High-impact jumping mitigates the short-term effects of low energy availability on bone resorption but not formation in regularly menstruating females

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High-impact jumping mitigates the short-term effects of low energy availability on bone resorption but not formation in regularly menstruating females: A randomized control trial

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Abstract

Low energy availability (LEA) is prevalent in active individuals and negatively impacts bone turnover in young females. High-impact exercise can promote bone health in an energy efficient manner and may benefit bone during periods of LEA. Nineteen regularly menstruating females (aged 18–31 years) participated in two three-day conditions providing 15 (LEA) and 45 kcals kg fat-free mass^{-1} day^{-1} (BAL) of energy availability, each beginning 3 ± 1 days following the self-reported onset of menses. Participants either did (LEA+J, $n = 10$) or did not (LEA, $n = 9$) perform 20 high-impact jumps twice per day during LEA, with P1NP, β-CTx (circulating biomarkers of bone formation and resorption, respectively) and other markers of LEA measured pre and post in a resting and fasted state. Data are presented as estimated marginal mean ± 95% CI. P1NP was significantly reduced in LEA (71.8 ± 6.1–60.4 ± 6.2 ng mL^{-1}, $p < 0.001$, $d = 2.36$) and LEA+J (93.9 ± 13.4–85.2 ± 12.3 ng mL^{-1}, $p < 0.001$, $d = 1.66$), and these effects were not significantly different (time by condition interaction: $p = 0.269$). β-CTx was significantly increased in LEA (0.39 ± 0.09–0.46 ± 0.10 ng mL^{-1}, $p = 0.002$, $d = 1.11$) but not in LEA+J (0.65 ± 0.08–0.65 ± 0.08 ng mL^{-1}, $p > 0.999$, $d = 0.19$), and these effects were significantly different (time by condition interaction: $p = 0.007$). Morning basal bone formation rate is reduced following 3 days LEA, induced via dietary restriction, with or without high-impact jumping in regularly menstruating young females. However, high-impact jumping can prevent an increase in morning basal bone resorption rate and may benefit long-term bone health in individuals repeatedly exposed to such bouts.

Section I: Physiology & Biochemistry.

KEYWORDS
biochemical markers of bone turnover, exercise intervention, female, low energy availability, nutrition

1 | INTRODUCTION

Low energy availability (LEA) describes the failure to consume sufficient energy to support the optimal function of all remaining bodily processes after accounting for exercise energy expenditure. Some athletes have high exercise energy expenditures and many (including endurance runners and cyclists) are under pressure to maintain a low body mass to optimize performance, increasing the risk of LEA.[1,2] Reported prevalence of LEA in female athletes varies greatly and is partly dependent on measurement method, but has often exceeded 50% of the sample and is understood to be greater than in male athletes.[3–6] Recreationally active females can also experience LEA, albeit less prevalent than in female athletes.[7]

Three to five days of LEA below 15 kcals kg fat-free mass^{-1} day^{-1} (kcals kgFFM^{-1} d^{-1}) is characterized by a plethora of endocrine and metabolic perturbations in exercising females, such as reduced triiodothyronine (T3), glucose, and leptin, and increased β-hydroxybutyrate (β-OHB).[8–11] Amino-terminal propeptide of type 1 collagen (P1NP) is cleaved off during collagen maturation and β-carboxyterminal telopeptide of type 1 collagen (β-CTx) form collagen cross-links and is cleaved off during collagen breakdown. P1NP and β-CTx are released into the circulation and are the recommended international reference standard markers for the processes of bone formation and resorption, respectively.[12] P1NP decreased and β-CTx increased in active females following 5 days of LEA at 15 compared to 45 kcals kgFFM^{-1} d^{-1}.[13] P1NP is similarly decreased following 3 days at 15 kcals kgFFM^{-1} d^{-1} induced via dietary restriction,[14] but not 1 day at ~10 kcals kgFFM^{-1} d^{-1} in a mixed group males and females.[15] These perturbations in markers of bone formation and resorption may contribute to the development of low bone mineral density and osteoporosis, altered bone architecture, reduced bone strength, and an increased rate of bone stress injury in female athletes with longer-term LEA.[16–20]

