

# The Impact of Low Energy Availability on Nonexercise Activity Thermogenesis and Physical Activity Behavior in Recreationally Trained Adults

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Energy availability describes the amount of dietary energy remaining for physiological functionality after the energy cost of exercise is deducted. The physiological and hormonal consequences of low energy availability (LEA) are well established, but the impact of LEA on physical activity behavior outside of exercise and, specifically, nonexercise activity thermogenesis (NEAT) has not been systematically examined. The authors conducted a secondary analysis of a repeated-measures crossover study in which recreationally trained young men ( $n=6$ ,  $25 \pm 1.0$  years) underwent two 4-day conditions of LEA ( $15 \text{ kcal} \cdot \text{kg fat-free mass}^{-1} \cdot \text{day}^{-1}$ ) with and without endurance exercise (LEA + EX and LEA EX) and two energy-balanced control conditions (CON + EX and CON EX). The duration and intensity of physical activity outside of prescribed exercise were assessed using the SenseWear Pro3 armband. LEA did not alter NEAT ( $p = .41$ ), nor time spent in moderate to vigorous ( $p = .20$ ) and low-intensity physical activity ( $p = .17$ ). However, time spent in low-intensity physical activity was lower in LEA + EX than LEA – EX ( $13.7 \pm 0.3$  vs.  $15.2 \pm 0.3 \text{ hr/day}$ ;  $p = .002$ ). Short-term LEA does not seem to impact NEAT per se, but the way it is attained may impact physical activity behavior outside of exercise. As the participants expended similar amounts of energy during NEAT ( $900\text{--}1,300 \text{ kcal/day} = 12.5\text{--}18.0 \text{ kcal} \cdot \text{kg fat-free mass}^{-1} \cdot \text{day}^{-1}$ ) and prescribed exercise bouts ( $15.0 \text{ kcal} \cdot \text{kg fat-free mass}^{-1} \cdot \text{day}^{-1}$ ), excluding it as a component of energy expenditure may skew the true energy available for physiological functionality in active populations.

**Keywords:** behavioral compensation, low-intensity physical activity, MVPA

Low energy availability (LEA) is considered the key etiological factor for the closely related concepts of the female and male athlete triad (De Souza et al., 2014; Tenforde et al., 2016) and the relative energy deficiency in sport syndrome (Mountjoy et al., 2018). LEA, which can occur as a result of high exercise energy expenditure (ExEE), low dietary energy intake (EI), or a combination of both, has been linked with acute hormonal, metabolic, and physiological dysregulations (De Souza et al., 2014; Mountjoy et al., 2014) that can lead to deleterious systemic consequences for long-term reproductive (Loucks & Heath, 1994; Loucks & Thuma, 2003) and bone health (Ihle & Loucks, 2004; Papageorgiou et al., 2017).

Energy availability (EA) is a concept that quantifies the energy available for sustained physiological functionality (Areta et al., 2020) after subtracting ExEE from EI. Unlike energy balance, which incorporates all components of total daily energy expenditure (TDEE), the EA concept only accounts for ExEE (Areta et al.,

2020), thereby excluding energy expended during any physical activity outside of organized exercise. Per definition, this exclusion eliminates the contribution of nonexercise activity thermogenesis (NEAT; Levine et al., 1999), with its most impactful expenditure originating from time spent in activities of daily living that contribute to moderate to vigorous physical activity (MVPA; cycling to work, stair climbing, etc.) or low-intensity physical activity (LPA; walking around the office, preparing food, etc.).

The elimination of NEAT simplifies the calculation of EA and may occur because LEA studies are conducted in trained individuals, with the focus on whether the remaining energy available is enough to prevent the commonly observed physiological disturbances in both female (Loucks & Horvath, 1985) and male athletes (Tenforde et al., 2016) in response to high training loads. It is postulated that high training loads, which increase ExEE and leave less time available for NEAT, make TDEE less susceptible to fluctuations in NEAT (Areta et al., 2020). However, this assumption negates that, from an energetic standpoint, there is no difference whether energy is expended during exercise or during activities of daily living, particularly during activities that can be classified as either (e.g., cycling; Torstveit et al., 2018), nor that there remains a substantial time each day wherein NEAT can occur (Sperlich et al., 2017). Furthermore, its exclusion ignores that NEAT can vary by up to approximately 2,000 kcal/day between two individuals of the same size (Levine, 2007). Therefore, eliminating NEAT as a potential contributor to the energy expenditure portion of the EA

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calculation, particularly when it occurs during MVPA (Guebels et al., 2014), may skew the true amount of energy available for normal physiological functionality.

