between-group differences were observed when comparing session RPE over the run-in to the intervention period (time: P = 0.114, group–time interaction: P = 0.417; Fig. 6D).

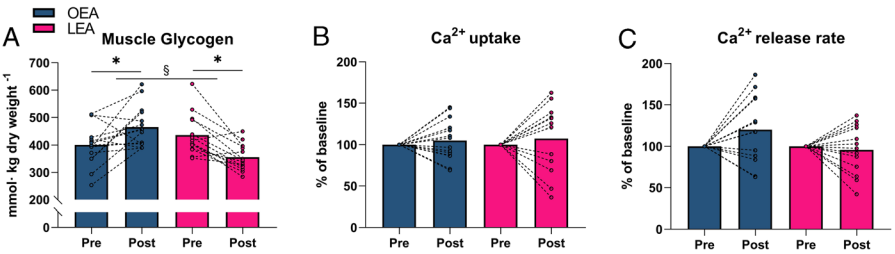

FIGURE 5—Changes in muscle glycogen content (A), sarcoplasmic reticulum Ca^{2+} uptake (B), and sarcoplasmic reticulum Ca^{2+} release rate (C), from before (Pre) and after a 10-d period with either optimal (OEA) or LEA in combination with training (Post). Data are presented as means with individual data points.

TABLE 2. Changes in accumulated training volume and training variables.

	Run-in		Early Intervention		Late Intervention	
	OEA	LEA	OEA	LEA	OEA	LEA
Upper body volume (kg)	2411 ± 456	2197 ± 436	2558* ± 501	2338* ± 532	2714* ± 527	2427* ± 501
Lower-body volume (kg)	4549 ± 1355	4678 ± 785	4986* ± 1289	4886* ± 722	5302* ± 1433	5196* ± 788
HIIT: power output (W)	207 ± 33	210 ± 30	206 ± 34	209 ± 30	212**,*** ± 33	209** ± 30
HIIT: RPE	17.6 ± 1.0	17.9 ± 0.8	17.3 ± 1.1	18.0 ± 0.8	17.5 ± 1.1	17.8 ± 1.2
HIIT: HR	173 ± 8	174 ± 9	170 ± 8	175 ± 9	172 ± 8	173 ± 9
HIIT: lactate	12.9 ± 2.2	12.9 ± 3.1	—	—	13.2 ± 1.4	13.0 ± 2.6
MIT: power output	145 ± 24	149 ± 18	145 ± 25	148 ± 20	145 ± 24	145 ± 19
MIT: RPE	13.6 ± 1.3	13.4 ± 1.5	14.0 ± 1.4	13.6 ± 1.2	14.0 ± 1.4	13.8 ± 1.8
MIT: HR	164 ± 9	163 ± 6	160 ± 8	162 ± 9	162 ± 7	164 ± 11

Values are presented as mean ± SD.
*Effect of time $P < 0.05$.
**$P < 0.05$ group–time interaction.
***$P < 0.05$ from Pre.
HIIT, high-intensity interval training; HR, heart rate; MIT, moderate-intensity training.

Energy and macronutrient intake. Compliance with the prescribed diet has been reported to be excellent in the run-in and intervention period (14). Similarly, compliance in the recovery period was 99.2% ± 1.5% for OEA and 97.8% ± 5.0% for LEA. The energy and macronutrient intake in the run-in period, intervention period, and recovery period are available in Supplemental Table 2 (Supplemental Digital Content, Energy and macronutrient intake, http://links.lww.com/MSS/C992). No differences between groups were observed in energy and macronutrient intake in the recovery period. In OEA, the mean daily energy and macronutrient intake were 2297 ± 297 kcal, 99 ± 12 g protein, 330 ± 42 g carbohydrate, and 59 ± 10 g fat. In LEA, the values were 2320 ± 281 kcal 102 ± 10 g protein, 334 ± 39 g carbohydrate, and 61 ± 11 g fat.