We have proposed that high-impact exercise may be useful in protecting bone health during periods of LEA, given that it can promote bone adaptation in a time and energy efficient manner.[21] Brief high-impact jumping exercise (as few as 10 vertical jumps per day, 3 days per week) can benefit bone structure and strength in young healthy women.[22–25] However, the bone marker response to high-impact jumping exercise (including that of P1NP and β-CTx) is not well understood, particularly in athletic populations in the context of LEA or energetic stress. Indeed, current evidence supporting an osteogenic benefit of bone-loading exercise during LEA either exists in overweight populations or is derived from cross-sectional or retrospective studies that have used indirect measures of LEA.[16,21,26] Therefore, the current study aimed to investigate the effects of a controlled bout of short-term LEA on P1NP and β-CTx in young, healthy, recreationally active women, and compare these responses to when high-impact jumping exercise is performed during LEA. It was hypothesized that high-impact jumping would mitigate the effects of LEA on these bone metabolic markers.

2 | METHODS

2.1 | Participants and ethics

Twenty-one regularly menstruating young females provided written informed consent to participate in this study—approved by the Ethics Review Sub-Committee of Loughborough University, conducted in accordance with the Declaration of Helsinki, and registered at www.clinicaltrials.gov (NCT04790019) prior to data collection. Participants were recruited from the local area via social media, flyer, and word-of-mouth. Eligibility was checked verbally prior to consent, then confirmed via questionnaire and body composition measurement, according to the inclusion and exclusion criteria shown in Table 1. All participants were considered moderately or highly physically active (at least 30 min of moderate intensity physical activity most days) according to the previously validated International Physical Activity Questionnaire.[27]

2.2 | Experimental design

This study utilized a two-armed randomized cross-over design. In both arms, participants completed balanced (BAL) and low energy availability (LEA) conditions following preliminary tests (Figure 1). In arm 2, participants completed jumping exercise during LEA (LEA+J condition). For the purposes of random allocation, four groups were created (refer to Figure 1) and block randomization

TABLE 1 Participant inclusion and exclusion criteria.

Inclusion criteria	Aged 18–40 years Self-reported regular menstrual cycles (21–35 days for at least the previous three cycles) Body mass index between 18.5 and 30 kg m^{-2} Injury free for the previous 6 months and bone injury free for the previous 12 months
Exclusion criteria	• Smoker • Vegan • Used hormonal contraception, hormonal replacement therapy, or any medication (other than vitamin or mineral supplements) known to effect bone metabolism (e.g., glucocorticoids, anticonvulsants, or anabolic steroids) within the previous 3 months • Currently dieting for weight loss • Previously diagnosed with an eating disorder • Regularly perform more than 3 vigorous, or 5 moderate, exercise sessions per week[a] • Compete in a high or multi-directional impact sport (e.g., gymnastics and soccer) at national level or higher[a,b] • Previously diagnosed with a medical condition known to impact bone health (e.g., hypothyroidism, hyperthyroidism, diabetes mellitus, hypercortisolism, and renal or gastrointestinal disease) or menstrual function (e.g., primary ovarian insufficiency, hyperprolactinemia, thyroid dysfunction, polycystic ovarian syndrome, and any other conditions of androgen excess)

[a]Minimizes risk of de-training effects during controlled conditions in which participants are asked to avoid structured exercise training.

[b]Long-term exposure to intense high and multi-directional impact exercise may influence the effects of high-impact jumping intervention.

was performed using pseudo-random online software (Sealed Envelope Ltd.) to ensure random allocation to a study arm and that condition order was counterbalanced. A CONSORT diagram (Appendix S1) documents participant flow through the study.

