Effects of 24-Hour Diet- or Exercise-Induced Energy Availability Manipulations on Substrate Utilization and Performance

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ABSTRACT

Purpose: To examine sex-based differences in substrate oxidation, postprandial metabolism, and performance in response to 24-hour manipulations in energy availability (EA), induced by manipulations to energy intake (EI) or exercise energy expenditure (EEE). **Methods:** In a Latin Square design, 20 endurance athletes (10 females using monophasic oral contraceptives and 10 males) undertook five trials, each comprising three consecutive days. Day one was a standardized period of high EA; EA was then manipulated on day two; post-intervention testing occurred on day three. Day two EA was low/high/higher EA (LEA/HEA/GEA) at 15/45/75 kcal·kg^{-1}FFM·day^{-1}, with conditions of LEA and HEA separately achieved by manipulations of either EI or EEE ($LEA_{REST/EX}$ vs. $HEA_{REST/EX}$). On day three, fasted peak fat oxidation during cycling and two-hour postprandial (high carbohydrate and energy meal) metabolism were assessed, alongside several performance tests: Wingate, countermovement jump (CMJ), squat jump (SJ), isometric mid-thigh pull (IMTP), and the Stroop Color and Word Test. **Results:** Highest peak fat oxidation occurred under LEA induced by exercise ($p<0.01$), with no difference between sexes. Postprandial glucose ($p<0.01$) and insulin ($p<0.05$) responses were highest across both sexes when LEA was induced by diet. Relative peak and mean power throughout the Wingate, alongside CMJ height did not differ between EA conditions ($p>0.05$), while SJ height was lower during GEA than both LEA_{REST} ($p=0.045$) and HEA_{EX} ($p=0.016$). IMTP peak force and the Stroop effect did not change with altered EA ($p>0.05$). **Conclusions:** Acute (24-hour) exercise-induced LEA influenced fasted substrate oxidation more than diet-induced LEA, while 24 hours of LEA did not impair strength/power, sprint capacity, or cognitive performance. Finally, the responses to EA manipulations did not differ between sexes.

Key Words: RELATIVE ENERGY DEFICIENCY IN SPORT, ENERGY INTAKE, ENERGY EXPENDITURE, ENERGY BALANCE, ATHLETES, CYCLING

INTRODUCTION

Energy availability (EA) is defined as the difference between dietary energy intake (EI) and exercise energy expenditure (EEE), expressed relative to fat free mass (FFM) and represents the energy remaining for physiological functions (1, 2). Originally, short-term (~5-day) studies considered optimal EA to be 45 kcal·kg^{-1}FFM·day^{-1}, while EA <30 kcal·kg^{-1}FFM·day^{-1} was considered low (LEA) and associated with health and performance impairments (1-3). However, these concepts evolved from laboratory-based studies on a small sample of sedentary women (4-6) and were not intended to be rigid or universally applied (7). Indeed, the EA "cut off" associated with health and performance impairments is moderated by factors such as sex and training history (7-10). Athletes commonly undertake both intentional and unintentional reductions in EA to facilitate performance goals. For example, intensified training blocks that increase EEE without a compensatory increase in EI, short periods of energy restriction to achieve optimal competition physique characteristics (11), alongside athletes in weight division sports who need to reduce body mass to meet competition weigh-in targets (12). Therefore, a more contemporary view is that while some short exposure to LEA may cause transient and minor metabolic adjustments and/or be associated with performance benefits ("adaptable" LEA), other LEA exposures are "problematic" because they are associated with negative health and performance outcomes that may result in Relative Energy Deficiency in Sport (7). However, characteristics of adaptable versus problematic LEA and moderating factors are not yet fully identified.

Short-term (3-6 day) LEA exposures <30 kcal·kg^{-1}FFM·day^{-1} may alter bone metabolism, reproductive function, metabolic hormones (insulin, leptin), fat oxidation and resting metabolic rate (RMR) in some populations (4, 5, 9, 13-15), but performance effects are either uninvestigated

or unclear. This is important for athletes needing to implement acute strategies, as aforementioned. Previous studies have reported maintenance of endurance capacity following three days at an EA of 19 kcal·kg^{-1}FFM·day^{-1} among Tier 2 (16) male runners, despite reductions in muscle glycogen (17). Meanwhile Burke et al. (18) demonstrated no impairments to 10,000 m race walk performance among Tier 4 athletes of both sexes following a slightly longer exposure (nine days at 15 kcal·kg^{-1}FFM·day^{-1}), when optimal pre-race fueling was implemented. However, to our knowledge, no studies have examined the influence of acute (<7 days) LEA exposure on strength/power performance outcomes <60 seconds in duration.

Cognition and decision-making are key aspects of sport. However, few studies have examined the influence of acute LEA on cognitive performance, though preliminary evidence suggests that women experiencing LEA may show some resilience in cognitive function. Martin et al. (19) reported no alterations to cognitive function among women following three days of exercise- (but not diet-) induced LEA (15 kcal·kg^{-1}FFM·day^{-1}). Moreover, Lieberman et al. (20) observed that the cognitive performance decline observed with two days of severe LEA (-3681±716 kcal·kg^{-1}FFM·day^{-1}) was less pronounced among women than men, although women accounted for only 26% of the sample. However, these studies were all conducted in non-athletic [≤Tier 1 (16)] populations.

Sex may mediate the response to EA manipulations, with women potentially more sensitive to acute LEA, and experiencing negative consequences at a higher EA compared to men (8, 13). LEA intervention studies in male athletes (8, 9, 21) have reported fewer perturbations to body systems (bone metabolism, immune, inflammatory, and iron-regulatory responses and metabolic

hormones) than shown in young untrained women (4, 5, 8). However, since few studies have examined performance indices, particularly among trained [≥Tier 2 (16)] athletes, or with specific designs that can contrast responses between the sexes, robust conclusions regarding sex differences are not possible.

The complete time course over which various maladaptations to LEA manifest requires clarification. It is also unknown whether the method of reducing EA (i.e., dietary restriction or increased EEE) has divergent effects on physiological outcomes. From a health standpoint, it appears that an EA of 15 kcal·kg^{-1}FFM·day^{-1} induced through dietary restriction, but not increased EEE, decreases bone formation; but that both methods alter hormonal profiles (6, 9, 22). There is also preliminary evidence that cognition may be more impaired by acute exercise-induced LEA (19), however physical performance has never been examined. This could be relevant when tailoring training/nutritional protocols to alter body composition whilst minimizing negative performance effects. Lastly, low carbohydrate (CHO) availability, independent of LEA, is associated with perturbations to iron (21) and bone metabolism (23, 24), however acute performance effects remain uninvestigated.

We therefore aimed to assess effects of acute (24-hour) manipulations in EA (induced via diet or exercise) on substrate utilization, postprandial metabolism, and physical/cognitive performance among trained males and females, evaluating results within individuals and between sexes. We hypothesized that increased fat oxidation would occur under LEA, with an augmented response among women, due to the concurrent reductions in CHO availability but the diet/exercise manipulation would be too brief alter the exercise intensity at which maximal fat oxidation occurs

(FATMAX) (25). We also expected that acute EA manipulations would not alter strength/power, but that men would experience a greater decline in cognitive performance with LEA compared to women.

METHODS

Participants

Ten female and ten male Tier 2-3 (16) endurance trained athletes (Table 1) participated in this study approved by the Australian Catholic University Human Ethics Research Committee (2022-2561H) in accordance with the Declaration of Helsinki. Participants were familiar with cycling even if not their primary sport (i.e., cross-training, or regular commuting). A sample size calculation (G*Power 3.1, Dusseldorf, Germany), using data from Chrzanowski-Smith (26), estimated that 10 males and 10 females were required to detect differences in peak fat oxidation (PFO) relative to FFM between the sexes, with 90% statistical power and an alpha of 0.05. To eliminate potential effects of menstrual status/phase, we recruited pre-menopausal females taking a combined oral contraceptive pill (OCP; see details in Supplemental Table 1, Supplemental Digital Content) for >3 months prior to study commencement (median usage time was 4 years). All participants provided informed consent prior to participating.

Experimental overview

The study design included a baseline/familiarization session, followed by five randomized experimental trials, each comprising three consecutive days (15 days of testing per participant; Figure 1). Trials were completed in a randomized order in a Latin square design, with an average of eight days separating trials (minimum four days, maximum 33 days). Doses of EA on trial day

two were: low (15 kcal·kg^{-1}FFM·day^{-1}; LEA), high (45 kcal·kg^{-1}FFM·day^{-1}; HEA) and higher EA for mass gain/growth (75 kcal·kg^{-1}FFM·day^{-1}; GEA), with conditions of high and low EA separately achieved via manipulations of EEE or EI (Figure 1). Participants undertook post-intervention testing on day three at the same time of day (± 8 min) across all five trials. Female participants completed each trial during the active (pill-taking) phase of the OCP cycle, when they were not experiencing a withdrawal bleed, to minimize fluctuations in both endogenous and exogenous ovarian hormones across trials. Women were instructed to take their daily OCP after post-intervention testing on day three to minimize effects of a bolus dose of exogenous hormones.

Baseline/Familiarization. Participants underwent a baseline/familiarization session 1-2 weeks prior to study commencement, which included a $\dot{V}O_2$max (to calculate subsequent EEE prescription), alongside familiarization to the FATMAX test and performance measures [Stroop, Wingate, isometric mid-thigh pull (IMTP), countermovement jump (CMJ) and squat jump (SJ)]. Dual-energy X-ray absorptiometry (DXA) and RMR measurements occurred to establish EI and EEE prescriptions for subsequent trials. Additionally, participants received an activity tracker [Oura ring (Generation 3, Oura Health, Oulu, Finland)], to wear during each of the five subsequent three-day trial periods to monitor step count and estimated energy expenditure.

Trial day 1 – Optimal EA standardization. Participants consumed a standardized diet, providing 45 kcal·kg^{-1}FFM·day^{-1} (CHO; 4.7±0.5 g·kg^{-1}, protein; 2.1±0.2 g·kg^{-1}, fat; 0.8±0.1 g·kg^{-1}) for 24 hours prior to EA manipulation to ensure each intervention began in a state of optimal EA. Exercise was permitted but was replicated within each participant across all five trials (verified through the

Oura ring), and dietary intake adjusted accordingly. As such, EI and EEE was identical for trial day one across all five trials.

Trial day 2 – EA manipulation. The five EA conditions are outlined in Figure 1: LEA [with and without exercise (LEA_{EX} and LEA_{REST})], HEA [with and without exercise (HEA_{EX} and HEA_{REST})], or GEA (without exercise) (3). For the two conditions involving exercise (LEA_{EX} and HEA_{EX}), participants completed two cycle sessions in the laboratory to achieve a total EEE of 30 $kcal \cdot kg^{-1}FFM \cdot day^{-1}$. Aside from prescribed exercise, participants remained inactive throughout the day, minimizing activities of daily living (verified via Oura ring). For the three conditions not involving exercise (LEA_{REST}, HEA_{REST} and GEA) participants did not come to the laboratory but adhered to the provided diet and remained inactive (Oura ring verification).

Trial day 3 – post-intervention measures. Upon laboratory arrival in a 10-hour rested and fasted state, body composition was measured via DXA. A cannula was then inserted, and blood sample collected, followed by the FATMAX test (20-30 min). After a mixed meal tolerance test (120 min), physical performance measures were obtained: IMTP, CMJ, SJ and Wingate, alongside questionnaire regarding perceived muscle soreness (27). Participants then rested for 30 minutes in a quiet, private room with *ad libitum* food, after which they underwent the Stroop Color and Word Test for cognitive performance. Each individual method is described below.