DISCUSSION

In the present study, we provide novel data on the effect of 10 d of LEA followed by 2 d of recovery with OEA on physical performance and physiological parameters in trained females. We found that 10 d of LEA resulted in a ~1.8-kg loss of body mass and impaired muscle function, repeated sprint ability, and 4-min time-trial performance (absolute values), with concomitant reductions in muscle glycogen content. Two days of recovery with OEA partially restored some of these impairments, but some aspects of physical performance remained inferior to the OEA group. Importantly, LEA had no greater effect (Pre vs Post and Pre vs Recovery) when physical performance was expressed relative to body mass. These findings highlight that LEA negatively affects physical performance, especially

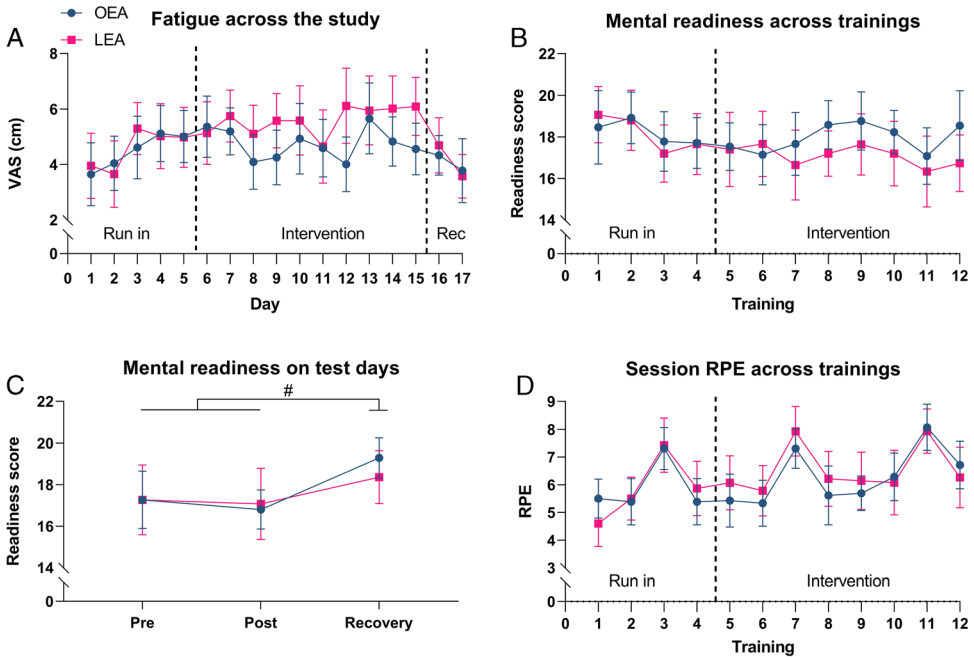

FIGURE 6—Changes in self-perceived fatigue across the study (days 1–17) (A), mental readiness across the training sessions (trainings 1–12) (B) and performance test days (days 6, 16, and 18) (C), and session RPE across training sessions (trainings 1–12) (D). Data are presented as means with 95% CI. #Effect of time $P < 0.05$.

if a period of LEA is not followed by a deliberate recovery period with OEA. Our data challenge the common thesis that a reduction in body mass always will lead to an improved power-to-mass ratio and thus increased physical performance.

Impact of LEA on physical performance. There is currently a lack of high-quality research investigating the impact of LEA on physical performance, particularly in females (17). We provide compelling evidence that 10 d of LEA impaired absolute values in physical performance. Specifically, we found impairments of RFD from 0 to 100 ms, repeated sprint peak and average power, and 4-min time-trial performance, whereas no change was observed in maximal isometric strength and CMJ height. These findings are supported by previous observational data in female athletes reporting associations between LEA (amenorrheic vs eumenorrheic athletes) and reduced dynamic strength (34). In addition, prospective data have reported associations between reduced EA (based on dietary records) and reduced explosive power (indicative of CMJ performance) after a 5-d intensive training camp in cross-country skiers (35) and diminished 400-m swimming performance after a 12-wk training season in junior elite swimmers with ovarian suppression (36). To the best of our knowledge, the present study is the first RCT to report the causal effects of LEA on physical performance in females.