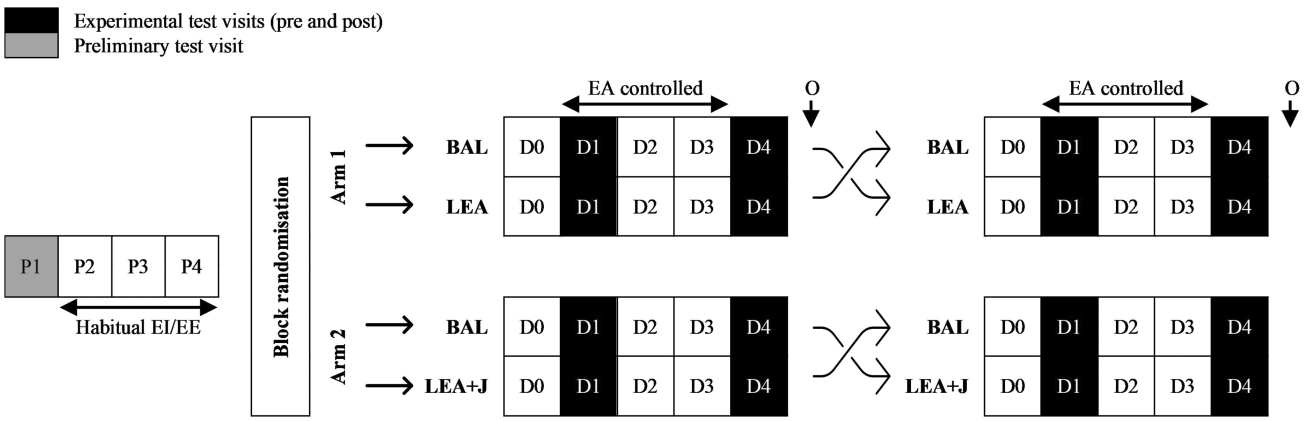
2.3 | Preliminary tests

Participants attended a preliminary visit (B1) to confirm eligibility and complete questionnaires. Questionnaires included a health screen form and the Low Energy Availability in Females Questionnaire (LEAF-Q).[28]

Habitual energy intake and physical activity were monitored for the following 3 days (B2–B4; Figure 1). Energy intake was measured using a three-day weighed food diary and analyzed using nutritional software (Nutritics v5.64, Dublin, Ireland). Physical activity was monitored using a triaxial accelerometer (ActiGraph wGT3X-BT, Pensacola, USA) worn on the non-dominant hip at all times except when washing and bathing. Data were collected at a sample rate of 90 Hz[29] and were analyzed (ActiLife v6.13.4, Pensacola, USA) for average daily activity energy expenditure and time spent in moderate to vigorous physical activity (MVPA), defined by validated thresholds.[30]

2.4 | Experimental conditions

All participants commenced D1 of each trial within 4 days following the self-reported onset of menses. Participants consumed diets providing 45 kcals kgFFM^{-1} d^{-1} throughout D1–D3 in BAL conditions and 15 kcals kgFFM^{-1} d^{-1} in LEA and LEA+J conditions. Participants were instructed to avoid planned and structured exercise, except prescribed jumping exercise, such that energy availability was equal to dietary energy provision. Omnivorous and vegetarian diets providing 45 kcals kgFFM^{-1} d^{-1} and composed of 50% carbohydrate, 20% protein, and 30% fat were created (Appendix S2) by a registered Sport and Exercise Nutritionist using nutritional software, and ingredient quantities were divided by three to provide 15 kcals kgFFM^{-1} d^{-1}. Diets were scaled to fat-free mass (FFM) measured on D1, resulting in average energy intakes of 2043 ± 144 and 687 ± 32 kcals d^{-1} in study arm 1, and 1985 ± 180 and 655 ± 36 kcals d^{-1} in study arm 2. Ingredients were weighed to within 1 g (Mettler Toledo PL601-S Electronic Scale). Omnivores and vegetarians were provided the diet that reflected their current dietary practices. Breakfast was consumed at the laboratory every morning and participants left with food packaged in containers. A multivitamin and multimineral supplement (Vitawell A-Z Multivitamins & Minerals, Lloyds Pharmacy, Loughborough, UK; nutritional information available



D: Day
P: Preliminary day
O: 8-day mid-menstrual cycle ovulation test
EI: Energy intake
EE: Energy expenditure
EA: Energy availability
BAL: Balanced condition including energy availability controlled at 45 kcal·kg^{-1}fat-free mass·day^{-1}
LEA: Experimental condition including energy availability controlled at 15 kcal·kg^{-1}fat-free mass·day^{-1}
LEA+J: LEA including a brief jumping intervention twice per day on D1, D2, and D3

FIGURE 1 Overview of two-armed study design. Participants completed preliminary testing (B1–B4) followed by two experimental trials: balanced energy availability (BAL) and low energy availability (LEA). Participants in arm 2 completed a jumping intervention during LEA (LEA+J) but participants in arm 1 did not.