Dietary manipulation

Participants received all food and drink individually pre-packaged and weighed prior to the start of each three-day trial. Diet prescription is outlined in detail by Kuikman et al., (28). In brief,

diets prescribed an EI of 15 (LEA_{REST}), 45 ($LEA_{EX,}$ HEA_{REST} [and day one standardization]) or 75 (HEA_{EX} and GEA) kcal·kg^{-1}FFM·day^{-1}. Macronutrient percentage distribution was equal between all EA conditions (alongside the day one standardization diet) at 25% of EI from protein, 20% from fat and 55% from CHO (providing an intake of 1.6±0.2 g CHO·kg^{-1}·day^{-1} for LEA_{REST}, 4.7±0.5 g CHO·kg^{-1}·day^{-1} for $LEA_{EX,}$ HEA_{REST} and day one standardization, and 7.5±1.2 g CHO·kg^{-1}·day^{-1}for HEA_{EX} and GEA). All EA conditions (alongside the day one standardization diet) provided participants with three meals and three snacks. Participants were instructed to space out meals and snacks by at least one hour, and to consume the last snack 10-hours prior to laboratory arrival on day three. Caffeine consumption was permitted on trial days one and two, but not three, and replicated across each of the five trials. Alcohol was prohibited throughout each three-day trial period. Participants verbally confirmed the consumption of all food/drink upon arrival to the laboratory on trial day three.

Exercise manipulation

For the two EA conditions involving exercise (LEA_{EX} and HEA_{EX}), participants completed two cycle sessions in the laboratory on a stationary load bike (Load Excalibur Sport, Groningen, Netherlands) to achieve an EEE of 30 kcal·kg^{-1}FFM·day^{-1}. The evening session was 60 minutes at 65% $\dot{V}O_2$max (males, 195±46 W; females, 131±19 W), concluding 12 hours prior to next day laboratory arrival. The remaining EEE was completed in the morning at 55% $\dot{V}O_2$max with exercise duration manipulated to achieve 30 kcal·kg^{-1}FFM·day^{-1} EEE (males, 157±40 W for 135±26 min; females, 103±16 W for 163±37 min). The EEE at each cycling intensity was determined from gas exchange data collected during baseline $\dot{V}O_2$max testing. Expired gases were used to calculate substrate oxidation rates and energy expenditure (EE) in accordance with the

stoichiometric equations outlined by Jeukendrup and Wallis (29), assuming negligible protein oxidation. An athlete's RMR was then excluded from EE to determine EEE.

Test Protocols

DXA and RMR: Both DXA and RMR were assessed at baseline, to calculate EI and EEE prescriptions. A DXA was performed at each laboratory visit to normalize results to FFM.

FATMAX and $\dot{V}O_2$max: The PFO and exercise intensity eliciting PFO (FATMAX) were assessed by an incremental cycling protocol on a load bike using the measured values approach (25). Starting at 30 W for females and 50 W for males, participants completed three-minute stages increasing by 25 W increments until RER >1.0. During familiarization testing, participants completed an additional maximal exercise bout ($\dot{V}O_2$max test) following completion of the FATMAX protocol. When RER >1.0, instead of ceasing the test, wattage continued to increase in 25 W increments every 60 s until volitional exhaustion, as indicated by the participant. $\dot{V}O_2$max was taken as the highest $\dot{V}O_2$ value observed across a 30 s period. Chest HR (Forerunner, Garmin International) and rating of perceived exertion (RPE, 6–20, Borg Scale) were recorded at the end of each stage. Expired gas was collected and analyzed using a custom built indirect calorimetry system with associated in-house software as previously described (30). The $\dot{V}O_2$ and $\dot{V}CO_2$ values from the last minute of each stage were used to calculate PFO using non-protein RER values (31).

Mixed meal tolerance test (MMTT): Participants consumed a breakfast meal of raisin toast, jam, and apple juice (males, 1035±148 kcal; females, 841±172 kcal; 2.00±0.00 $g \cdot kg^{-1}$ CHO; 0.27±0.03 $g \cdot kg^{-1}$ protein; 0.13±0.01 $g \cdot kg^{-1}$ fat) followed by a two-hour resting period. Meal consumption began at 0 min and finished within 15 min.

Blood sampling**:** At the start of each lab visit, a cannula was inserted into the antecubital vein by a trained phlebotomist while the athlete was in a rested and fasted state. A total of eight 1 ml blood samples were collected per trial: baseline (rested and fasted), alongside the following timepoints during the MMTT: 0 (pre-meal), 15, 30, 45, 60, 90 and 120 min. Blood tubes clotted at room temperature for 30 min before being centrifuged at 2200 G for 10 min at 4°C. The serum was split into aliquots and stored at -80°C until batch analysis. Glucose was measured via an automated colorimetric assay (AU480 chemistry analyzer, Beckman Coulter, Brea, California, USA) with intra-assay coefficient of variations (CV) of 1.0%. Insulin was analyzed via chemiluminescent immunoassay (Access 2 immunoassay system, Beckman Coulter, Brea, California, USA) with CV of 8.9%. Incremental area under the curve was calculated for glucose and insulin concentration using an automated tool (32).

Countermovement/squat jumps and isometric mid-thigh pull*:* Following a standardized warm-up and wearing the same shoes on all five occasions, participants completed the CMJ, SJ, and IMTP on a dual force plate system sampling at 1000 Hz (0.60 x 0.40 m; Model 10 kN 9286B, Kistler Instrument AG, Winterthur, Switzerland). Participants first completed three repetitions each of the CMJ and then SJ with ~60 seconds rest between jumps. Participants were instructed to "jump as high and powerfully as possible" with their hands remaining on hips. For the SJ,

participants jumped from a 90° squat (or as close as possible) without countermovement. Squat depth was standardized within participants between trials using a plastic pole that participants lowered themselves to, and an additional effort was performed if any countermovement was observed via the force-time trace. The highest jump repetition was analyzed; if jump height was equal, then peak power was used to determine the "best" effort. Outcome measures included jump height (calculated through impulse-momentum), mean and peak concentric force, velocity, and power, alongside impulse and rate of force development at 50/100/150/200 ms, as well as contraction time, concentric time, eccentric time, and center of mass displacement (33). Jump initiation was identified using the criterion method (34).

Following two sub maximal warm-up efforts, participants performed two maximal repetitions of the IMTP separated by two minutes rest. Participants pulled at maximal effort for three seconds on an immovable bar fixed to a customized power rack. The bar was set during the familiarization visit, such that joint angles at the knee and hip were between 125-145° and 140-150°, respectively (35). Participants were instructed to "push the ground away as hard and as fast as possible". Verbal encouragement was maintained throughout. A third effort was performed if: >200 N difference was observed between the peak force of the two efforts; there was variability >50 N in the quiet period; there was a countermovement prior to the lift, excessive pre-tension, or leaning on the bar (35). The highest relative peak force effort was analyzed. Pull initiation was identified as the moment when force exceeded five standard deviations (SD) of a participant's body mass (35), established through a one-second stable weighing period. Peak force, time to peak force, rate of force development and impulse at 50/100/150/200/250 ms were calculated.

All ground reaction force-time data for the CMJ, SJ and IMTP were recorded using ForceDecks software (VALD ForceDecks, 2.0.8587) and then exported for analysis via a customized R script. CMJ and SJ jump heights were also used to calculate the eccentric utilization ratio (EUR) and reactive strength index (RSI), while the dynamic strength index (DSI) was calculated from CMJ peak concentric force and IMTP peak force.

Wingate: Participants performed a five-minute standardized cycling warm-up, which included three six-second sub-maximal sprints. Participants then completed a 30 second all-out cycling effort (Wattbike Pro, Nottingham, England) at maximal speed against a high braking force from a rolling start. Participants were instructed to "pedal as hard as possible from the start without pacing the effort but remaining in the saddle". Verbal encouragement was maintained throughout. Outcome measures were peak power, mean power, and fatigue index.

***Stroop Color and Word Test*:** Participants were shown colored words on a laptop and asked to indicate the word's color (and not it's meaning) by pressing a key as fast as possible whilst minimizing errors (36). Colored labels were placed on keyboard keys to signify the corresponding color. Three types of trials were presented: control (colored rectangles), congruent (words of matched color and meaning) and incongruent (words with mismatched color and meaning). A red "X" flashed onto the screen when an incorrect response occurred. Each test had 180 trials, taking approximately three minutes to complete. The Stroop test was administered using Inquisit 6 (6.6.1 64bit, [Windows 10], (2020) Retrieved from https://www.millisecond.com). The Stroop effect was calculated as the difference between responses (proportion correct and reaction time) in the incongruent versus congruent trials.

Muscle soreness. A seven-point Likert scale for lower limb muscle soreness (27) (Supplemental Table 2, Supplemental Digital Content) was completed at 0, 60 and 120 min during the MMTT, with the mean score used in analysis.

Statistical analyses

Results were compared across EA conditions and between sexes using linear mixed models. Fixed effects were "condition" (LEA_{REST}, LEA_{EX}, HEA_{REST}, HEA_{EX}, GEA), and "sex" (female or male), with "subject identification" as a random effect. Statistical significance of fixed effects occurred using type II Wald tests with Kenward–Roger degrees of freedom. Where significant fixed effects were established, pairwise comparisons were performed with Tukey *post hoc* adjustments. Significance was accepted at $p<0.05$. Data are presented as means ± SD with non-normal data (assessed via histogram inspection) log-transformed prior to analysis. Outliers >3 SD beyond the group mean were removed (37). Muscle soreness data are missing for one condition (LEA_{REST} and HEA_{REST}) for two females due to a failure in the server administering questionnaires. Squat jump results are missing for a single condition (LEA_{REST} and HEA_{EX}) for two females due to technical data collection issues. Two females are missing one timepoint in the LEA_{REST} condition (15 and 90 min) for glucose and insulin because of cannula blockage; linear interpolation was used to address this when calculating iAUC (32).

RESULTS

Energy availability: As intended, the LEA_{REST} and LEA_{EX} conditions, alongside HEA_{REST} and HEA_{EX} conditions were matched for EA (all $p=1.000$), with differences observed between all other conditions (all $p<0.001$, Table 2). Similarly, LEA_{EX} and HEA_{REST}, alongside HEA_{EX} and GEA,

were matched for dietary EI, CHO, protein, and fat (all $p>0.050$). EEE was also matched for LEA_{EX} and HEA_{EX} within sexes ($p=1.000$). Males had a higher EEE than females in both exercise conditions ($p<0.001$) and higher EI than females in HEA_{EX} and GEA ($p<0.001$). There was no difference in step counts between trials ($p=0.128$).

Day two exercise during LEA_{EX} and HEA_{EX} conditions: Heart rate and RPE were higher during the second exercise bout at 65% $\dot{V}O_2$max (144±13 b·min^{-1} and 14±1) than the first bout at 55% $\dot{V}O_2$max (131±15 b·min^{-1} and 11±2, both $p<0.001$). There were no differences between sexes or condition, nor any interactions (all $p>0.050$). Self-reported/ perceived muscle soreness on trial day three was higher in the exercise conditions (LEA_{EX} and HEA_{EX}) than all other conditions ($p<0.010$, Table 3), with no difference between sexes ($p=0.668$).

Fat oxidation: There was a main effect of condition ($p<0.001$) but not sex or interaction *(*all *$p>0.050$)* for both absolute PFO (Figure 2A) and PFO relative to FFM (Figure 2B, FFM reported in Supplemental Table 3, Supplemental Digital Content). Absolute PFO was greatest in LEA_{EX} (0.60±0.17 g·min^{-1}) and lowest in GEA (0.37±0.13 g·min^{-1}). Differences between conditions were the same for absolute and relative PFO. Relative PFO peaked under LEA_{EX} (10.9±2.5 mg·(kg·FFM^{-1})·min^{-1}), 48% greater than GEA (6.7±2.0 mg·(kg·FFM^{-1})·min^{-1}, $p<0.001$), 34% greater than HEA_{REST} (7.7±2.1 mg·(kg·FFM^{-1})·min^{-1}, $p<0.001$) and 17% greater than LEA_{REST} (9.2±2.2 mg·(kg·FFM^{-1})·min^{-1}, $p=0.006$). Relative PFO was lower in GEA than HEA_{EX} (37%, 9.7±2.3 mg·(kg·FFM^{-1})·min^{-1}, $p<0.001$) and LEA_{REST} (31%, $p<0.001$), as well as in HEA_{REST} compared to HEA_{EX} (23%, $p<0.001$) and LEA_{REST} (18%, $p=0.022$). There was no difference in relative (or absolute) PFO between exercising conditions (LEA_{EX} and HEA_{EX}, $p=0.092$), nor

between LEA_{REST} and HEA_{EX} (*p=0.846*) or GEA and HEA_{REST} (*p=0.218*). Mean total CHO oxidation across all conditions during the exercise test was 29.5±14.0 $g \cdot min^{-1}$, and when expressed relative to FFM, did not differ between conditions (*p=0.459*) or sexes (*p=0.065,* Supplemental Table 4, Supplemental Digital Content).