Previous reports in male long-distance runners have shown that 3 d of exposure to LEA (~19 kcal·kg $FFM^{-1}\cdot d^{-1}$) compared with OEA (~53 kcal·kg $FFM^{-1}\cdot d^{-1}$) did not impact time to exhaustion at 90% $\dot{V}O_{2max}$ (15). Nevertheless, in well-trained male cyclists, 14 d of LEA (based on dietary records) at three different levels (~22, ~17, and ~9 kcal·kg $FFM^{-1}\cdot d^{-1}$) resulted in reduced explosive power (CMJ) and power output on a bike ergometer (37). A recent elegantly conducted study in highly trained race walkers (19 males and 3 females) used an exposure time of LEA comparable to the present study (9 vs 10 d), but different EA levels (15 and 40 vs 25 and 50 kcal·kg $FFM^{-1}\cdot d^{-1}$). They also included a short recovery with refueling (24 vs 48 h in the present study) (18). Their findings showed that, despite an increased perception of fatigue and a loss of training quality, that following 24-h refueling, the LEA group was able to improve 10-km race performance equal to the group receiving a diet with higher EA (40 kcal·kg $FFM^{-1}\cdot d^{-1}$) and carbohydrate availability. In our study, 10 d of LEA (25 kcal·kg $FFM^{-1}\cdot d^{-1}$) reduced absolute values in both repeated sprint ability and 4-min time-trial performance after LEA, and these impairments were still inferior to OEA after 2 d of recovery with refueling. The small discrepancies between studies may be explained by the differences in performance test modality (power walking vs cycling), intensity/duration (aerobic based vs anaerobic based), and/or the level of athlete (elite vs subelite). However, considering the proposed role of sex in the response to LEA (22), it is possible that the more pronounced performance reductions observed in our study are a result of greater sensitivity to LEA in females compared with males.

Manipulating body composition through reduced EA exposure to improve the power-to-mass ratio is a well-documented practice among endurance athletes (1,2). Nevertheless, despite its common practice close to competition or as a periodized approach across the season, there is a lack of causal evidence to support its effectiveness in optimizing performance. Some case studies (19) and prospective studies (38) in elite endurance athletes demonstrate that weight cycling may be undertaken successfully to achieve an optimal physique for race performance. However, our findings demonstrating no superior effect on performance outcomes normalized to body mass after 10 d of LEA and 2 d of recovery with refueling in trained females challenge this practice, at least in subelite athletes. Our findings are supported by the recent study by Burke et al. (18), demonstrating that 10-km race performance in male race walkers was improved equally and independently of a ~1.1-kg group difference in weight change. Together, these findings show that the relationship between body composition and performance is complex and that performance improvements achieved through a weight loss of ~1–2 kg may be smaller than anticipated. Consequently, the increased risk of athletes experiencing problematic LEA, associated with impaired muscle protein synthesis, and increased risk of injury, overtraining syndrome, and disordered eating behavior (3,14,39), may outweigh any potential performance benefit. Athletes and coaches should be mindful of these risks and acknowledge the limited performance improvement that they may gain. Accordingly, body mass manipulation should not be prioritized before other performance optimization strategies such as sleep, diet composition, and training programming are already in place, and if undertaken, it should be carefully planned and supervised to reduce the risks involved.