TABLE 2 Structure of morning high-impact jumping sessions performed in LEA and LEA+J conditions.

	D1	D2	D3
Set 1	5× LDJ (L)	5× LDJ (R)	5× LDJ (L)
Set 2	5× LDJ (R)	5× LDJ (L)	5× LDJ (R)
Set 3	5× CMJ	5× CMJ	5× CMJ
Set 4	5× CMJ	5× CMJ	5× CMJ

Abbreviations: CMJ, countermovement jump; D, day of intervention; L, left direction; LDJ, lateral drop jump; R, right direction.

online: https://lloydspharmacy.com/products/vitawell-a-z-multivitamin-mega-pack) was taken each day with breakfast during LEA and LEA+J conditions to replicate previous research and provide adequate micronutrient intake.[14] Adherence to diets was confirmed verbally each day. Participants were encouraged to bring back any leftovers and report additional items consumed. Participants were permitted to drink black coffee, black tea, and green tea to improve adherence. An accelerometer was worn for the duration of D1–D3 to measure MVPA.

In the LEA+J condition, participants completed high-impact jumping exercise every morning and evening (morning session on D1 completed at the end of the laboratory visit). Morning sessions were supervised at the laboratory and completed in bare feet on a force plate sampling at 2000 Hz (Kistler Type 9286B, Winterthur, Switzerland), and data were analyzed using commercial software (Kistler BioWare v5.4.3.0, Winterthur, Switzerland). Morning session structure is shown in Table 2 and comprised four sets of five lateral drop jumps (LDJ; shown in Appendix S3) or countermovement jumps (CMJ) with 10-sec rest between jumps and 1-min rest between sets. Participants were familiarized with each jump during the preliminary test visit and performed two LDJ and two CMJ, with feedback, for re-familiarization before beginning the morning session on D1. Encouragement was provided throughout each morning session to promote maximum effort. For evening sessions, participants were instructed to repeat the morning session, but CMJ were used in all four sets such that no LDJ were performed. This was done at home, on a hard floor, at least 8 h later to allow time for bone to re-sensitize to loading.[31] Participants reported when evening sessions were concluded via email, text message, or verbally in-person the following morning. Energy expended during jumping exercise was considered negligible and was not compensated for via an increase in dietary energy provision.

2.5 | Experimental test visits

Participants weighed and recorded their diet during D0 of the first trial and replicated it during D0 of the second trial and were instructed to avoid strenuous exercise, alcohol, and caffeine from midday on D0 and D3. Each participant was provided with a pizza on D0 and D3 (scaled on D3 according to target energy availability and FFM, as per the

experimental conditions) and instructed to eat it between 19:00 and 20:00 and ingest nothing but water afterwards. On the morning of D1 and D4, participants were instructed to drink 500 mL of water immediately upon waking and arrive at the laboratory in a fasted state between 07:00 and 09:00 (the same time on both days, in both trials). Ambient laboratory conditions (temperature, humidity, and pressure) were recorded on arrival. Participants completed The Pittsburgh Sleep Quality Index (PSQI) which assesses sleep quality and disturbances during the previous month and produces a cumulative sleep quality score.[32] Fat-free mass was measured to determine dietary provisions using bioelectrical impedance scales (Seca MBCA 515, Hamburg, Germany), and a blood sample was drawn. The equipment and equation used to measure and convert impedance to FFM has been previously validated against a four-compartment body composition model measured using gold standard methods.[33]

2.5.1 | Ovulation status

For each menstrual cycle within which a trial was completed, participants took one ovulation (luteinising hormone) test (One Step, AI DE Diagnostic Co. Ltd.) per day for eight consecutive mid-cycle days, or until a photograph of a positive test was sent to the researchers.