FATMAX, both absolute and relative to body mass, did not differ between EA conditions (*p>0.050,* Figures 3A and 3B). There was a main effect of sex for absolute FATMAX *(p=0.022,* Figure 3A*)*, with males reaching FATMAX at a higher power than females (150±75 W *vs* 105±75 W). However, when expressed relative to body mass, there was no effect of sex *(p=0.119,* Figure 3B*).* The HR at FATMAX, as a percentage of maximal HR, was higher in LEA_{EX} (72±8%, Figure 3C) versus HEA_{REST} (64±10%, *p=0.016)* and GEA (64±9%, *p=0.033*). The RPE at FATMAX was lower during GEA (9±2, Figure 3D) than LEA_{EX} (11±2, *p=0.006*), LEA_{REST} (11±2, *p=0.005*) and HEA_{EX} (11±2, *p=0.010*). The $\dot{V}O_2$ at FATMAX, as a percentage of $\dot{V}O_2$max, was lower during HEA_{REST} (49±16%, Figure 3E) than LEA_{EX} (58±12%, *p=0.008*), LEA_{REST} (56±11%, *p=0.047*) and HEA_{EX} (56±10%, *p=0.039*), and was also lower during GEA (48±11%) than LEA_{EX} *(p=0.026*). Time to reach FATMAX was lower in HEA_{REST} (11.0±7.2 min, Figure 3F) versus LEA_{REST} (13.8±5.5, *p=0.027*) and LEA_{EX} (14.0±5.8, *p=0.024*). There was no effect of sex or sex*condition interaction on HR, RPE or time to FATMAX *(p>0.050).*

MMTT: There was no clear difference in rested, fasted glucose concentration between conditions (*p=0.050*), but baseline fasted insulin concentration was higher in GEA (4.36±2.04 $uIU \cdot mL^{-1}$) than LEA_{REST} (2.66±1.32 $uIU \cdot mL^{-1}$, *p<0.001*), LEA_{EX} (2.96±1.66 $uIU \cdot mL^{-1}$, *p<0.001*) and HEA_{EX} (3.24±1.72 $uIU \cdot mL^{-1}$, *p=0.007*). Mean postprandial glucose concentration was higher in LEA_{REST}

(6.01±1.27 mmol·L^{-1}, Figure 4A) than GEA (5.56±1.28 mmol·L^{-1}, *p<0.001*) and HEA_{REST} (5.60±1.20 mmol·L^{-1}, *p=0.007*). There was no alteration in glucose iAUC or maximum/minimum glucose concentration across conditions (all *p>0.050*, Figure 4B). Mean postprandial insulin concentration was lower in LEA_{EX} (20.0±15.1 uIU·mL^{-1}, Figure 4C) than GEA (24.4±16.3 uIU·mL^{-1}, *p=0.013*) and LEA_{REST} (23.0±14.2 uIU·mL^{-1}, *p=0.035*). Minimum insulin concentration was also lower in LEA_{EX} (4.16±2.43 uIU·mL^{-1}) than HEA_{REST} (5.30±2.46 uIU·mL^{-1}, *p=0.045*) and GEA (5.59±2.56 uIU·mL^{-1}, *p=0.011*). Insulin iAUC was higher in LEA_{REST} (2440±803 uIU·120min·mL^{-1}, Figure 4D) than LEA_{EX} (2068±1214 uIU·120min·mL^{-1}, *p=0.003*) and HEA_{EX} (2018±1086 uIU·120min·mL^{-1}, *p=0.007*). There were no alterations in peak insulin concentration between conditions (*p>0.050*) and no differences between sexes for glucose or insulin responses.

CMJ: There was a main effect of sex for jump height, take-off velocity, peak and mean velocity and relative power, CM displacement, RFD at 200ms, impulse at 50-200ms, and total impulse (all *p<0.050*, Supplemental Table 5, Supplemental Digital Content). Males jumped higher and produced greater velocity, power, CM displacement, RFD and impulse. There was also a main effect of sex for RSI (*p=0.045*), calculated from CMJ and SJ height, with males displaying higher values than females. The CM displacement was lower during GEA than HEA_{REST} (*p=0.007*) and HEA_{EX} (*p=0.025*), and also lower during LEA_{EX} (*p=0.043*) than HEA_{REST}. Mean velocity was lower during GEA than LEA_{REST} (*p=0.041*). There was no sex*condition interaction for any outcome measure.

SJ: There was a main effect of sex for jump height, velocity at take-off, velocity, power, RFD at 150 and 200ms, and impulse at 50-200ms and total impulse (all $p<0.050$, Supplemental Table 6, Supplemental Digital Content), with males jumping higher and producing greater velocity, power, RFD, and impulse. During GEA, jump height and peak velocity were both lower compared to LEA_{REST} ($p=0.045$ and $p=0.043$) and HEA_{EX} ($p=0.016$ and $p=0.023$), whilst take-off velocity was also lower compared to HEA_{EX} ($p=0.040$). There was no sex*condition interaction for any variable.

IMTP: There was a main effect of sex for impulse between 50-250ms (all $p<0.010$) and RFD between 150-250ms (all $p<0.050$), with males producing a greater RFD and impulse (Supplemental Table 7, Supplemental Digital Content). There was no main effect of condition, nor sex*condition interaction for any outcome.

Wingate: There was a main effect of sex for relative peak power ($p=0.001$) and relative mean power $p=0.007$), with males producing a peak power 26% greater than females (12.0±2.1 $W \cdot kg^{-1}$ *vs* 8.9±1.6 $W \cdot kg^{-1}$, Figure 5A) and a mean power 21% greater (7.8±1.5 $W \cdot kg^{-1}$ *vs* 6.2±0.9 $W \cdot kg^{-1}$, Figure 5B). There was no main effect of condition, nor condition*sex interaction for either relative peak or mean power (all $p>0.050$). Fatigue index was also not different between sexes or across conditions ($p>0.050$).

Stroop Color and Word: There was no effect of sex, condition, nor their interaction on the Stroop effect (either proportion of correct responses or reaction time, all $p>0.050$, Supplemental Table 8, Supplemental Digital Content).

DISCUSSION

This study examined the effects of an acute 24-hour EA manipulation, induced by either diet or exercise, on substrate oxidation, postprandial metabolism and physical/cognitive performance among endurance trained males and females. Our primary findings demonstrate that the highest peak fat oxidation rate occurred under conditions of LEA induced by exercise (LEA_{EX}), with no differences in FATMAX between EA conditions. However, HR and RPE at FATMAX were both lowest under conditions of high EA for mass gain (GEA). Postprandial mean glucose concentration was higher in LEA_{REST} than other resting conditions (GEA and HEA_{REST}), and the insulin response was augmented in LEA_{REST} compared to the exercise conditions (LEA_{EX}/HEA_{EX}). There was no effect of EA manipulation on performance during the CMJ/IMTP, Wingate or Stroop Test, although SJ height was impaired with GEA. Lastly, the response to EA manipulations did not differ between sexes. Our findings therefore suggest that 24 hours of LEA is not a sufficient exposure to impair strength/power, sprint capacity, or cognitive performance, at least when measured post-prandially, but that 24 hours of exercise-induced LEA appears to influence substrate oxidation more than LEA induced by diet alone.

The elevated PFO and increased reliance on fat oxidation following LEA supports the predicted outcome of reduced EI and resultant decline in CHO availability, noting a three-fold decrease in CHO intake with LEA_{REST} (compared to LEA_{EX} and HEA_{REST}) and increased CHO utilization with the increased EEE during LEA_{EX} . Conversely, the increased CHO availability in the HEA_{REST} and GEA conditions decreased PFO and increased CHO oxidation. Interestingly, both conditions involving exercise (LEA_{EX} and HEA_{EX}) elicited a higher PFO compared to their respective EA-matched conditions without exercise (LEA_{REST} and HEA_{REST}), while PFO did not

differ between LEA_{EX} and HEA_{EX}. This increase in PFO following prior day exercise is likely underpinned by an acute decline in muscle and liver glycogen rather than a chronic adaptation from muscle retooling to increase fat oxidation via changes in fat mobilization and transport as observed following a ketogenic diet, since this requires >3-6 days of exposure (38). Indeed, depleted muscle glycogen has been demonstrated following three days of 19-20 $kcal \cdot kg^{-1}FFM \cdot day^{-1}$ among Tier 2 male runners (15, 17).

Our data suggest that 24 hours of LEA derived from energy/CHO restriction depletes muscle glycogen by preventing restoration from prior exercise, but this effect is amplified when further exercise contributes to the manipulation of EA. Thus, despite controlling for the overall EA reduction, acute achievement of the energy mismatch via exercise has a greater effect on CHO availability than energy restriction. In contrast, previous work by Loucks et al. (6) in young sedentary females demonstrated a decline in CHO oxidation during exercise following five days of LEA compared to optimal EA (10-15 vs 45-50 $kcal \cdot kg^{-1}FFM \cdot day^{-1}$, both 55% CHO), with both conditions involving exercise (30 $kcal \cdot kg^{-1}LBM \cdot day^{-1}$). Moreover, Loucks et al. (6) observed greater perturbations to luteinizing hormone pulsatility with diet- versus exercise-induced LEA. Here, exercise achieved an augmented decline in relative CHO availability which was attributed to a within-exercise glycogen-sparing achieved with a longer LEA exposure of four days. The influence of CHO availability is important; other work has demonstrated that low CHO availability, independent of LEA, is associated with perturbations to iron (21) and bone metabolism (23, 24). Therefore, more research is needed to differentiate the effects of exercise increase and dietary restriction on CHO availability, independently of EA outcomes. At present, the disparity between our results regarding CHO availability and that of Loucks et al. (6, 39) might

be explained by a higher training status of participants and shorter duration of EA manipulation in the current study.

The metabolic response to breakfast was associated with a 0.41-0.45 mmol·L^{-1} (7-8%) increase in mean postprandial glucose concentration in LEA_{REST} comparative to the other resting conditions (GEA and HEA_{REST}). Insulin iAUC and/or mean postprandial insulin concentrations were also 14-19% higher in LEA_{REST} compared to LEA_{EX} and HEA_{EX}. This increase in both postprandial glucose and insulin concentration under LEA_{REST} may suggest an over-compensatory metabolic response to the first high CHO meal following 24 hours of substantial underfeeding. Nevertheless, the mean postprandial glucose concentration in LEA_{REST} was 6.01±1.27 mmol·L^{-1} and glucose concentration returned to baseline by 90 minutes, indicating excellent glucose control (40). The elevated insulin concentration in LEA_{REST} versus exercise conditions (LEA_{EX} and HEA_{EX}) may reflect the influence of exercise in increasing insulin sensitivity (41). As insulin-stimulated peripheral tissue glucose uptake is considered the primary driver of post-prandial glucose tolerance (42), an increased insulin sensitivity following prior day exercise in LEA_{EX} and HEA_{EX} may result in a lower insulin concentration required to regulate postprandial glucose control compared to LEA_{REST}.

Total CHO oxidation during the FATMAX test immediately before the MMTT was identical across conditions (Table S4) and is therefore unlikely to explain the altered postprandial response. Due to increased hepatic glucose output during exercise, both glucose and insulin concentrations increased following the FATMAX test across all conditions. However, the magnitude of this increase was 4-10% larger in LEA_{REST} versus other conditions. It is possible that

an elevated glucose/insulin concentration immediately prior to the MMTT during LEA_{REST} somewhat accounted for the higher mean postprandial glucose/insulin concentrations. There were no differences in blood glucose concentration in the rested and fasted state between conditions, whereas baseline insulin concentration was 0.9-1.7 $uIU \cdot mL^{-1}$ lower in LEA_{REST}, LEA_{EX} and HEA_{EX} versus GEA. Lower fasted blood glucose/insulin has been reported following five days of LEA <20 $kcal \cdot kg^{-1}FFM \cdot day^{-1}$ (4, 9). Our results suggest that just 24 hours of LEA, in addition to independent effects of exercise, may lead to lower next day fasted insulin.