NEAT has previously been shown to respond to changes in energy status (Levine et al., 1999), as demonstrated by the compensatory reduction in NEAT during calorie-restricted weight loss (Hunter et al., 2015; Verdich et al., 2001). However, no prospective studies to our knowledge have examined the impact of LEA on NEAT outside of training thus far. Therefore, the primary purpose of the present study was to determine whether LEA, when compared with EA at levels required for normal physiological functionality, alters NEAT and, more specifically, time spent in MVPA and LPA. Our secondary goal was to determine whether NEAT is impacted differently when LEA is achieved solely by a reduction in EI or in combination with increased ExEE. **We hypothesized that NEAT would decrease in response to LEA attained by calorie restriction alone and, to a lesser degree, when combined with exercise.** As an exploratory goal, we further sought to determine whether LEA alters the subjective perception of energy status.

## Methods

### Study Design

To address the study aims, we conducted a secondary analysis of a previously published, randomized controlled trial on the impact of LEA on hormonal markers (Koehler et al., 2016a). Using a repeated-measures four-way crossover design, the study involved two LEA conditions at 15 kcal/kg fat-free mass (FFM) and two energy-balanced control conditions (CON) at 40 kcal/kg FFM. The reduction in EA to 15 kcal/kg FFM was chosen based on previous experiments demonstrating metabolic and hormonal adaptations during short-term interventions (Hilton & Loucks, 2000; Ihle & Loucks, 2004; Loucks & Thuma, 2003). The participants performed supervised exercise during one LEA (LEA + EX) and one CON (CON + EX) condition. No exercise was performed during the other conditions (LEA – EX and CON – EX). The experimental sequence was randomized, with each condition lasting 4 days, starting Monday and ending Friday. Between conditions, the participants completed washout periods of ad libitum diet and exercise. A washout period of at least 10 days was installed to recover tissue losses following LEA conditions. As no weight loss was expected during CON, a washout period of at least 4 days was deemed sufficient after the CON conditions. The study was approved by the ethical committee of the German Sport University Cologne (Koehler et al., 2016a) and was in accordance with the Declaration of Helsinki.

### Participants

Physically active and recreationally trained healthy men were recruited from the local campus community using the following inclusion criteria: male, 18–30 years of age, at least 3 hr/week of purposeful endurance exercise, normal weight (body mass index between 19 and 25 kg/m<sup>2</sup>), and stable body weight ( $\pm 3$  kg) during the past 6 months. Volunteers were excluded if they smoked; used any medication that could interfere with study outcomes; had a recent infectious, cardiovascular, metabolic disease or orthopedic impairment that prohibited exercise; or had a history of a clinical eating disorder. All participants provided written informed consent prior to the study enrollment.

### Diet Prescriptions

During each condition, dietary EI was manipulated depending on the expected ExEE to achieve target EA levels of 15 or 40 kcal/kg FFM<sup>-1</sup>·day<sup>-1</sup>. To achieve the required EI, the participants were given a detailed meal plan, listing the amount and types of foods they were permitted to eat during each condition (Koehler et al., 2016a). Meal plans were developed individually based on each participant's dietary habits and food preferences, which were assessed during an initial dietary interview with an experienced nutritionist prior to the study start. EI was distributed across four to six meals and snacks per day, depending on the participants' habitual meal and snack patterns. Meal sizes were adjusted so that the daily EI matched prescriptions for each condition. Efforts were made to meet the macronutrient composition recommendations of 50–55% carbohydrates, 30–35% fat, and 10–15% protein. All participants were trained to weigh all food and leftovers and report any deviation from their prescribed diet. Dietary EI was analyzed daily, using EBIS (2005, version 7.0; University of Hohenheim, Stuttgart, Germany). If the actual EI differed from the prescribed EI by  $\pm 50$  kcal on any day, the meal plans for the remaining condition days were adjusted so that the target EI for the condition was attained.

### Exercise Prescriptions

Aerobic fitness, assessed as peak oxygen uptake, was determined during an incremental bicycle ergometer test at a preliminary visit, as described previously (Koehler et al., 2016a). During both exercising conditions, the participants exercised on a cycle ergometer (SRM GmbH, Jülich, Germany) at the workload equivalent of 60% of their peak oxygen uptake until the target ExEE of 15 kcal/kg FFM<sup>-1</sup>·day<sup>-1</sup> was reached (Koehler et al., 2016a). The participants were instructed to not conduct any additional purposeful exercise outside of the supervised exercise sessions.