2.5.2 | Blood sampling and storage

Blood was drawn from an antecubital forearm vein at the same time of day (all samples taken prior to 10:30) at D1 and D4. Samples were collected in tubes containing K2EDTA and serum separation gel (BD Vacutainer®). Percentage plasma volume change was calculated using hematocrit and hemoglobin concentration, measured in whole blood (drawn into a separate K2EDTA tube) on the same day using the cyanmethemoglobin method.[34] Samples were stored on ice (EDTA plasma) or allowed to clot at room temperature (serum) for 30-min before centrifugation at a maximum of 2058 G for 15-min at 4°C. Aliquots of plasma and serum were stored at −80°C for later analysis.

2.6 | Biochemical analysis

β-CTx, P1NP, and total triiodothyronine (T3) were measured in serum using an automated electrochemiluminescence immunoassay (ECLIA) analyzer (Cobas e411; Roche Diagnostics, Burgess Hill, UK). Inter-assay coefficient of variations (CV) were all <3.5%, and low detection limits were 0.01 (β-CTx), 5 (P1NP), and 0.2 ng mL^{-1} (T3). 17β-estradiol was measured in serum in duplicate using an enzyme-linked immunosorbent assay (IBL International GmbH). Intra-assay CVs were 8.3 and 7.8%, and inter-assay CV was 8.0%, low detection limit was 2.1 pg mL^{-1}. β-OHB was measured in plasma in duplicate using an enzymatic spectrophotometric assay (Randox, Co. Antrim, UK) as per the manufacturers' instructions. Inter-assay CV was 10.2%, and low detection limit was 0.1 mmol L^{-1}. Glucose, calcium, magnesium, and phosphorus were measured in serum by enzymatic, colorimetric methods using a benchtop analyzer (Pentra C400; HORIBA Medical). Inter-assay CVs were 0.6%, 0.6%, 4.2%, and 1.2%, and low detection limits were 0.11, 0.37, 0.07, and 0.09 mmoL L^{-1}.

2.7 | Statistical analysis

A generalized estimating equation (GEE) model was built to investigate main effect of time (D1 vs. D4), condition (LEA vs. LEA+J), and interaction to explore the effect jumping during LEA. Further within-participant GEE models were built within each study arm (BAL vs. LEA in arm 1, and BAL vs. LEA+J in arm 2) to investigate the effects of LEA and LEA+J compared to a more balanced energy availability condition. Model residuals and D1 to D4 change data in each condition were screened for outliers, and those considered physiologically implausible or erroneous were excluded. Unstructured or autoregressive (AR [1]) correlation structures were used depending upon which produced the best model fit according to QIC (quasi-likelihood under independence model criterion) value. Gamma distribution and log link function was applied for models with positively skewed residuals; otherwise, normal distribution and identity link functions were used. Within-condition post hoc pairwise comparisons (D1 vs. D4) were made and adjusted for multiple comparisons using Bonferroni correction. Cohen's d (mean difference ÷ SD of differences) was calculated for these comparisons and interpreted considering 0.2 small, 0.5 moderate, and 0.8 large effect sizes.[35] An a priori power calculation using a partial eta squared of 0.37 taken from previous research (utilizing a comparable protocol in a similar sample) estimated that a minimum of 9 participants would be required to detect a significant effect of LEA on P1NP with >80% power.[14] Pearson correlations (r) were used to check whether age or T3 were related to P1NP or β-CTx. Morning CMJs and LDJs were analyzed for peak ground reaction force during landing (from box for LDJ) and rate of force development from landing contact to peak force. Data were analyzed using SPSS version 27 (IBM) and are presented as estimated marginal mean ± 95% confidence interval, unless stated otherwise. Alpha was set at <0.05.

3 | RESULTS

Participant characteristics during preliminary testing are presented in Table 3. Nine and ten participants completed LEA and LEA+J conditions, respectively, as well as the corresponding BAL condition (Appendix S1). D1 of LEA and LEA+J commenced 2 ± 1 days following self-reported onset of menses and did not differ between conditions ($p = 0.675$). D1 of BAL in study arms 1 and 2 commenced 3 ± 1 and 2 ± 1 days following self-reported onset of menses, respectively, and this did not differ from corresponding LEA ($p = 0.651$) or LEA+J condition ($p = 0.834$). Ambient laboratory temperature, humidity, and pressure were (mean ± SD): 21.6 ± 1.2°C, 35.6 ± 11.1%, and 1016 ± 12.7 hPa.