There were no alterations to peak or mean power output during the Wingate across conditions, and no effect of EA on CMJ height or IMTP peak force. During the SJ, participants jumped an average of 1.2-1.3 cm (5%) lower under conditions of GEA versus LEA_{REST} and HEA_{EX}. When considering kinetic/kinematic outcomes, there was little alteration across EA conditions. The exceptions were a 2.9-3.3 cm (~10%) lower CM displacement in the CMJ under GEA versus HEA_{REST} and HEA_{EX}, alongside a 2.7 cm (~9%) decline in CM displacement in LEA_{EX} versus HEA_{REST}. Mean velocity was also 0.04 $m \cdot s^{-1}$ (3%) lower during the CMJ in GEA versus LEA_{REST}. The SJ peak velocity was 0.06 $m \cdot s^{-1}$ (3%) lower in GEA versus both LEA_{REST} and HEA_{EX}, while take-off velocity was also 0.06 $m \cdot s^{-1}$ (3%) lower in GEA compared to HEA_{EX}. The decreased jump height, CM displacement, and velocity with GEA may be the result of an elevated body mass in GEA (Table S3). A lack of change in overall performance (jump height, power output, peak force production) following LEA suggests a 24-hour exposure is too brief to impair strength/power performance. However, as these measures were not a primary outcome variable, we may lack statistical power. Additionally, while lower limb muscle soreness on day three was higher in exercise versus non-exercise conditions, the absolute soreness ratings were low

for both ("two" out of six; moderate soreness/slight persistent ache vs "one"; light soreness/ vague ache, respectively). As physical performance was not altered between conditions of rest and exercise, it appears that prior day exercise-induced muscle soreness was not substantial to influence overall performance.

To our knowledge, this is the first study to examine acute (24-hour) manipulation in EA on strength/power or sprint capacity. Acute (five day) periods of rapid weight loss can impair dynamic force expression (e.g., punching force) (43). However, such studies are typically free living, examining EI/EEE through dietary recall/training logs without directly manipulating EA, challenging comparisons to our study, which implemented a high degree of control. A longer (14 day) period of exercise-induced EAs ranging from 9-22 $kcal \cdot kg^{-1}FFM \cdot day^{-1}$ among Tier 2-3 males all reduced CMJ height by ~3 cm (44, 45). However, EA was altered in the field by monitoring participant self-selected EI and then altering EEE accordingly, rather than the more precise method of prescribing a standardized EI/EEE as in the present study.

Manipulations to EA did not influence the Stroop effect (proportion of correct responses or reaction time). Interestingly, prior work has observed declines in other aspects of cognitive function when exercise (but not diet) is used to induce LEA (19, 20, 46). Moreover, we observed no differences to cognitive performance between the sexes, in contrast to Lieberman et al. (20), who observed a decline in cognitive function among women, but not men, experiencing LEA. However, women accounted for only 26% (n=6) of the sample in this study, and energy intake was severely restricted [266 $kcal \cdot day^{-1}$ combined with a 4-hour exercise bout (20)], which is unlikely to represent practices in elite athletes.

Sex is thought to moderate the response to EA manipulations, with women potentially more sensitive to acute LEA, and experiencing negative consequences at a higher EA compared to men (8). Our findings do not support this theory; we observed no differences in the response to EA manipulations between the sexes in performance, substrate oxidation, or postprandial metabolism. Given innate differences in substrate oxidation, with women demonstrating a lesser reliance on whole-body CHO oxidation to support fuel requirements for endurance activities (47), and reaching FATMAX at greater exercise intensities than males (26), it may be that reduced muscle glycogen influences substrate oxidation in males more than in females. However, the response to substrate oxidation with altered EA did not differ between the sexes, whilst there was also no difference in the exercise intensity eliciting FATMAX. This may be explained in part by a lower $\dot{V}O_2max$ in our female participants compared to males (although not statistically different *p=0.145*), perhaps suggesting a reduced capability of our female athletes to oxidize fats as a fuel source relative to more endurance-trained athletes (48), however this is speculative.

Sex-based differences in substrate oxidation/performance are hypothesized to be mediated by endogenous estrogen concentrations (47). Therefore, a lack of differential response may be a result of studying females using OCP, eliminating the cyclical fluctuations in endogenous estrogen observed in naturally menstruating female athletes. Indeed, there are some reports of altered CHO/fat oxidation during 45-90 min submaximal cycling, alongside power output across repeated sprints, between the follicular and luteal phases (47, 49-51), with no such effects observed between active and withdrawal pill-taking phases among OCP users (50). However, the directionalities of such alterations are conflicting. Moreover, because adequate classification and control of menstrual status was implemented in only one of these studies (50), conclusions regarding an effect

of menstrual status on glycogen utilization and muscular power are difficult. A lack of sex-based differences may also be due to a shorter, more severe EA restriction in the present study, with conditions of LEA providing 15 kcal·kg^{-1}FFM·day^{-1}. It is possible that different responses would be observed across longer time periods or at a higher LEA threshold (~20-25 kcal·kg^{-1}FFM·day^{-1}). However, this remains speculative.

Our findings should be considered in light of potential limitations. First, because we recruited female athletes utilizing OCP to facilitate the standardization of ovarian hormones, our results directly apply only to a subset of women. Second, during the conditions involving exercise (LEA_{EX} and HEA_{EX}), participants were not provided explicit instructions about the timing of food intake around exercise. Given the effect of nutrient timing on post-exercise muscle glycogen resynthesis (52, 53), there may have been small differences in post-exercise muscle glycogen repletion that may have consequently altered next-day substrate oxidation. Finally, only whole-body substrate oxidation was measured and future research examining the rate of appearance and disappearance of glucose and lipids would provide greater mechanistic detail.

CONCLUSIONS AND FUTURE RESEARCH

A brief (24-hour) period of LEA appeared not to impair strength/power, sprint capacity, or cognitive performance, at least when measured post-prandially. However, in trained individuals, a 24-hour LEA exposure induced by exercise appears to influence substrate oxidation to a greater extent that LEA induced by diet alone. Whether this translates to more prolonged EA manipulation is of interest, as is the potential for independent alterations to CHO availability to affect various

body systems. Lastly, future research may consider examining LEA between ~20-25 kcal·kg^{-1}FFM·day^{-1} to elucidate potential sex differences that may occur.

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FIGURE LEGENDS

Figure 1. Project overview. Timings on day three were consistent within participants (± 8 min) and varied ± 1.5 h between participants according to participants' habitual wake time (assessed via the Morningness-Eveningness Questionnaire (2)). *RMR,* resting metabolic rate; *DXA*, dual-energy X-ray absorptiometry; *FATMAX*; exercise intensity at which the maximal rate of fat oxidation occurs; *EA,* energy availability; *EI*, energy intake; *FFM,* fat free mass; *LEA*, low energy availability; *HEA*, high energy availability; *GEA,* high energy availability for mass gain/growth; *MMTT,* mixed meal tolerance test; *IMTP*, isometric mid-thigh pull; *CMJ*, countermovement jump; *SJ*, squat jump; *CHO*, carbohydrate. Figure created with BioRender.com.

Figure 2. Peak fat oxidation (A) absolute values and, (B) values expressed relative to FFM. Colors denote sex. *FFM,* fat free mass; *LEA*, low energy availability; *HEA*, high energy availability; *GEA,* high energy availability for mass gain/growth. *denotes significance $p<0.05$, **denotes significance $p<0.01$, ***denotes significance $p<0.0001$.

Figure 3. (A) Absolute power output at FATMAX, (B) power output at FATMAX relative to body mass, (C) heart rate at FATMAX as a percentage of HRmax, (D) RPE at FATMAX, (E) $\dot{V}O_2$ at FATMAX as a percentage or $\dot{V}O_2$max, and (F) time to reach FATMAX from the start of exercise. *FATMAX,* the exercise intensity at which maximal fat oxidation occurs; *HRmax,* maximal heart rate*; LEA*, low energy availability; *HEA*, high energy availability; *GEA,* high energy availability for mass gain/growth. Colors denote sex. *denotes significance $p<0.05$, **denotes significance $p<0.01$.

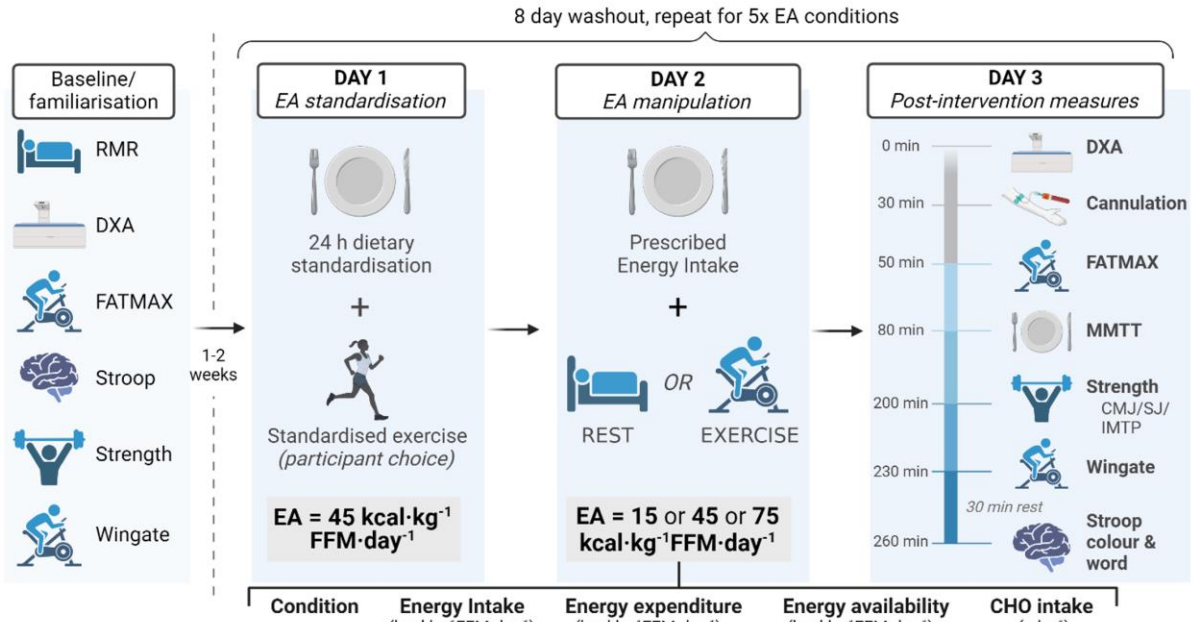
Figure 4. (A) Postprandial serum glucose concentration, (B) serum glucose incremental area under the curve, (C) postprandial serum insulin concentration, (D) serum insulin incremental area under the curve during the mixed meal tolerance test. Data is expressed as mean ± standard deviation. Dashed lines in figures 4A and 4C represent the standard deviations. *iAUC*; incremental area under the curve, *LEA*, low energy availability; *HEA*, high energy availability; *GEA,* high energy availability for mass gain/growth. **denotes significance $p<0.01$.

Figure 5. (A) relative peak power, and (B) relative mean power during a 30 sec Wingate performance task. Data is expressed as mean ± standard deviation, displayed across conditions. Colors denote sex. *LEA*, low energy availability; *HEA*, high energy availability; *GEA,* high energy availability for mass gain/growth.