### Physical Activity Assessment

Physical activity was assessed throughout the study employing a multisensor armband (SenseWear Pro3; BodyMedia, Pittsburgh, PA), which enabled a direct observation of time spent in different intensities of physical activity and quantification of overall NEAT (Arvidsson et al., 2019). The armband has been previously validated against doubly labeled water for energy expenditure (intraclass correlation coefficient [ICC]: .81–.89; St-Onge et al., 2007) and against the Intelligent Device for Estimating Energy Expenditure and Activity monitor for time spent engaging in physical activity (ICC: .90; Welk et al., 2007).

The participants wore the armband per manufacturer instructions on the right arm over the triceps brachii and were instructed to wear it continuously throughout each condition, removing it only while showering and bathing. After each condition, the armband data were downloaded and analyzed using SenseWear Professional software (version 8.0; BodyMedia). The armband data were considered incomplete if the wear time was <90% on any condition day. Incomplete days were rejected and the remaining days were included in the analysis as long as the data from at least two conditions days (50% of the condition length) were available. Otherwise, the condition was rejected for further analysis.

Using the manufacturer algorithm, data were analyzed to quantify the time spent in different activity categories (MVPA, LPA, and sleep), NEAT, overall physical activity, and steps. In agreement with our focus on physical activity outside of exercise,

the data collected during the supervised exercise were eliminated before analysis. The time spent in MVPA was defined as activities with a metabolic equivalent (MET)  $\geq 3$ , while the time spent in LPA was defined as activities with a MET  $< 3$  (US Department of Health and Human Services, 2008), excluding sleep. In alignment with the current EA calculations (Areta et al., 2020), NEAT was quantified as active energy expenditure outside of the prescribed exercise bouts that exceeded resting energy expenditure (REE). REE was predicted using the Cunningham equation (Cunningham, 1991), which predicts REE most accurately in exercising individuals (Thompson & Manore, 1996).

Time spent in MVPA, LPA, sleep, and prescribed exercise was expressed in the percentage of total wear time, and overall physical activity was expressed in METs. Total NEAT and its subdivisions, overall physical activity, and daily steps were expressed as raw values and were adjusted for hours of nonexercise wear time and upright time. The adjustment for nonexercise wear time was made because of the presence of prescribed exercise in only two of the four conditions (LEA + EX and CON + EX), which resulted in different nonexercise wear times. The adjustment for time spent in an upright position was made to represent the time when physical activity is energetically relevant and when nonexercise activity contributes to TDEE (Amaro-Gahete et al., 2019). Adjustments were made by multiplying raw data by the quotient of total condition length and nonexercise wear time or upright time, respectively.

## Body Weight and Body Composition

Body weight and body composition were estimated using a bioimpedance scale (Tanita BC 418 MA; Tanita, Amsterdam, The Netherlands) following an overnight fast of at least 10 hr at baseline, at the beginning of each condition (Day 1), and upon completion (Day 5). The participants were instructed to hydrate adequately, and hydration status was assessed upon arrival in the lab by determining urine specific gravity (Koehler et al., 2016a).

## Subjective Perception of Energy Status

At the beginning (Day 1) and end (Day 5) of each condition, the participants completed electronic surveys to assess their perceived physical state, as described previously (Schneider et al., 2009), in the morning while fasted and before any exercise. The participants were asked to indicate how well 32 adjectives matched their current state on a scale from 0 (*not at all*) to 5 (*completely*), with 0.5 increments. The time allowed for each question was 5 s, to discourage rational deliberation. The present analysis only includes the subdimension of physical energy (physical fitness, health, recovery, flexibility, and positive mood). The survey was provided

in German, and the variables were translated to English for this publication.

## Statistical Analysis

Statistical analyses were performed using R (version 3.3.2; The R Foundation for Statistical Computing, Vienna, Austria). The data are reported as mean  $\pm$  standard error of the mean (SEM). To account for the repeated-measures crossover design, linear mixed-model analyses were used to identify the differences in study outcomes as a result of LEA and exercise (main effects) and the interaction between LEA and exercise. When main or interaction effects were detected ( $p < .1$ ), post hoc analyses were conducted using paired  $t$  tests or Wilcoxon signed-rank tests based on normality, which was assessed using the Shapiro–Wilk’s test. Significance was set at  $p < .05$  and was adjusted for multiple testing using Bonferroni correction. **Post hoc sensitivity analyses revealed that this secondary analysis was powered to detect an effect size of 0.85 with a power of 0.8 as significant.** Using the pooled  $SD$  for the nonexercise conditions, this would translate into differences of 248 kcal/day for NEAT and 2.6% or approximately 38 min/day for MVPA and LPA.