PSQI score and daily MVPA during conditions were (mean ± SD): 4.6 ± 2.3 and 54.7 ± 31.5 min, and there were no significant differences between LEA and LEA+J conditions (both $p > 0.419$), BAL and LEA conditions in study arm 1 (both $p > 0.081$), or BAL and LEA+J conditions in study arm 2 (both $p > 0.862$). Six participants did not register a positive ovulation result in both testing windows (four completed LEA and two completed LEA+J). All thirteen remaining participants registered a positive result following the BAL condition; however, seven of these did not following the corresponding LEA condition (three completed LEA and four completed LEA+J).

3.1 | Jump performance

Jumping performance data are presented in Table 4. All supervised morning jumps were completed. One participant reported that evening jumps were not completed on D1; otherwise, all other evening jumps were reported as completed.

3.2 | Bone metabolic markers.

There was a significant main effect of time (Wald $\chi^2 = 66.88$, $p < 0.001$), but no significant interaction (Wald $\chi^2 = 1.220$, $p = 0.269$) for P1NP when comparing LEA and LEA+J conditions, suggesting that decreases in P1NP from D1 to D4 in LEA (71.8 ± 6.1–60.4 ± 6.2 ng mL^{-1}, $p < 0.001$, $d = 2.36$) and LEA+J (93.9 ± 13.4–85.2 ± 12.3 ng mL^{-1}, $p < 0.001$, $d = 1.66$) conditions were not significantly different. Furthermore, there was a significant interaction for P1NP when comparing BAL to LEA in study arm 1 (Wald $\chi^2 = 7.75$, $p = 0.005$), whereby P1NP decreased by a greater amount from D1 to D4 in LEA than in BAL (67.4 ± 8.0–62.4 ± 8.5 ng mL^{-1}, $p = 0.007$, $d = 0.98$). There was also a significant interaction when comparing BAL to LEA+J in study arm 2 (Wald $\chi^2 = 4.58$, $p = 0.032$), whereby P1NP decreased from D1 to D4 in LEA+J and remained stable in BAL (99.2 ± 15.6–96.3 ± 17.0 ng mL^{-1}, $p = 0.410$, $d = 0.41$).

There was a significant interaction for β-CTx when comparing LEA and LEA+J conditions (Wald $\chi^2 = 7.24$, $p = 0.007$), suggesting that the increase in β-CTx from D1 to D4 in LEA (0.39 ± 0.09–0.46 ± 0.10 ng mL^{-1}, $p = 0.002$, $d = 1.11$) was significantly greater than in LEA+J (0.65 ± 0.08–0.65 ± 0.08 ng mL^{-1}, $p > 0.999$, $d = 0.19$). Data regarding percentage change in P1NP and β-CTx in LEA and LEA+J conditions are presented in Figure 2. There was no significant interaction for β-CTx when comparing BAL to LEA in study arm 1 (Wald $\chi^2 = 0.29$, $p = 0.592$), although β-CTx increased from D1 to D4 in LEA and did not change significantly in BAL (0.45 ± 0.09–0.50 ± 0.10 ng mL^{-1}, $p = 0.077$, $d = 0.72$). There was also no significant interaction when comparing BAL to LEA+J in study arm 2, whereby β-CTx remained stable in LEA+J and BAL (0.58 ± 0.11–0.62 ± 0.09 ng mL^{-1}, $p = 0.252$, $d = 0.51$). Age was significantly different between participants who completed LEA versus LEA+J conditions (Table 3); however, age was not significantly correlated with change in P1NP

TABLE 3 Descriptive characteristics measured during preliminary testing for participants that completed low energy availability (LEA; study arm 1) and low energy availability plus jumping (LEA+J; study arm 2) conditions and significance of group differences from GEE models.

	LEA (arm 1) $n = 9$	LEA+J (arm 2) $n = 10$	p-value for group difference
Age (years)	25.0 ± 3.5	19.0 ± 4.8[a]	**0.008**
Height (m)	165.6 ± 4.7	162.9 ± 7.2	0.345
Body mass (kg)	63.5 ± 6.6	59.0 ± 9.0	0.144
LEAF-Q score	4.0 ± 2.9	3.0 ± 1.3	0.910
Habitual daily EI (kcals)	2030 ± 502	1664 ± 486	0.173
Habitual daily AEE (kcals)	515 ± 270	392 ± 206	0.284
Habitual daily MVPA (min)	58.0 ± 30.9	48.5 ± 34.4	0.636

Abbreviations: AEE, activity energy expenditure; EI, energy intake; GEE, generalized estimating equation; LEAF-Q, low energy availability in females questionnaire; MVPA, moderate to vigorous physical activity.