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Supplementary.docx

Figure 1



Condition	Energy Intake (kcal·kg⁻¹FFM·day⁻¹)	Energy expenditure (kcal·kg⁻¹FFM·day⁻¹)	Energy availability (kcal·kg⁻¹FFM·day⁻¹)	CHO intake (g·kg⁻¹)
LEA_{REST}	15	0	15	1.6±0.2
LEA_{EX}	45	30	15	4.7±0.5
HEA_{REST}	45	0	45	4.7±0.5
HEA_{EX}	75	30	45	7.8±0.8
GEA	75	0	75	7.8±0.8

Figure 2

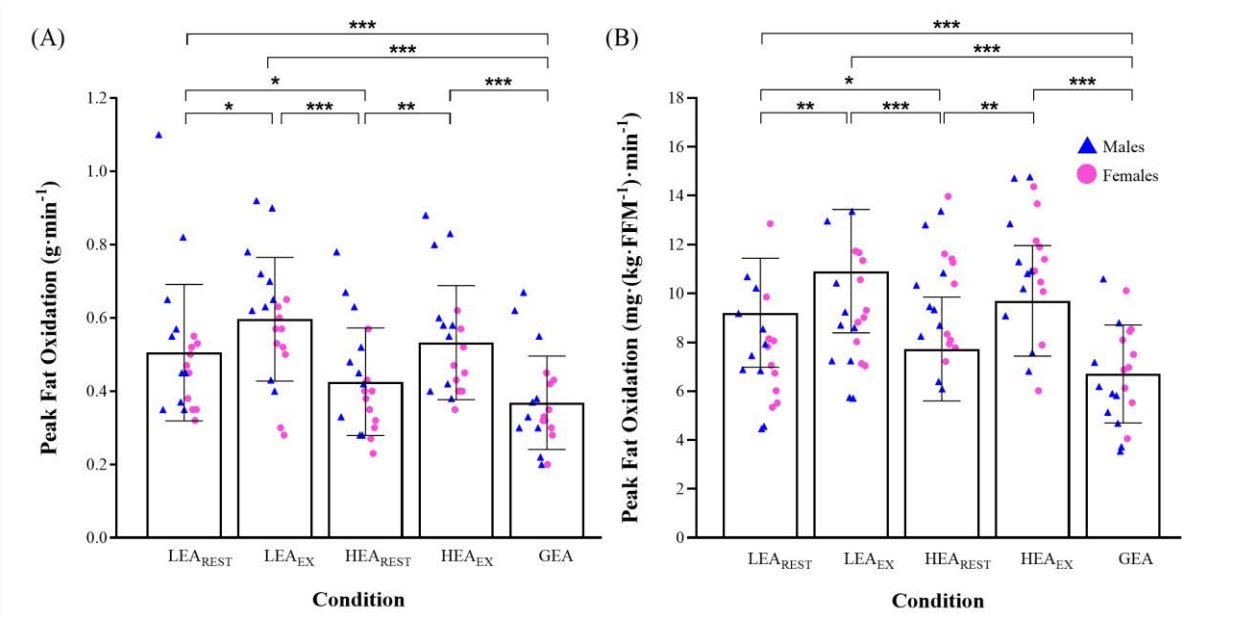

(A)
(B)
Peak Fat Oxidation (g·min-1)
Peak Fat Oxidation (mg·(kg·FFM-1)·min-1)
Males
Females
LEAREST
LEAEX
HEAREST
HEAEX
GEA
Condition
***
**
*

Figure 3

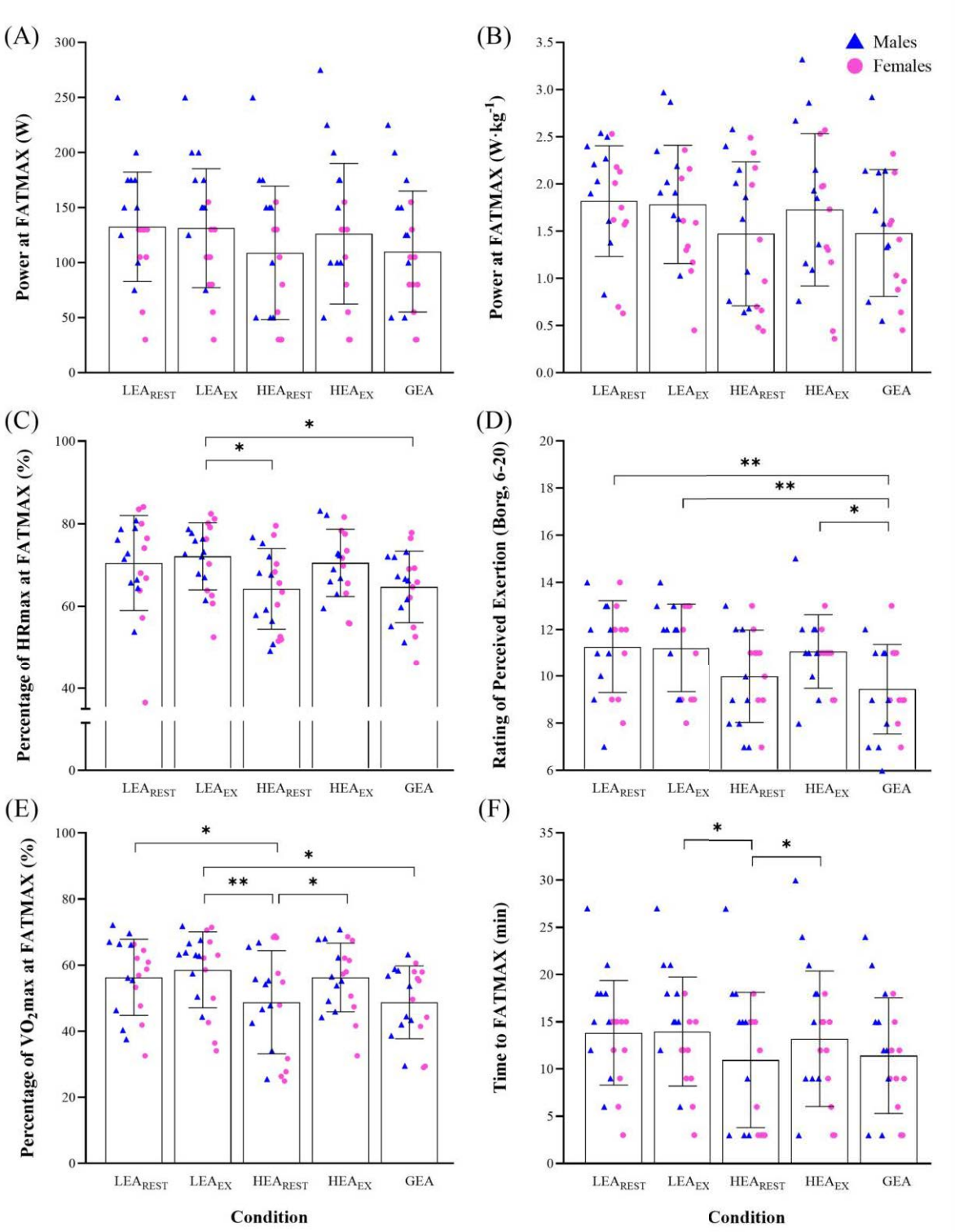

(A)
Power at FATMAX (W)
(B)
Power at FATMAX (W·kg-1)
Males
Females
(C)
Percentage of HRmax at FATMAX (%)
(D)
Rating of Perceived Exertion (Borg, 6-20)
(E)
Percentage of VO2max at FATMAX (%)
(F)
Time to FATMAX (min)
LEAREST
LEAEX
HEAREST
HEAEX
GEA
Condition

Figure 4

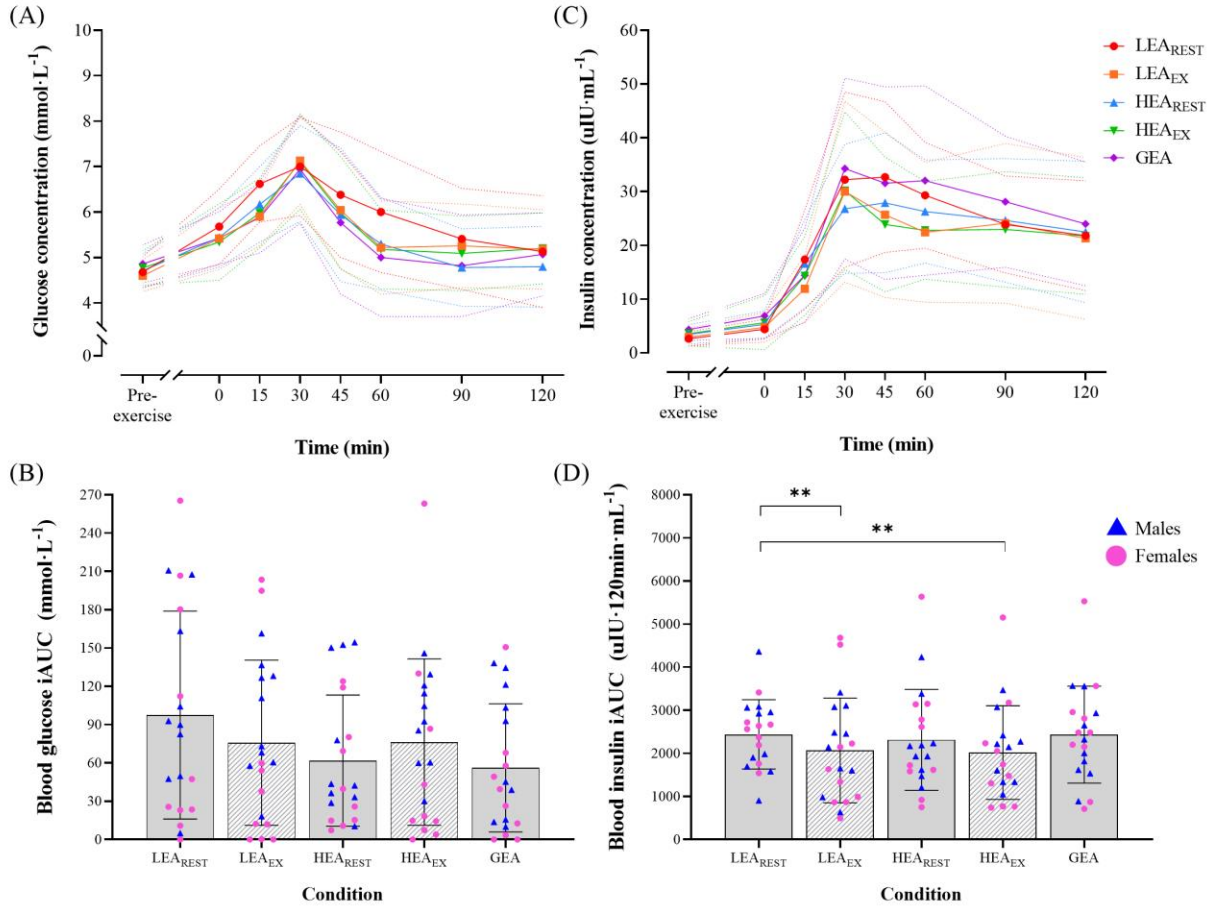

(A)
Glucose concentration (mmol·L-1)
Pre-exercise
0
15
30
45
60
90
120
Time (min)
(C)
Insulin concentration (uIU·mL-1)
LEAREST
LEAEX
HEAREST
HEAEX
GEA
(B)
Blood glucose iAUC (mmol·L-1)
Condition
(D)
Blood insulin iAUC (uIU·120min·mL-1)
**
**
Males
Females

Figure 5

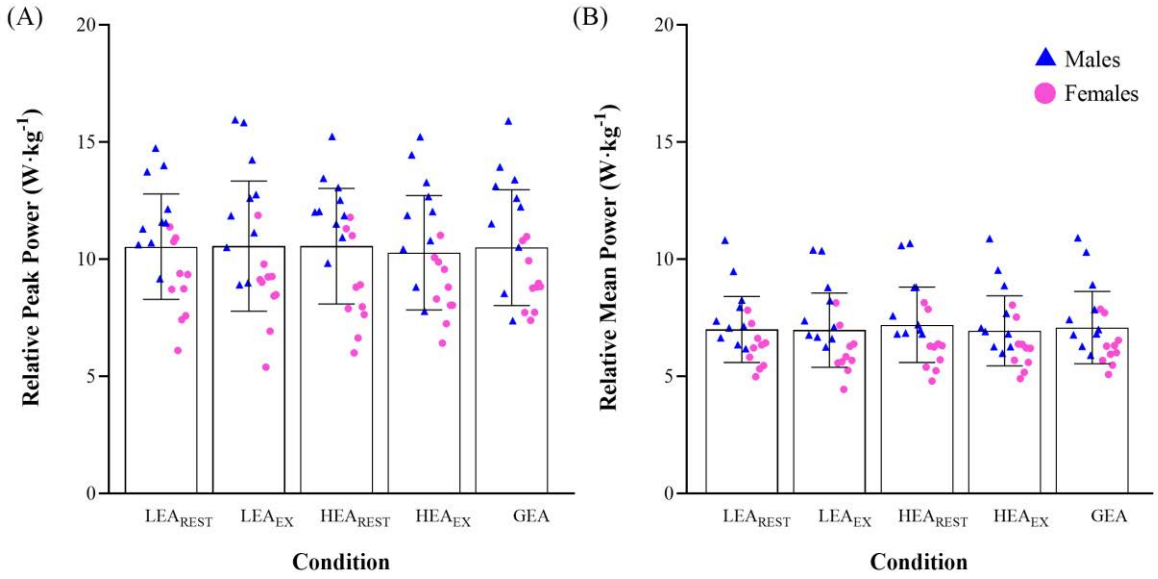

(A)
Relative Peak Power (W·kg-1)
20
15
10
5
0
LEAREST
LEAEX
HEAREST
HEAEX
GEA
Condition
(B)
Relative Mean Power (W·kg-1)
Males
Females
Condition

Table 1. Participant characteristics.