Mechanisms underpinning the effects of LEA on physical performance. We measured various physiological variables to elucidate the underlying mechanisms responsible for the observed impairments in muscle function, repeated sprint ability, and 4-min time-trial performance (absolute values) after LEA. One of the most physiologically compelling mechanisms is changes in carbohydrate availability, the primary fuel during strenuous exercise. Not surprisingly, we found a ~0.5 mM reduction in fasting blood glucose and an ~82 mmol·kg dry weight $^{-1}$ reduction in muscle glycogen content after LEA. Maintaining stable blood glucose levels during exercise is critical to counteract exercise-induced CNS fatigue (40). However, the reduction observed in blood glucose is not considered to have impacted performance, based on the premise that endogenous glucose production was sufficient to maintain blood glucose homeostasis above critically low levels (40). In contrast, muscle glycogen content is critical for single and repeated high-intensity exercise tolerance, where maximal rates of glycogenolysis are required (41). High-intensity physical performance is consistently impaired when muscle glycogen content (based on muscle homogenates) is reduced below a critical threshold, which seems apparent at around ~250 to 300 mmol·kg dry $weight^{-1}$ (41). In the present study, most females approached this threshold after LEA, but none were below it. Thus, mechanisms other than muscle glycogen reduction appear responsible for the reductions seen in LEA in most

performance test. However, the threshold is based on studies in males, and we cannot exclude the possibility that the threshold may differ in females. Apart from providing substrate for glycolysis, muscle glycogen has been linked to changes in muscle Ca^{2+} handling, NA^{+}-K^{+}-ATPase activity, and myofibrillar function, all of which may contribute to impairments of the excitation–contraction coupling (41). Accordingly, we carried out *in vitro* analysis of sarcoplasmic reticulum function to investigate the effect of LEA on muscle Ca^{2+} handling kinetics. Here, we found no changes with LEA but increased Ca^{2+} release rate in OEA. Previous research has shown a link between reduced Ca^{2+} release rate from the sarcoplasmic reticulum and reduced muscle glycogen content and suggested that the reduction in Ca^{2+} release contributed to the observed reduction in peak power output during repeated high-intensity exercise in elite endurance athletes (33). Thus, we speculate that the changes observed in Ca^{2+} release rate with OEA may be linked to the higher muscle glycogen content after the intervention period. However, our data indicate no direct effect of LEA on sarcoplasmic reticulum function, at least when measured in the rested state.

Another possible mechanism driving performance impairments during LEA could be the loss of muscle mass. Muscle mass is a strong predictor of strength and power, given that the amount of muscle one carries is linked to the amount of contractile tissue available and thus the muscles' contractile capacity (42). In the present study, we observed a 1.8-kg body mass loss after LEA, which was attributed to a loss of ~0.4 kg of lean mass, whereas OEA, in contrast, gained ~0.4 kg of lean mass (14). Furthermore, as we have shown previously, this reduction in lean mass was linked to reductions in daily integrated myofibrillar and sarcoplasmic muscle protein synthesis, despite resistance exercise and high dietary protein available to stimulate anabolic processes (14). However, we recognize that a change in DXA-derived lean mass is a proxy measure for changes in muscle mass and also includes other tissues such as organs (43). Furthermore, the measure of lean mass is influenced by factors such as hydration status and intramuscular fluid stores (i.e., muscle glycogen) (44). In perspective to physical performance, we believe that the loss of lean mass observed in the present study is small enough to only trivially affect physical performance. However, it is plausible that a greater loss of lean mass, due to a more severe or longer period of LEA, or the absence of resistance exercise and sufficient dietary protein to stimulate anabolism may negatively impact physical performance. Importantly, the loss of lean mass cannot be reversed within days like energy substrate stores and may have longer-term consequences for physical performance.

A final mechanism that may have affected physical performance is changes in psychological parameters. Previous studies have reported associations between LEA and mood disturbances such as anger, confusion, cognitive restriction, and tension in athletes (17,45,46). Furthermore, in the recent investigation by Burke et al. (18), self-reports from the RESTQ-Sport-76, a validated athlete self-report measuring tool, showed marked increases in stress and fatigue and reductions in recovery at the end of the training camp in the LEA group. In the present study, we observed no significant change in fatigue during LEA. We expect that the lack of significant findings regarding fatigue may be explained by the methodology used or sex differences compared with the study by Burke et al. (18). We did not use validated questionnaires in our data collection. Although these questionnaires were quick and feasible to complete for our participants, their sensitivity is likely lower than validated questionnaires such as the RESTQ-Sport-76. Thus, we acknowledge that LEA, in many cases, results in a change in psychological parameters and that these changes may contribute to impaired physical performance. In this context, it is worth noting that physical performance outcomes are affected by an individual's drive to perform, which can be difficult to control in laboratory-based studies. To tackle this issue, sports performance can be assessed in an authentic manner in a real-world setting, such as a simulated competitive event (18). However, this setup demands significant resources, making it quite challenging to do.