## Results

All six participants initially enrolled in the study completed all study conditions. At baseline, the participants were  $25.2 \pm 1.0$  years old, with a body mass index of  $23.5 \pm 0.6$  kg/m<sup>2</sup> and a peak oxygen uptake of  $49.3 \pm 2.4$  ml·kg<sup>-1</sup>·min<sup>-1</sup>. All supervised exercise sessions were completed as prescribed, resulting in an ExEE of 15.0 kcal/kg FFM during both exercise conditions (Table 1). After subtracting ExEE from the reported dietary EI, the actual EA did not differ between LEA ( $p = .31$ ) or CON conditions ( $p = .56$ ). Both LEA conditions resulted in significant weight loss (LEA + EX:  $-1.8 \pm 0.4$  kg; LEA – EX:  $-2.4 \pm 0.3$  kg; both  $p < .001$ ; see [Supplementary Table 1](#) [available online]). Changes in body composition failed to achieve statistical significance in LEA – EX (fat mass:  $-0.7 \pm 0.7$  kg,  $p = .17$ ; FFM:  $-1.6 \pm 0.8$  kg,  $p = .06$ ) or in LEA + EX (fat mass:  $-0.8 \pm 0.5$  kg,  $p = .07$ ; FFM:  $-1.0 \pm 0.8$  kg,  $p = .12$ ). There were no noteworthy changes in body composition in CON conditions.

After accounting for the prescribed exercise (1:14–2:17 hr: min/day), the nonwear time ranged on average between 18 and 35 min/day (1.3–2.4%) during all conditions (see [Supplementary Table 2](#) [available online]). In four instances, the daily data were rejected because the wear time was  $< 90\%$ , but in all cases, there were sufficient data to not reject a whole condition. In one condition, no data were recorded due to a device malfunction.

**Table 1** Energy Intake, Exercise Expenditure, and Energy Availability in Exercising Men ( $n = 6$ ) During Each of the Four Study Conditions

Condition	Energy intake		Exercise expenditure		Energy availability	
	kcal/kg FFM	kcal/day	kcal/kg FFM	kcal/day	kcal/kg FFM	kcal/day
LEA – EX	$15.9 \pm 0.4$	$1,137 \pm 36$	$0 \pm 0$	$0 \pm 0$	$15.9 \pm 0.4$	$1,137 \pm 36$
LEA + EX	$29.6 \pm 1.3$	$2,144 \pm 92$	$15.0 \pm 0$	$1,088 \pm 37$	$14.6 \pm 1.3$	$1,056 \pm 82$
CON – EX	$39.9 \pm 0.6$	$2,873 \pm 102$	$0 \pm 0$	$0 \pm 0$	$39.9 \pm 0.6$	$2,873 \pm 102$
CON + EX	$52.0 \pm 2.4$	$3,732 \pm 227$	$15.0 \pm 0$	$1,076 \pm 36$	$37.0 \pm 1.4$	$2,656 \pm 206$

Note. LEA + EX = low energy availability with exercise; LEA – EX = low energy availability without exercise; CON + EX = energy-balanced control condition with exercise; CON – EX = energy-balanced control condition without exercise; FFM = fat-free mass.



However, because this was a CON condition, the participant's remaining conditions were included in the analysis.

The time spent in MVPA, LPA, sleep, and prescribed exercise is shown in Figure 1. Per study design, the participants only engaged in prescribed exercise during exercise conditions, and exercise duration did not differ between LEA + EX and CON + EX. The time spent in total MVPA, including prescribed exercise, was significantly higher during exercise versus nonexercise conditions (LEA + EX vs. LEA - EX:  $p = .02$ ; CON + EX vs. CON - EX:  $p = .01$ ). After deducting prescribed exercise from MVPA, there was a main effect for exercise ( $p = .03$ ), but no main effect for LEA ( $p = .20$ ) or the interaction effect ( $p = .24$ ). There were no significant differences in nonexercise MVPA between conditions, though nonexercise MVPA trended toward being greater in CON + EX than in CON - EX ( $p = .08$ ). The time spent in LPA was significantly lower during LEA + EX when compared with LEA - EX ( $p < .01$ ). The difference in LPA between CON + EX and CON - EX did not reach statistical significance ( $p = .054$ ). There were no main or interaction effects for time spent lying ( $p \geq .79$ ) or sleeping ( $p \geq .37$ ).