	Females (n=10)	Males (n=10)
Age (yrs)	33±7	38±9
Athletic tier	Tier 2 (n=8)	Tier 2 (n=10)
	Tier 3 (n=2)	
Primary sport	Cycling (n=3)	Cycling (n=5)
	Mountain bike (n=1)	Mountain bike (n=3)
	Triathlon (n=4)	Triathlon (n=1)
	Running (n=2)	Running (n=1)
Body mass (kg)	65.6±10.9	81.0±12.4
Fat free mass (kg)	47.7±6.1	62.4±9.7
Body fat percentage (%)	26.7±5.6	22.6±8.3
Body mass index	23.6±3.3	25.2±3.4
Absolute $\dot{V}O_2max$ ($L \cdot min^{-1}$)	2.8±0.4	4.1±0.8
Relative $\dot{V}O_2max$ ($ml \cdot kg \cdot min^{-1}$)	44.4±8.1	50.9±10.8
Absolute Wmax (W)	263±24	350±71
Relative Wmax ($W \cdot kg^{-1}$)	4.1±0.6	4.4±1.0

Values displayed as mean ± standard deviation. Athletic tier as defined by McKay et al. (1)

Wmax; maximal power output in Watts

Table 2. Energy availability, energy intake, exercise energy expenditure, and macronutrient composition of all five experimental conditions, for both males and females.

	Males (n=10)					Females (n=10)				
	LEA_{REST}	LEA_{EX}	HEA_{REST}	HEA_{EX}	GEA	LEA_{REST}	LEA_{EX}	HEA_{REST}	HEA_{EX}	GEA
Energy availability ($kcal \cdot kg^{-1}FFM \cdot day^{-1}$)	15.0±0.2^	15.0±0.4^	44.8±0.8°	44.7±0.9°	74.0±1.1*	15.1±0.3^	15.1±0.4^	45.0±0.9°	44.9±0.6°	75.1±0.7*
Energy intake (kcal)	934±139*	2799±436†	2799±436†	4678±733&#	4679±733&#	712±93*	2145±269†	2145±269†	3578±466&#	3576±465&#
Exercise energy expenditure (kcal)	-	1867±282#	-	1867±282#	-	-	1430±184#	-	1430±184#	-
Carbohydrate intake ($g \cdot kg^{-1}$)	1.6±0.2*	4.8±0.5†	4.8±0.5†	7.4±0.9&	7.9±0.9&	1.6±0.3*	4.6±0.4†	4.6±0.4†	7.1±1.9&	7.5±0.6&
Protein intake ($g \cdot kg^{-1}$)	0.8±0.1*	2.2±0.2†	2.3±0.3†	3.6±0.4&	3.6±0.4&	0.7±0.1*	2.1±0.2†	2.1±0.2†	3.4±0.3&	3.4±0.3&
Fat intake	0.2±0.0*	0.8±0.1†	0.8±0.1†	1.3±0.1&	1.2±0.1&	0.2±0.0*	0.7±0.1†	0.7±0.1†	1.2±0.1&	1.3±0.1&

($g \cdot kg^{-1}$)

Values displayed as mean ± standard deviation. LEA, low energy availability; *HEA*, high energy availability; *GEA,* high energy availability for mass gain/growth. **significantly different from all other conditions. ^significantly different from HEA_{REST}, HEA_{EX}, and GEA, °significantly different from LEA_{REST}, LEA_{EX}, and GEA, †significantly different from LEA_{REST}, HEA_{EX}, and GEA, &significantly different from LEA_{REST}, LEA_{EX} and HEA_{REST}. #significantly different from same condition in the opposite sex.*

Table 3. Self-reported/ perceived muscle soreness on trial day three averaged across all timepoints for each condition, where "0" represents a complete absence of soreness, and "6" denotes a severe muscle soreness, stiffness or weakness that limits the ability to move.

Condition	Soreness (0-6)	*p value*	
		vs LEA_{EX}	vs HEA_{EX}
LEA_{REST}	1.1 + 1.4	***<0.0001****	***<0.0001****
LEA_{EX}	1.9 + 1.5	-	*0.993*
HEA_{REST}	1.2 + 1.4	***0.0003****	***0.0001****
HEA_{EX}	2.0 + 1.6	*0.993*	-
GEA	0.8 + 1.1	***<0.0001****	***<0.0001****

Values displayed as mean ± SD. LEA, low energy availability; *HEA*, high energy availability; *GEA,* high energy availability for mass gain/growth. ***denotes significance p<0.01, ****denotes significance p<0.0001.*

Table S1. The type, formulation, brand, and length of usage of oral contraceptive pill across n=10 female participants.

Pill brand	Pill type (mono-/bi-/tri-phasic)	Pill formulation	Length of usage (years)
Estelle 35 ED	Monophasic	35 µg ethinyloestradiol, 2 mg cyproterone acetate	6
Evelyn ED 150/30	Monophasic	30 µg ethinyloestradiol, 150 µg levonorgestrel	24
Femme-Tab 20/100	Monophasic	20 µg ethinyloestradiol, 100 µg levonorgestrel	1
Isabelle	Monophasic	30 µg ethinyloestradiol, 3mg drospirenone	0.3
Levlen ED 150/30	Monophasic	30 µg ethinyloestradiol, 150 µg levonorgestrel	10
Levlen ED 150/30	Monophasic	30 µg ethinyloestradiol, 150 µg levonorgestrel	5
Levlen ED 150/30	Monophasic	30 µg ethinyloestradiol, 150 µg levonorgestrel	3
Micronelle 30 ED	Monophasic	30 µg ethinyloestradiol, 150 µg levonorgestrel	20
Yang 20/3	Monophasic	20 µg ethinyloestradiol, 3mg drospirenone	0.3
Yasmin	Monophasic	30 µg ethinyloestradiol, 3mg drospirenone	1.5

	Median	**4.0**
	Min	**0.3**
	Max	**24.0**

Length of usage taken from the time of commencing the study.

Table S2. The 7-point Likert scale used to assess lower limb muscle soreness (27).

Please indicate how your leg muscles feel at this present moment.

0	A complete absence of soreness
1	A light soreness in the muscle felt only when touched/ a vague ache
2	A moderate soreness/ pain felt only when touched/ a slight persistent ache/ pain
3	A light muscle soreness/ pain when walking up or down stairs
4	A light muscle soreness, pain, stiffness or weakness when walking on a flat surface
5	A moderate muscle soreness, pain, stiffness or weakness when walking
6	A severe muscle soreness, stiffness or weakness that limits my ability to move

Table S3. Participant fat-free mass as measured by DXA scan (participants fasted), and body mass as measured on the force plate by ForceDecks software (participants fed), across all five conditions of energy availability. Results are averaged across sexes.

Condition	Fat-free mass (kg)	*p value (vs LEA_{REST})*	*p value (vs LEA_{EX})*	Body mass (kg)	*p value (vs LEA_{REST})*
LEA_{REST}	54.6 ± 11.0	-	*0.733*	73.3 ± 13.7	-
LEA_{EX}	54.8 ± 11.1	*0.733*	-	73.7 ± 14.0	*0.447*
HEA_{REST}	55.2 ± 11.0	***0.022****	*0.339*	74.0 ± 14.1	***0.030****
HEA_{EX}	55.5 ± 11.2	***0.0004*****	***0.021****	73.9 ± 13.8	*0.056*
GEA	55.5 ± 11.3	***0.0002*****	***0.011****	74.2 ± 14.1	***0.0006*****

*Values displayed as mean ± standard deviation. LEA, low energy availability; HEA, high energy availability; GEA, high energy availability for mass gain/growth. *denotes significances $p<0.05$, ***denotes significance $p<0.001$.*

Table S4. Energy expenditure, carbohydrate oxidation and test duration of the FATMAX test across all five conditions of energy availability. Results are averaged across sexes.

Condition	Energy expenditure (kcal)	Carbohydrate oxidation ($g \cdot min^{-1}$)	Test duration (min: seconds)
LEA_{REST}	200±90 [c]	29.2±13.7	22:12 [c e]
LEA_{EX}	217±96 [c e]	28.9±14.0	22:48 [c e]
HEA_{REST}	178±96 [a b d]	28.2±14.6	20:30 [a b]
HEA_{EX}	205±101 [c]	29.5±13.7	22:00 [e]
GEA	182±89 [b]	31.7±15.0	20:12 [a b d]

Values displayed as mean ± standard deviation. LEA, low energy availability; HEA, high energy availability; GEA, high energy availability for mass gain/growth. [a] significantly different from LEA_{REST} condition, [b] significantly different from LEA_{EX} condition, [c] significantly different from HEA_{REST} condition, [d] significantly different from HEA_{EX} condition, [e] significantly different from GEA condition.

Table S5. Outcome measures assessed during the countermovement jump across all five conditions of energy availability and between sexes.

		LEA_{REST}	LEA_{EX}	HEA_{REST}	HEA_{EX}	GEA	*p value (main effect of condition)*	*p value (main effect of sex)*	*p value (sex*condition interaction)*
Jump height (cm)							*0.158*	***0.002****	*0.503*
	Females	21.1±4.3	21.8±4.9	21.3±5.0	21.3±5.2	20.8±4.9			
	Males	29.9±5.9	29.4±5.6	29.6±5.4	29.5±5.2	28.8±5.6			
	Average	25.5±6.7	25.4±6.6	25.4±6.6	25.4±6.6	24.8±6.6			
Velocity at take-off ($m \cdot s^{-1}$)							*0.217*	***0.003****	*0.567*
	Females	2.03±0.22	2.06±0.25	2.05±0.25	2.03±0.28	2.01±0.24			
	Males	2.42±0.24	2.40±0.23	2.40±0.23	2.41±0.21	2.38±0.23			
	Average	2.22±0.30	2.23±0.29	2.23±0.30	2.22±0.31	2.20±0.30			
Peak velocity ($m \cdot s^{-1}$)							*0.069*	***0.001****	*0.530*
	Females	2.18±0.19	2.20±0.22	2.20±0.21	2.18±0.23	2.16±0.21			
	Males	2.56±0.21	2.53±0.21	2.54±0.20	2.54±0.19	2.51±0.21			
	Average	2.37±0.27	2.36±0.27	2.37±0.27	2.36±0.28	2.33±0.27			
Mean velocity ($m \cdot s^{-1}$)							***0.029****	***<0.0004****	*0.602*
	Females	1.17±0.12	1.12±0.09	1.12±0.09	1.14±0.16	1.13±0.16			
	Males	1.37±0.10	1.37±0.07	1.37±0.07	1.37±0.08	1.32±0.08			
	Average	**1.27±0.15^**	1.25±0.15	1.25±0.15	1.25±0.17	**1.23±0.15^**			
Relative peak power ($W \cdot kg^{-1}$)							***0.033***$^{@}$	***0.014****	*0.780*