[a]Data are presented as mean ± SD or median ± IQR.

TABLE 4 Ground reaction force (GRF) and rate of force development (RFD) for countermovement jumps (CMJ) and lateral drop jumps (LDJ) performed in LEA+J.

	CMJ			LDJ		
Force direction	**GRF (*N*)**	**GRF (*bw)**	**RFD ($N ms^{-1}$)**	**GRF (*N*)**	**GRF (*bw)**	**RFD ($N ms^{-1}$)**
Vertical	3460 ± 157	6.6 ± 1.9	62.5 ± 67.5[a]	3254 ± 161	6.1 ± 1.7	61.1 ± 28.6
Lateral				331 ± 12	0.6 ± 0.1	7.4 ± 2.9

Note: GRF is also presented as a multiple of body weight (*bw).

[a]Data are presented as mean ± SD across all sessions, or median ± IQR.

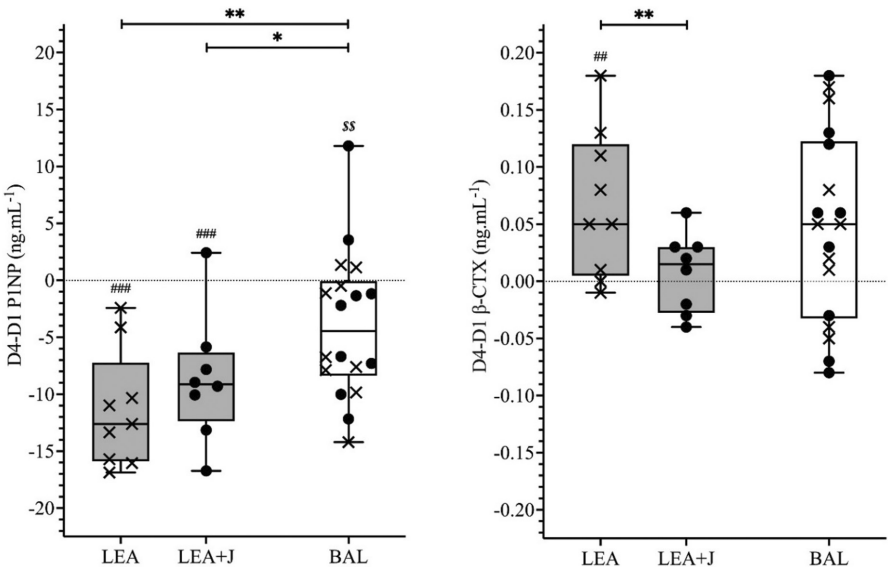


FIGURE 2 Change in P1NP and β-CTx concentration from D1 to D4 in low energy availability (LEA) and low energy availability plus jumping (LEA+J) conditions. Data are presented as mean change (bars) and 95% confidence interval (error bars). Significant time (D1 vs. D4) by condition interactions ($^{\#}$) and Bonferroni-adjusted D1–D4 post hoc comparisons (*) from GEE models are presented.

TABLE 5 Hormonal and metabolic marker data at D1 and D4 in low energy availability (LEA) and low energy availability plus jumping (LEA+J) conditions, presented as estimated marginal mean ± 95% confidence interval.