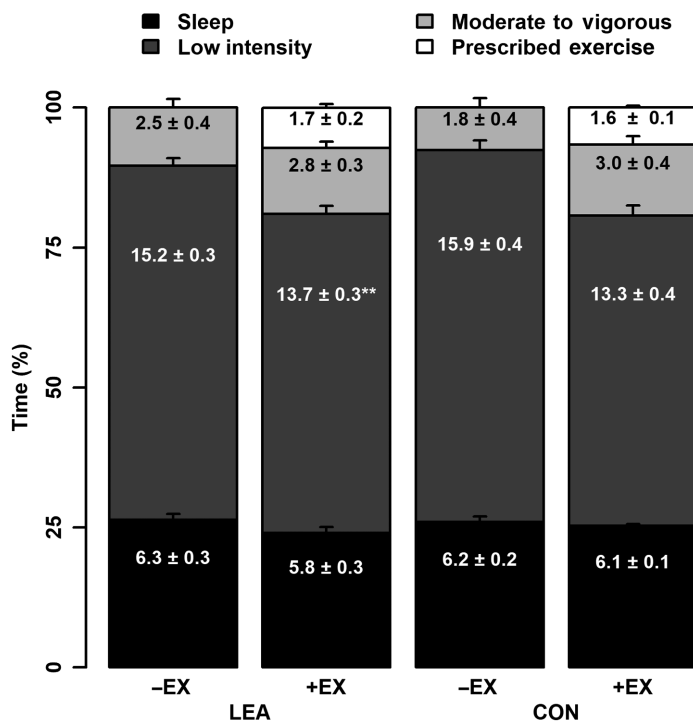
Total NEAT (Figure 2) was not significantly different between conditions (LEA - EX:  $1,049 \pm 142$ ; LEA + EX:  $1,224 \pm 107$ ; CON - EX:  $891 \pm 140$ ; CON + EX:  $1,294 \pm 142$  kcal/day). However, when adjusted for upright time, total NEAT was significantly greater during LEA + EX than LEA - EX ( $p = .05$ ). There were no significant differences between conditions in unadjusted NEAT

during MVPA and LPA, but after adjusting NEAT for upright time, both NEAT expended during MVPA ( $p = .05$ ) and during LPA ( $p = .03$ ) were significantly greater during LEA + EX than LEA - EX. The overall physical activity levels did not differ between conditions prior to adjustments, although there was a trend toward a greater physical activity level in CON + EX than in CON - EX ( $p = .06$ ). However, after adjustment for upright time, the physical activity levels were significantly greater in LEA + EX than LEA - EX ( $p = .02$ ). The daily step counts did not differ significantly between conditions (LEA - EX:  $10,389 \pm 1,850$ ; LEA + EX:  $10,069 \pm 1,499$ ; CON - EX:  $7,625 \pm 1,480$ ; CON + EX:  $9,852 \pm 1,922$  kcal/day), even after adjustment for nonexercise wear or upright time.

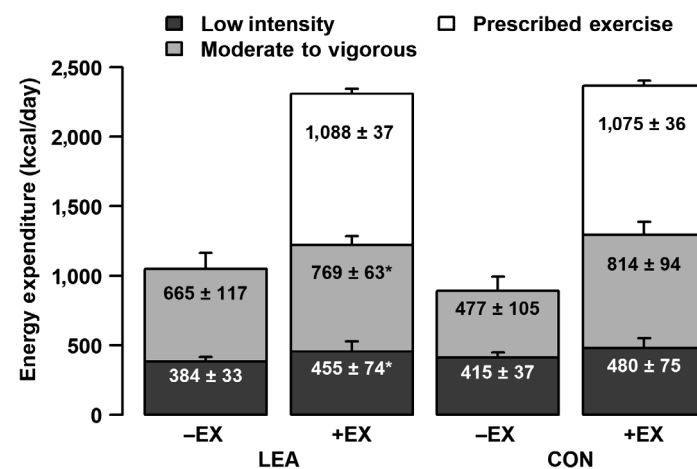
Several subjective indices of energy state, including sense of fitness, physical energy, and recovery, decreased during LEA - EX ( $p < .05$ ), but not during LEA + EX (Figure 3). Positive mood also tended to decline during LEA - EX ( $p < .1$ ) but not during LEA + EX.