	Females	36.6±6.5	36.5±5.8	35.6±5.4	35.6±5.6	35.4±5.7			
	Males	44.2±6.7	43.9±6.7	43.4±6.4	43.5±6.7	43.2±6.7			
	Average	40.4±7.5	40.2±7.2	39.7±7.0	39.5±7.3	39.5±7.3			
Relative mean power ($W \cdot kg^{-1}$)							*0.109*	***0.009*****	*0.984*
	Females	2.03±0.80	1.83±0.60	1.58±0.58	1.82±0.70	1.66±0.78			
	Males	2.68±0.74	2.54±0.63	2.38±0.53	2.55±0.79	2.45±0.74			
	Average	2.35±0.82	2.19±0.70	1.98±0.68	2.18±0.82	2.06±0.84			
Relative peak force ($N \cdot kg^{-1}$)							*0.715*	*0.075*	*0.676*
	Females	19.6±1.3	20.0±2.0	19.3±1.8	19.4±2.1	19.9±2.1			
	Males	21.0±1.7	21.3±1.6	21.4±1.3	21.0±1.1	20.8±1.9			
	Average	20.3±1.7	20.7±1.9	20.4±1.8	20.2±1.8	20.4±2.0			
Relative mean force ($N \cdot kg^{-1}$)							*0.136*	*0.383*	*0.377*
	Females	12.4±0.7	12.2±0.5	12.1±0.4	12.1±0.5	12.2±0.4			
	Males	12.5±0.6	12.3±0.6	12.3±0.5	12.5±0.6	12.3±0.6			
	Average	12.4±0.7	12.2±0.5	12.2±0.4	12.3±0.6	12.2±0.5			
Rate of force development at 50 ms ($N \cdot s^{-1}$)							*0.265*	*0.798*	*0.962*
	Females	3590±1804	3056±2234	3370±1997	3635±1959	4477±3247			
	Males	5086±3589	3494±2465	2892±1412	4127±2282	3385±1838			
	Average	4377±2912	3275±2301	3131±1701	3881±2086	3931±2628			
Rate of force development at 100 ms ($N \cdot s^{-1}$)							*0.435*	*0.274*	*0.647*
	Females	3143±1178	2952±1839	2854±1326	3038±1567	3746±2438			

	Males	4608±2509	3547±1961	3332±1438	3975±1771	3550±1864		
	Average	3914±2081	3249±1875	3093±1369	3507±1697	3648±2114		
Rate of force development at 150 ms ($N \cdot s^{-1}$)							*0.847*	*0.050*	*0.861*
	Females	3359±1667	2706±1480	2749±1180	2842±1296	3149±1693		
	Males	4003±1605	4042±2108	3619±1450	4116±1417	3733±1749		
	Average	3681±1627	3374±1901	3184±1362	3479±1474	3441±1702		
Rate of force development at 200 ms ($N \cdot s^{-1}$)							*0.896*	***0.0003****	*0.929*
	Females	2519±746	2324±979	2281±942	2476±878	2414±1126		
	Males	3685±957	3854±1825	3648±1010	4005±988	3457±1597		
	Average	3102±1027	3089±1627	2965±1181	3240±1202	2936±1448		
Impulse at 50 ms (N·s)							*0.403*	***0.0007****	*0.397*
	Females	64.0±17.9	57.2±7.5	59.7±10.4	58.6±10.6	59.2±10.8		
	Males	79.7±11.5	80.5±16.9	80.6±13.2	80.5±12.5	78.6±11.0		
	Average	71.8±16.8	68.9±17.5	70.1±15.7	69.6±15.9	68.9±14.5		
Impulse at 100 ms (N·s)							*0.324*	***0.0006****	*0.186*
	Females	126.7±30.1	114.4±14.2	117.3±19.7	116.6±19.6	119.1±20.9		
	Males	158.7±25.2	160.5±32.7	159.8±26.0	159.8±25.6	157.5±23.3		
	Average	142.7±31.6	137.4±34.1	138.5±31.3	138.2±31.4	138.3±29.2		
Impulse at 150 ms (N·s)							*0.418*	***0.0007****	*0.483*
	Females	182.8±33.1	171.8±22.5	173.6±28.6	172.8±27.0	177.4±31.1		
	Males	234.2±38.0	234.7±45.8	233.1±38.6	234.5±38.7	233.0±35.3		

	Average	208.5±43.6	203.2±47.7	203.3±45.0	203.6±45.3	205.2±43.2			
Impulse at 200 ms (N·s)							*0.421*	***0.001****	*0.766*
	Females	234.2±36.8	231.6±34.0	230.4±38.5	229.7±34.7	237.9±42.5			
	Males	306.9±50.3	303.6±49.9	303.5±50.2	306.4±50.7	306.4±46.8			
	Average	272.5±57.1	267.6±55.6	266.9±57.5	268.1±57.8	272.2±55.9			
Total impulse (N·s)							*0.081*	***0.002****	*0.608*
	Females	320.3±77.1	334.8±72.8	341.0±68.4	343.3±77.6	325.4±57.9			
	Males	448.6±91.5	452.8±102.1	461.1±91.8	454.8±81.4	453.5±97.5			
	Average	384.4±105.4	393.8±105.4	401.1±100.0	399.0±96.3	389.5±102.0			
Flight time: contraction time (s)							*0.097*	*0.493*	*0.293*
	Females	0.55±0.14	0.50±0.10	0.49±0.09	0.48±0.11	0.51±0.09			
	Males	0.56±0.12	0.52±0.12	0.52±0.10	0.56±0.12	0.53±0.12			
	Average	0.55±0.13	0.51±0.11	0.50±0.09	0.52±0.12	0.52±0.11			
Contraction time (s)							*0.208*	*0.188*	*0.336*
	Females	0.82±0.13	0.89±0.16	0.89±0.11	0.92±0.12	0.85±0.09			
	Males	0.93±0.17	0.94±0.19	1.00±0.17	0.91±0.14	0.93±0.16			
	Average	0.87±0.16	0.91±0.17	0.94±0.15	0.92±0.13	0.89±0.13			
Concentric time (s)							*0.209*	*0.118*	*0.203*
	Females	0.53±0.08	0.58±0.13	0.58±0.07	0.60±0.09	0.56±0.08			
	Males	0.61±0.11	0.67±0.20	0.66±0.13	0.59±0.08	0.67±0.19			
	Average	0.57±0.10	0.63±0.17	0.62±0.11	0.59±0.09	0.61±0.15			

Eccentric time (s)							*0.098*	*0.430*	*0.675*
	Females	0.29±0.06	0.31±0.05	0.32±0.05	0.32±0.05	0.30±0.03			
	Males	0.32±0.06	0.32±0.07	0.33±0.07	0.33±0.06	0.32±0.07			
	Average	0.30±0.06	0.32±0.06	0.32±0.06	0.32±0.05	0.31±0.06			
Centre of mass displacement (cm)							***0.003****	***0.018****	*0.933*
	Females	-29.1±4.6	-28.3±3.6	-30.6±4.5	-29.9±3.8	-27.7±2.9			
	Males	-36.1±7.4	-35.1±9.0	-38.2±9.5	-38.1±9.1	-34.5±8.5			
	Average	-32.8±7.1	**-31.7±7.6**$^{\#}$	**-34.4±8.2**$^{\wedge\#}$	**-34.0±8.0**$^{\lambda}$	**-31.1±7.1**$^{\wedge\lambda}$			
Eccentric utilization ratio							*0.205*	*0.232*	*0.452*
	Females	1.06±0.07	1.08±0.13	1.06±0.07	1.01±0.07	1.06±0.07			
	Males	1.06±0.09	1.09±0.09	1.12±0.14	1.09±0.07	1.10±0.07			
	Average	1.06±0.08	1.09±0.11	1.09±0.11	1.04±0.08	1.08±0.07			
Reactive strength index							*0.171*	***0.045****	*0.691*
	Females	0.010±0.01	0.012±0.02	0.012±0.01	0.003±0.02	0.011±0.01			
	Males	0.019±0.03	0.024±0.03	0.032±0.03	0.021±0.02	0.028±0.02			
	Average	0.015±0.02	0.018±0.02	0.022±0.03	0.012±0.02	0.020±0.02			
Dynamic strength index							*0.634*	*0.504*	*0.318*
	Females	0.73±0.19	0.70±0.14	0.70±0.11	0.68±0.11	0.70±0.12			
	Males	0.65±0.15	0.65±0.15	0.67±0.17	0.66±0.13	0.69±0.17			
	Average	0.69±0.17	0.67±0.14	0.69±0.14	0.67±0.12	0.70±0.14			

*Values displayed as mean ± standard deviation. LEA, low energy availability; HEA, high energy availability; GEA, high energy availability for mass gain/growth. *denotes significance between the sexes/conditions p<0.05, **denotes significant difference between the sexes/conditions p<0.01, ***denotes significant difference between the sexes/conditions p<0.001. [@]denotes no significant differences between conditions in post-hoc testing. ^/[#]/[λ] significantly different from other condition with a matching symbol.*

Table S6. Outcome measures assessed during the squat jump across all five conditions of energy availability and between sexes.

		LEA_{REST}	LEA_{EX}	HEA_{REST}	HEA_{EX}	GEA	*p value (main effect of condition)*	*p value (main effect of sex)*	*p value (sex*condition interaction)*
Jump height (cm)							***0.008****	***0.004****	*0.282*
	Females	19.8±4.7	21.0±6.0	20.1±4.4	21.0±4.8	19.7±5.0			
	Males	28.0±4.2	27.0±4.5	26.4±4.3	27.4±4.3	26.0±4.1			
	Average	**24.1±6.0^**	24.2±6.0	23.2±5.3	**24.2±5.5$^{\#}$**	**22.9±5.5^$^{\#}$**			
Velocity at take-off ($m \cdot s^{-1}$)							***0.039****	***0.006****	*0.541*
	Females	1.97±0.25	2.01±0.32	1.98±0.23	2.02±0.27	1.96±0.25			
	Males	2.35±0.17	2.30±0.19	2.28±0.18	2.32±0.18	2.27±0.17			
	Average	2.17±0.28	2.17±0.29	2.13±0.25	**2.17±0.27^**	**2.11±0.26^**			
Peak velocity ($m \cdot s^{-1}$)							***0.016****	***0.002****	*0.354*
	Females	2.12±0.20	2.18±0.28	2.14±0.20	2.18±0.21	2.12±0.21			
	Males	2.49±0.15	2.44±0.18	2.43±10.7	2.46±0.16	2.41±0.15			
	Average	**2.32±0.26^**	2.32±0.26	2.29±0.23	**2.32±0.23$^{\#}$**	**2.26±0.23^$^{\#}$**			
Mean velocity ($m \cdot s^{-1}$)							*0.400*	***0.0006*****	*0.575*
	Females	0.76±0.10	0.78±0.20	0.80±0.10	0.83±0.15	0.75±0.12			
	Males	0.97±0.09	0.95±0.06	0.92±0.13	0.97±0.11	0.93±0.06			
	Average	0.87±0.14	0.87±0.16	0.86±0.13	0.90±0.15	0.84±0.13			
Relative peak power ($W \cdot kg^{-1}$)							*0.051*	***0.040****	*0.216*