We propose that the mechanism(s) leading to performance impairments during LEA are likely multifactorial, including changes in energy substrate reserves, body composition, and psychological parameters. It is possible that an equal loss of body mass achieved over an extended period with less drastic reductions in EA could mitigate the impact of lowered EA on physical performance (47). Such an approach could increase carbohydrate availability to increase fuel stores, increase protein intake to limit lean mass loss, and possibly counteract changes in psychological parameters. In this perspective, it is relevant to discuss the feasibility of undertaking an aggressive weight loss (approximately 1.5–2 kg) over a short period, as in the present and previous studies (18). Although this practice has been documented among athletes (19,48), the aggressive reduction in EA may result in acute fatigue, and feelings of being overwhelmed or demotivated for performing intense exercise. Therefore, for some athletes, a less aggressive reduction in EA over a prolonged period may be easier and still achieve comparable weight loss. However, this approach may be challenging in real-world sports settings, where time is valuable, and an extended period of weight loss may interfere with a periodized training plan. Future studies are needed to establish best practice guidelines for body mass manipulation with a focus on feasibility and reducing the risks associated with problematic LEA (3).

Recovery of physiological parameters after LEA. Despite the high reported prevalence of LEA in athletic populations, the timeline of recovery from a period of LEA has received very little attention from the scientific community. A previous study showed a lack of restoration of T_3 levels and only partially restored LH pulsatility after 5 d of LEA (~10 kcal·kg $FFM^{-1}\cdot d^{-1}$) and 1 d of aggressive refeeding (~77 kcal·kg $FFM^{-1}\cdot d^{-1}$) (16). In the present study, we observed reductions in REE, TSH, T_3, glucose, androgen index, and increased cortisol/insulin ratio and SHBG after 10 d of LEA (14). However, after undergoing 2 d of recovery with

OEA, all markers except SHBG and REE returned to their prevalues without any significant difference. This observation could be a manifestation of the new term adaptable LEA, which is presented in the new 2023 IOC statement (3). Adaptable LEA describes the exposure to a reduction in EA that has benign effects, including mild and quickly reversible changes in the biomarkers of various body systems. These changes signal an adaptive partitioning of energy and the plasticity of human physiology (3). Therefore, our findings may illustrate a situation of adaptable LEA, where the changes observed in different physiological systems in healthy eumenorrheic females after 10 d of LEA are quickly reversible after 2 d of refueling, at least in the context of the blood biomarkers measured. In contrast, studies on females with problematic LEA indicate that recovery from clinical symptoms such as amenorrhea/oligomenorrhea takes much longer (49). In a 12-month RCT, De Souza et al. (49) reported that a modest increase in energy intake of 330 $kcal \cdot d^{-1}$ in exercising women enabled a large majority to recover menses by month 6 of the intervention and that T_3 levels increased but were not restored until the end of the 12-month intervention period (49).

Taken together, these findings illustrate that exposure to 10 d of LEA in eumenorrheic females results in the alteration of physiological systems. However, these changes are reversed by 2 d of recovery with OEA, showcasing a situation of adaptable LEA. On the other hand, if females already exhibit clinical symptoms of problematic LEA, such as amenorrhea/oligomenorrhea, the recovery time for metabolic and hormonal systems may be significantly prolonged. Consequently, we strongly emphasize that if coaches and athletes intend to undertake a well-planned period of LEA, a screening assessment of LEA symptoms is needed; otherwise, the risk of problematic LEA may increase substantially.