## Discussion

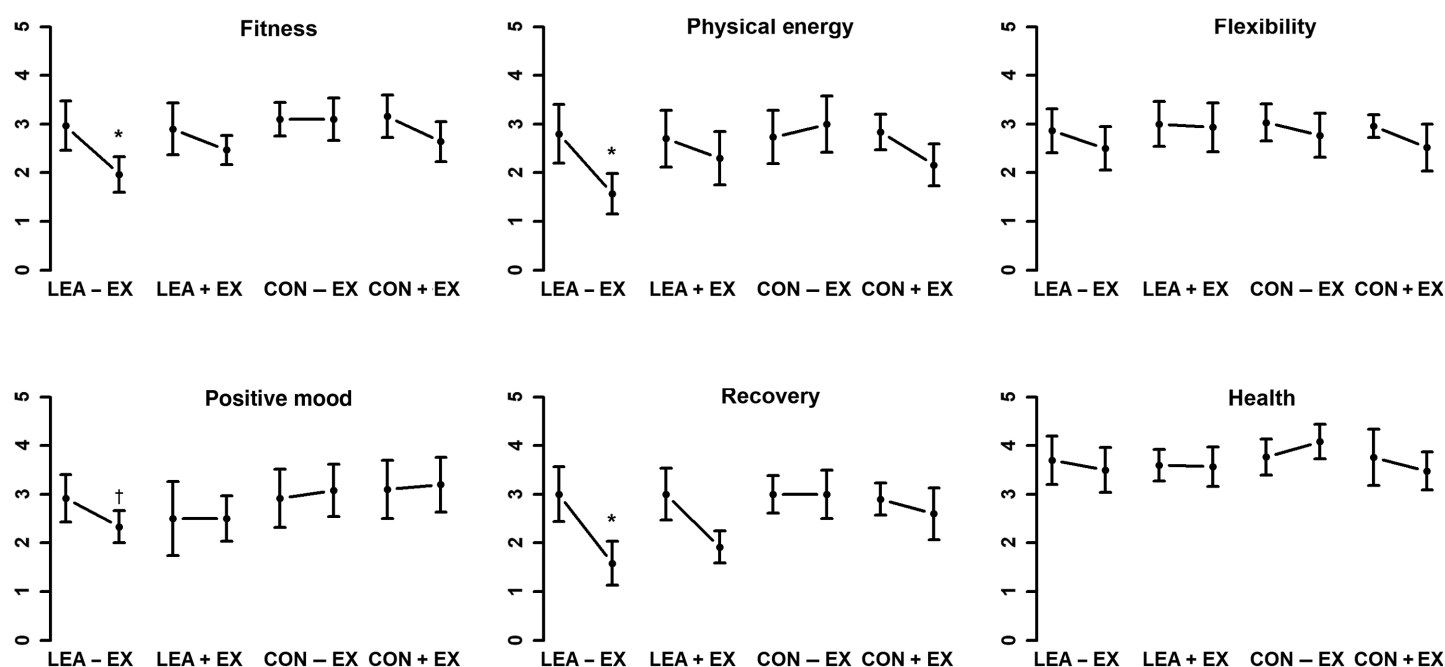
The present analysis demonstrates that exposing recreationally trained young men to LEA ( $15 \text{ kcal} \cdot \text{kg FFM}^{-1} \cdot \text{day}^{-1}$ ) for 4 days does not alter NEAT nor time spent in MVPA and LPA per se. However, time spent in LPA was lower when LEA was achieved through a combination of dietary restriction and exercise (LEA + EX) when compared with dietary restriction alone (LEA - EX). Taken together, these findings indicate that the study participants engaged in the same amount of activity and expended the same amount of energy during periods outside of prescribed exercise, independent of the time available for nonexercise activities. These differential effects of LEA on LPA, depending on how LEA was achieved, were paralleled by changes in subjective perceptions of



**Figure 1** — Average distribution of moderate to vigorous activities and low-intensity activities in exercising men ( $n = 6$ ) over the course of each 4-day intervention, consisting of LEA + EX, LEA - EX, CON + EX, and CON - EX. Values within the bars represent the average number of hours spent per day within each activity category. Control condition:  $n = 5$ . LEA + EX = low energy availability with exercise; LEA - EX = low energy availability without exercise; CON + EX = energy-balanced control condition with exercise; CON - EX = energy-balanced control condition without exercise; \* and \*\* denote significant differences from LEA - EX (\* $p < .05$ , \*\* $p < .01$ )