	Females	35.3±5.9	36.4±6.7	35.7±5.8	36.4±5.8	35.0±5.7			
	Males	42.3±5.3	41.4±5.8	41.0±4.8	40.8±5.0	40.6±5.7			
	Average	39.0±6.5	39.0±6.6	38.4±5.9	38.6±5.7	37.8±6.3			
Relative mean power ($W \cdot kg^{-1}$)							*0.269*	***0.004*****	*0.571*
	Females	10.8±1.8	11.3±3.9	11.5±2.0	12.0±3.2	10.5±2.3			
	Males	14.3±1.5	14.0±1.2	13.4±2.4	14.3±2.0	13.6±1.1			
	Average	12.7±2.4	12.7±3.1	12.5±2.4	13.2±2.8	12.1±2.4			
Relative peak force ($N \cdot kg^{-1}$)							*0.619*	*0.624*	*0.583*
	Females	19.6±2.4	19.8±1.7	19.7±2.5	19.7±2.6	19.3±1.8			
	Males	19.4±1.7	19.3±1.6	19.2±1.3	18.9±1.4	19.2±1.8			
	Average	19.5±2.0	19.5±1.6	19.4±2.0	19.3±2.1	19.2±1.8			
Relative mean force ($N \cdot kg^{-1}$)							*0.406*	*0.259*	*0.379*
	Females	14.0±1.2	14.0±1.6	14.2±1.3	14.3±1.7	13.7±1.2			
	Males	14.7±0.8	14.6±0.7	14.4±0.8	14.6±0.7	14.5±0.7			
	Average	14.3±1.0	14.3±1.2	14.3±1.1	14.4±1.3	14.1±1.0			
Rate of force development at 50 ms ($N \cdot s^{-1}$)							*0.913*	*0.198*	*0.233*
	Females	1351±1188	1604±1747	1552±1013	1942±1631	1109±867			
	Males	1930±1302	2070±1313	1304±885	1836±1686	2108±1539			
	Average	1655±1250	1849±1509	1434±936	1892±1612	1609±1319			
Rate of force development at 100 ms ($N \cdot s^{-1}$)							*0.854*	*0.058*	*0.328*
	Females	1666±1374	1868±1857	1954±1017	2337±1852	1331±1043			

	Males	2709±1464	2696±1490	2307±1592	2776±1766	2692±1570		
	Average	2215±1482	2304±1680	2131±1313	2556±1776	2011±1473		
Rate of force development at 150 ms ($N \cdot s^{-1}$)							*0.692*	***0.044****	*0.449*
	Females	1638±1060	1667±1334	1988±960	2151±1592	1490±982		
	Males	2773±1069	2639±997	2356±1135	2662±1087	2567±921		
	Average	2236±1187	2179±1240	2172±1040	2406±1352	2028±1079		
Rate of force development at 200 ms ($N \cdot s^{-1}$)							*0.919*	***0.009****	*0.448*
	Females	1357±631	1461±936	1685±834	1661±973	1448±873		
	Males	2305±768	2343±564	2076±734	2318±550	2324±565		
	Average	1856±842	1925±868	1881±790	1990±840	1886±845		
Impulse at 50 ms (N·s)							*0.281*	***0.005****	*0.836*
	Females	33.8±4.0	33.3±5.4	33.5±4.8	34.2±4.8	33.3±4.7		
	Males	40.8±6.1	41.9±6.6	41.8±6.9	41.9±7.0	41.8±6.9		
	Average	37.5±6.2	37.8±7.4	37.7±7.2	38.1±7.1	37.6±7.2		
Impulse at 100 ms (N·s)							*0.525*	***0.003****	*0.521*
	Females	73.0±7.0	71.9±13.6	72.9±7.8	75.2±10.3	70.6±9.0		
	Males	90.1±14.0	91.5±14.9	89.7±16.2	92.1±16.7	91.9±16.0		
	Average	82.0±14.0	82.2±17.2	81.3±15.1	83.7±16.0	81.2±16.7		
Impulse at 150 ms (N·s)							*0.604*	***0.002****	*0.628*
	Females	116.3±11.1	115.2±24.3	117.8±10.9	122.0±18.3	112.2±14.6		
	Males	147.3±23.7	158.7±25.7	145.1±26.8	149.3±27.0	148.7±26.8		

	Average	132.6±24.3	132.8±29.8	131.4±24.4	135.7±26.5	130.5±28.1			
Impulse at 200 ms (N·s)							*0.654*	***0.001****	*0.519*
	Females	163.1±17.3	160.5±35.0	166.6±17.1	171.6±28.3	157.7±21.7			
	Males	209.5±33.7	210.9±36.3	204.4±36.9	211.5±36.0	210.7±36.3			
	Average	187.6±35.6	187.0±43.2	185.5±34.0	191.5±37.6	184.2±39.8			
Total impulse (N·s)							*0.934*	***0.029****	*0.205*
	Females	463.6±114.1	464.4±143.5	443.9±108.4	458.3±124.1	470.5±125.9			
	Males	581.3±122.7	572.3±109.2	591.0±120.3	577.8±108.0	579.2±103.6			
	Average	525.5±130.3	521.2±134.8	517.4±134.6	518.0±128.8	524.8±125.3			
Flight time: contraction time (s)							*0.547*	*0.185*	*0.256*
	Females	0.86±0.23	0.87±0.32	0.91±0.27	0.92±0.33	0.83±0.24			
	Males	0.99±0.14	0.99±0.14	0.94±0.16	0.98±0.13	0.97±0.14			
	Average	0.93±0.20	0.93±0.24	0.93±0.21	0.95±0.25	0.90±0.21			
Contraction time (s)							*0.803*	*0.813*	*0.174*
	Females	0.50±0.10	0.52±0.14	0.47±0.10	0.49±0.13	0.52±0.10			
	Males	0.49±0.08	0.48±0.07	0.50±0.07	0.48±0.06	0.49±0.07			
	Average	0.49±0.08	0.50±0.11	0.49±0.09	0.49±0.10	0.50±0.08			

*Values displayed as mean ± standard deviation. LEA, low energy availability; HEA, high energy availability; GEA, high energy availability for mass gain/growth. *denotes significance between the sexes/conditions p<0.05, **denotes significant difference between the sexes/conditions p<0.01, ***denotes significant difference between the sexes/conditions p<0.001. @denotes no significant differences between conditions in post-hoc testing. ^/ # significantly different from other condition with a matching symbol.*

Table S7. Outcome measures assessed during the isometric mid-thigh pull across all five conditions of energy availability and between sexes.

		LEA_{REST}	LEA_{EX}	HEA_{REST}	HEA_{EX}	GEA	*p value (main effect of condition)*	*p value (main effect of sex)*	*p value (sex*condition interaction)*
Relative peak force ($N \cdot kg^{-1}$)							*0.874*	*0.346*	*0.731*
	Females	25.4±3.3	25.2±4.2	24.7±3.5	25.7±3.7	25.3±4.5			
	Males	28.1±7.6	27.7±6.6	28.5±7.3	27.9±6.1	27.4±7.3			
	Average	26.7±5.9	26.4±5.5	26.6±5.9	26.8±5.0	26.3±6.0			
Time to peak force (s)							*0.969*	*0.850*	*0.213*
	Females	2.56±1.44	2.31±1.12	2.22±1.39	2.46±1.15	2.01±1.15			
	Males	2.22±1.00	2.54±1.16	2.49±1.38	2.01±1.33	2.69±0.09			
	Average	2.39±1.22	2.42±1.11	2.36±1.35	2.24±1.23	2.35±1.06			
Rate of force development at 50 ms ($N \cdot s^{-1}$)							*0.919*	*0.439*	*0.702*
	Females	2813±2600	2646±2323	2650±2008	2801±2810	3750±2819			
	Males	4208±3434	3991±2815	3635±3350	4677±3079	3499±2345			
	Average	3511±3050	3318±2605	3117±2694	3739±3026	3624±2527			
Rate of force development at 100 ms ($N \cdot s^{-1}$)							*0.755*	*0.064*	*0.876*
	Females	2983±2394	2547±1964	3477±2049	2691±2387	3572±2002			
	Males	4326±3116	3898±2554	4982±3426	4612±2922	3933±2274			
	Average	3654±2791	3222±2323	4229±2854	3652±2778	3753±2093			
Rate of force development at 150 ms ($N \cdot s^{-1}$)							*0.887*	***0.030****	*0.873*

	Females	2833±2129	2566±1721	3167±1544	2603±2010	3287±1660			
	Males	4146±2821	3951±2486	4710±2904	4717±2837	3966±2303			
	Average	3489±2524	3259±2199	3938±2398	3660±2627	3627±1985			
Rate of force development at 200 ms ($N \cdot s^{-1}$)							*0.947*	***0.018****	*0.924*
	Females	2666±1883	2716±1444	3059±1432	2677±1721	3113±1446			
	Males	4044±2500	3913±2220	4412±2445	4568±2502	3905±2130			
	Average	3355±2267	3314±1923	3736±2070	3622±2304	3509±1818			
Rate of force development at 250 ms ($N \cdot s^{-1}$)							*0.942*	***0.024****	*0.985*
	Females	2439±1567	2601±1243	2867±1072	2512±1469	2751±1195			
	Males	3608±2088	3472±1874	3936±2005	3784±2065	3518±1761			
	Average	3024±1894	3036±1611	3401±1658	3115±1844	3134±1517			
Impulse at 50 ms (N·s)							*0.421*	***0.008****	*0.735*
	Females	41.3±8.1	41.4±7.0	40.2±5.5	41.1±6.7	43.2±7.9			
	Males	54.4±12.1	55.4±13.5	54.7±13.3	53.2±11.3	55.1±13.2			
	Average	47.9±12.1	48.4±12.7	47.4±12.4	47.1±11.0	49.2±12.2			
Impulse at 100 ms (N·s)							*0.800*	***0.004****	*0.756*
	Females	91.0±20.7	90.1±15.4	89.7±11.8	89.5±17.0	96.4±16.7			
	Males	121.0±26.8	121.3±29.0	123.1±31.5	120.1±25.3	121.1±28.2			
	Average	106.0±27.9	105.7±27.7	106.4±28.8	104.8±26.2	108.7±25.9			
Impulse at 150 ms (N·s)							*0.819*	***0.003****	*0.809*
	Females	147.1±36.3	144.2±25.5	147.6±21.3	143.5±30.4	156.5±27.2			

	Males	197.4±44.8	196.7±47.9	202.6±50.0	197.0±42.6	197.2±47.9			
	Average	172.3±47.4	170.4±46.0	175.1±46.8	170.3±45.3	176.9±43.3			
Impulse at 200 ms (N·s)							*0.862*	***0.002****	*0.805*
	Females	195.6±47.5	193.2±33.8	195.6±26.4	191.7±39.7	208.4±36.6			
	Males	262.5±58.6	262.5±62.0	269.1±64.7	263.4±56.5	262.6±62.9			
	Average	229.1±62.2	227.8±60.2	232.4±61.1	227.5±60.1	235.5±57.3			
Impulse at 250 ms (N·s)							*0.957*	***0.0007*****	*0.942*
	Females	276.1±74.9	274.4±50.8	282.6±42.8	271.7±64.5	295.6±54.8			
	Males	376.0±88.4	374.2±90.3	387.8±88.8	383.3±89.5	376.0±95.8			
	Average	326.0±94.8	324.3±87.8	335.2±86.7	327.5±95.1	335.8±86.4			

*Values displayed as mean ± standard deviation. LEA, low energy availability; HEA, high energy availability; GEA, high energy availability for mass gain/growth. *denotes significance between the sexes/conditions p<0.05, **denotes significant difference between the sexes/conditions p<0.01, ***denotes significant difference between the sexes/conditions p<0.001.*

Table S8. Outcome measures assessed during the Stroop Color and Word Test across all five conditions of energy availability and between sexes.

		LEA_{REST}	LEA_{EX}	HEA_{REST}	HEA_{EX}	GEA	*p value (main effect of condition)*	*p value (main effect of sex)*	*p value (sex*condition interaction)*
Stroop effect, accuracy (%)							*0.520*	*0.115*	*0.836*
	Females	0.2±2.0	-0.7±2.7	-1.5±2.9	-0.2±1.7	-0.2±1.7			
	Males	-1.2±2.2	-1.1±1.7	-2.0±2.6	-1.3±3.4	-2.2±3.9			
	Average	-0.5±2.2	-0.9±2.2	-1.7±2.7	-0.7±2.7	-1.2±3.1			
Stroop effect, reaction time (ms)							*0.913*	*0.798*	*0.452*
	Females	84±48	75±44	63±38	76±41	78±45			
	Males	69±50	95±62	90±61	78±43	70±45			
	Average	76±49	85±53	76±51	77±41	74±44			

Values displayed as mean ± standard deviation. LEA, low energy availability; HEA, high energy availability; GEA, high energy availability for mass gain/growth.