Strength, limitations, and future directions. We conducted a rigorously controlled RCT, which enabled us to minimize confounding factors, such as diet and exercise training variations. However, we acknowledge that findings from laboratory-based studies may not be directly extrapolated to real-life athlete scenarios (50). Moreover, although we used fixed energy targets regarding EA to gain insights into the physiological response to those targets, this scenario is rarely encountered in free-living situations where EA can vary on a day-to-day basis and during the day because of significant differences in training volume and timing of energy intake relative to training sessions (50,51).

It is important to acknowledge that our study shows the physiological response to LEA in trained females. Therefore, the findings may not necessarily apply to elite female athletes with greater training volumes and lower body fat. Therefore, we strongly encourage future studies to investigate the causal effects of LEA in groups of elite athletes within real-world settings while maintaining high-quality methodology and incorporating sport-specific testing. These studies could help uncover the risks and possible benefits of LEA within athletic populations that are most susceptible to this issue. Conducting studies of this nature may help eliminate potential "training effects" that can arise when study participants are not well accustomed to a specific test, despite prior efforts to familiarize them. In fact, the observed improvements in isometric knee extensor strength and isokinetic peak torque from Pre to Recovery could possibly be attributed to a training effect in the present study. Therefore, even though we carried out a thorough familiarization procedure, we cannot disregard the possibility that the "true" impact of LEA on other performance parameters might have been concealed by this training effect.

We also acknowledge that our physical performance test battery focused on shorter-term performance outcomes, which limits the interpretation of our results to longer-term endurance performance. Therefore, we recommend that future studies investigate the effects of LEA on longer-term endurance performance in females, with a focus on weight-bearing disciplines.

CONCLUSIONS

We found that 10 d of LEA in trained females resulted in impaired muscle function, repeated sprint ability, and 4-min time-trial performance (absolute values), with concomitant reductions in muscle glycogen. Two days of recovery with OEA only partially restored these impairments, although some measures of physical performance (absolute values) remained inferior to being in OEA the whole period. Importantly, when performance outcomes were normalized to body mass, no superior effect was observed after a period of LEA. Together, these results challenge the common perception that a small reduction in body mass will lead to an improved power-to-mass ratio and thus increased physical performance.

We want to express our gratitude to all the participants who spent time and dedication on the project. Thanks to Janni Moesgaard Jensen and Gitte Kaiser Hartvigsen for your technical assistance. Thanks to Søren Skriver, Jeppe Jensen, Mathias Jessen, Søren Petersen, Stine Mathiasen, Sarah Stougaard, and Josephine Pora for your assistance with the training intervention.

S. M. P. reports grants or research contracts from the US National Dairy Council, Canadian Institutes for Health Research, Dairy Farmers of Canada, Roquette Freres, Ontario Centre of Innovation, Nestle Health Sciences, Myos, National Science and Engineering Research Council, Friesland Campina, and the US National Institutes of Health during the conduct of the study; personal fees from Nestle Health Sciences, nonfinancial support from Enhanced Recovery, outside the submitted work. S. M. P. has patents licensed to Exerkine but reports no financial gains from patents or related work.

The Faculty of Health, Aarhus University, The Danish Ministry of Culture, and Team Danmark (the Danish national association for elite athletes) financially supported the study. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

Data availability: The data that support the findings of the present study are available from the corresponding author upon reasonable request.

Author contributions: Conceptualization and design: M. O., M. H., P. M. C., A. K. M., S. M. P., J. S. J., Y. H.; Funding acquisition: M. O., M. H.; Data acquisition: M. O., D. M., O. E. A., F. T. J., M. B., J. R.; Formal analysis: M. O., D. M., N. Ø.; Original draft: M. O., M. H.; Review and editing: M. O., D. M., P. M. C., O. E. A., F. T. J., M. B., J. R., J. S. J., Y. H., S. M. P., A. K. M., NØ, M. H.

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