**Figure 2** — Average daily total nonexercise activity thermogenesis (NEAT) in exercising men ( $n = 6$ ) during each intervention, consisting of LEA + EX, LEA - EX, CON + EX, and CON - EX. Total NEAT was further broken into expenditure during moderate to vigorous and low-intensity activity. Total NEAT when adjusted for upright time was significantly greater in LEA + EX than LEA - EX ( $p = .05$ ). Control condition:  $n = 5$ . LEA + EX = low energy availability with exercise; LEA - EX = low energy availability without exercise; CON + EX = energy-balanced control condition with exercise; CON - EX = energy-balanced control condition without exercise; \* denotes a significant difference from LEA - EX when adjusted for upright time ( $p < .05$ ).



**Figure 3** — Changes in indices of perceived physical and psychological state in exercising men ( $n=6$ ) over the course of each 4-day intervention, consisting of LEA + EX, LEA - EX, CON + EX, and CON - EX. Participants matched their current state on a scale from 0 (*not at all*) to 5 (*completely*), with 0.5 increments. LEA + EX = low energy availability with exercise; LEA - EX = low energy availability without exercise; CON + EX = energy-balanced control condition with exercise; CON - EX = energy-balanced control condition without exercise. †, \*, and \*\* denote differences from pre-condition ( $†p < .1$ ,  $*p < .05$ ,  $**p < .01$ )

energy state, which declined significantly only in LEA without exercise.

To our knowledge, the present study is the first to prospectively examine the relationship between LEA and physical activity outside of prescribed exercise. Although other studies have examined the relationship between NEAT and proxy indicators of LEA in a cross-sectional setting (Torstveit et al., 2018), we are the first to quantify NEAT and time spent in MVPA and LPA in a controlled trial within the same individuals to account for the large interindividual variability in NEAT (Levine, 2007). Independent of the condition, we quantified NEAT in the range of 900–1,300 kcal/day, which is comparable to previously reported NEAT of approximately 1,250 kcal/day in endurance-trained athletes (Drenowatz et al., 2013). Furthermore, expressed relative to FFM, the denominator used when quantifying EA, NEAT amounted to 12.5–18.0 kcal·kg FFM<sup>-1</sup>·day<sup>-1</sup>, indicating that the participants expended comparable amounts of energy during activities of daily living when compared with prescribed exercise bouts (15 kcal·kg FFM<sup>-1</sup>·day<sup>-1</sup>).

While there are certainly periods where ExEE may exceed NEAT, such as during high-volume training or competition in endurance events, there are other parts of the training cycle where this may not be true. For example, in a previous study in endurance-trained athletes, ExEE was comparable with NEAT during high-volume training (>13 hr/week: ExEE = 1,285 ± 268 kcal/day; NEAT = 1,291 ± 285 kcal/day), but only approximately 50% during low-volume training (<7 hr/week: ExEE = 639 ± 267 kcal/day; NEAT = 1,262 ± 350 kcal/day; Drenowatz et al., 2013). These findings collectively challenge the general assumption that NEAT is always minimal when compared with ExEE and can therefore be neglected in the calculation of EA (Areta et al., 2020).

In contrast to previous weight loss studies in which calorie restriction resulted in reductions in NEAT (Hunter et al., 2015; Verdich et al., 2001), we did not observe significant effects of

LEA on NEAT or time spent in MVPA and LPA. In weight loss populations, reductions in energy expenditure have been reported as both behavioral adaptations (Hunter et al., 2015) and physiologically through reductions in REE (Camps et al., 2013), both as compensatory mechanisms opposing the initial energy deficit (Doucet et al., 2018). While physiological conservation has also been reported in exercising individuals in LEA (Koehler et al., 2016b; Loucks et al., 2011), our data suggest that reductions in NEAT in response to LEA do not occur, at least over the short term, in physically active and trained individuals. Considering our results and a recent report of—albeit not significantly higher—NEAT in male athletes with suppressed REE (Torstveit et al., 2018), it is possible that the lack of behavioral compensation makes physiological adaptations (e.g., reductions in REE) more prominent in exercising individuals in an LEA state.

Another important finding is that the perceived energy status (sense of fitness, physical energy, recovery, and positive mood) declined only in LEA - EX. Severe reductions in EI have been shown to reduce physical energy and increase lethargy (Keys et al., 1950), so the increased food intake in LEA + EX may have prevented decrements in the perceived energy state, even when the participants were at the same EA. Furthermore, the reductions in the perceived energy status in LEA - EX but not LEA + EX paralleled the differences in physical activity behavior, as the additional time gained during nonexercise conditions was spent in LPA.

While the present analysis is, to our knowledge, the first to assess the impact of LEA on NEAT in a prospective study, it is not without limitations. While our sample size was relatively small ( $n=6$ ), we utilized a repeated-measures crossover design to account for the high variability in NEAT (Levine, 2007). Furthermore, the exercise and nonexercise conditions were energy matched to achieve the same EA level, and exercise and food intake were supervised and controlled.

The condition duration of 4 days was chosen to ensure high compliance and has been shown to be sufficient to induce meaningful metabolic changes and weight loss (Hilton & Loucks, 2000; Koehler et al., 2016a), but it may have been too short for behavioral compensation (Melanson, 2017). However, the inclusion of non-exercise conditions made a longer study duration impractical as trained individuals engage in three or more sessions per week (De Pauw et al., 2013).

To clearly visualize whether substantial differences in LEA and ExEE could affect NEAT differently, we chose a severe LEA level (15 kcal/kg FFM) to emphasize differences in EI, even though EA values  $\leq 30$  kcal/kg FFM are considered the threshold for physiological disruptions (Areta et al., 2020; Ihle & Loucks, 2004). While LEA of 15 kcal·kg FFM<sup>-1</sup>·day<sup>-1</sup> did not alter NEAT in the short term, longer studies are needed to confirm whether NEAT remains stable within a person exposed to LEA. Considering the magnitude and interindividual variability of NEAT, further research is needed to determine the possible contribution of NEAT to the interindividual variability in the responsiveness of physiological markers to a specific LEA dose (Lieberman et al., 2018). While women were excluded from participation in the present study, future studies should explore sex-based differences with regard to behavioral compensation.

NEAT and physical activity were assessed using the SenseWear3 armband, which provides valid and accurate estimates of light to moderate physical activity (St-Onge et al., 2007). Though the armband has been shown to underestimate ExEE during high-intensity bouts (Koehler & Drenowatz, 2017), our analysis focused on expenditure during nonexercise periods. Due to measurement frequency, body composition was assessed via bioimpedance analysis. Future studies should utilize more advanced body composition methods to reflect the importance of FFM for EA prescriptions.

The present study did not consider the impact of postexercise oxygen consumption (EPOC) on NEAT, which we believe is minor when compared with ExEE (Torstveit et al., 2018), especially during continuous, moderate-intensity exercise (LaForgia et al., 2006). Furthermore, EPOC is not measured well by the Sensewear armband (Gastin et al., 2018) and, anecdotally, most participants removed their armband immediately postexercise to shower. It is also unlikely that EPOC would have increased energy expenditure sufficiently to alter MET classifications, which is also supported by our findings that there were no differences in MVPA or sleep between the exercise and nonexercise conditions. Still, future studies should quantify EPOC separately to ensure that it does not falsely inflate NEAT.

Finally, it should be noted that the participants in the present study were asked not to engage in purposeful exercise outside of the prescribed bouts, so it is unclear whether the participants would have engaged in outside exercise to compensate for the lack of training in nonexercise conditions. However, previous research has shown that even elite athletes display considerable sedentary off-training behavior (Sperlich et al., 2017), often for recovery, and no stipulations were made suggesting that the participants deviate from their habitual activity routines (e.g., commuting, social activities).

## Conclusion

The present analysis is the first to our knowledge to prospectively examine the relationship between LEA and NEAT. LEA, attained with dietary restriction alone or in combination with exercise, did

not result in adaptive reductions in NEAT when compared with adequate EA. However, when LEA was attained through a combination of dietary restriction and exercise, the time spent in LPA was approximately 10% lower, suggesting that NEAT was preserved as the participants conducted equal amounts of MVPA in less time per day. Reductions in subjective perceptions of energy state occurred only during LEA – EX, but not LEA + EX. Taken together, LEA does not seem to impact NEAT in the short term, but the way in which it is attained may impact objectively measured physical activity behavior outside of exercise and subjective perceptions of energy state. Given the potentially meaningful contribution of NEAT in the range of approximately 1,200 kcal/day to TDEE, future LEA studies should consider quantifying NEAT, particularly when examining the long-term ramifications of LEA.

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