	LEA			LEA+J				
	D1	**D4**	***d***	**D1**	**D4**	***d***	**Time (*p*)**	**Int (*p*)**
Triiodothyronine ($ng mL^{-1}$)	1.58 ± 0.19	**1.27 ± 0.13[c]**	>1	1.51 ± 0.13	1.33 ± 0.21	0.59	**<0.001**	0.360
17β-estradiol ($pg mL^{-1}$)	96.9 ± 9.8	86.1 ± 17.9	0.42	85.3 ± 14.7	97.5 ± 23.7	0.14	0.640	0.360
Glucose ($mmol L^{-1}$)	4.81 ± 0.16	**4.37 ± 0.31[c]**	>3	4.73 ± 0.08	**4.44 ± 0.08[c]**	>1	**<0.001**	0.227
β-OHB ($mmol L^{-1}$)	0.26 ± 0.06	**0.58 ± 0.28[a]**	0.88	0.15 ± 0.04	**0.52 ± 0.15[c]**	>1	**<0.001**	0.116
Calcium ($mmol L^{-1}$)	2.46 ± 0.03	2.46 ± 0.04	0.18	2.47 ± 0.04	2.46 ± 0.03	0.16	0.863	0.645
Magnesium ($mmol L^{-1}$)	0.85 ± 0.02	0.88 ± 0.03	0.78	0.89 ± 0.04	0.90 ± 0.03	0.28	**0.043**	0.142
Phosphorus ($mmol L^{1}$)	1.26 ± 0.06	1.25 ± 0.05	0.29	1.39 ± 0.05	1.41 ± 0.05	0.02	0.961	0.479

Note: Associated *p*-values (*p*) for main effects of time and interactions (Int) are shown. Significant Bonferroni-adjusted post hoc comparisons (D1 vs. D4): [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$. Cohen's *d* (*d*) reflects within-condition pairwise comparison. Bold indicates statistically significant difference or *p*-value.

Abbreviations: β-OHB, β-hydroxybutyrate; $kcals kg FFM^{-1} d^{-1}$, kilocalories per kg of fat-free mass per day.

($r = 0.283$, $p = 0.270$) or β-CTx ($r = 0.214$, $p = 0.410$) during LEA and LEA+J conditions.

3.3 | Hormonal and metabolic markers

The concentrations of hormonal and metabolic markers at D1 and D4 in LEA and LEA+J conditions, associated *p*-values for main effects of time and time by condition interactions, and relevant post hoc comparisons are presented in Table 5. T3 did not exhibit a significant post hoc decrease in LEA+J and, unexpectedly, did not decrease in four cases (Figure 3); however, change in T3 was not correlated with change in P1NP ($r = -0.19$, $p = 0.655$) or β-CTx ($r = -0.20$, $p = 0.632$). The effects of LEA+J compared to LEA (on bone outcomes) are of primary interest. Therefore, the effect of these conditions on hormonal and metabolic markers compared to BAL are not described

in detail. However, the significant interactions for glucose when comparing BAL to LEA in study arm 1 (Wald $\chi^2 = 8.28$, $p = 0.004$) and BAL to LEA+J in study arm 2 (Wald $\chi^2 = 6.61$, $p = 0.010$) are noteworthy. Glucose significantly decreased from D1 to D4 during BAL in study arms 1 (4.86 ± 0.20–4.72 ± 0.24 mmoL L^{-1}, $p = 0.023$, $d = 0.85$) and 2 (4.83 ± 0.10–4.70 ± 0.12 mmoL L^{-1}, $p = 0.049$, $d = 0.81$), albeit to a lesser extent than during LEA and LEA+J conditions. All other measured metabolic and hormonal markers remained stable during BAL conditions, as indicated by no significant D1–D4 post hoc comparisons in study arms 1 or 2 (data shown in Appendix S4).

3.4 | Plasma volume

There was no main effect of condition on percentage plasma volume change from D1 to D4 (Wald $\chi^2 = 0.65$, $p = 0.420$), which was $-1.3 \pm 3.1\%$ in LEA and $0.6 \pm 3.3\%$ in LEA+J.

4 | DISCUSSION

This study is the first to investigate the effect of high-impact exercise on bone formation and resorption during short-term LEA in regularly menstruating young females. P1NP concentrations were significantly decreased following 3 days of LEA (15 kcals kgFFM^{-1} d^{-1} induced via dietary restriction) with and without high-impact jumping exercise; however, performing high-impact jumping exercise twice daily during LEA significantly mitigated the increase in β-CTx concentration shown during LEA alone.

Findings regarding the effect of LEA on P1NP are supported by previous research which showed that 3 days at 15 kcals kgFFM^{-1} d^{-1}, induced via dietary restriction, decreased P1NP concentrations by 17% in a similar population.[14] We observe a similar 16% reduction in P1NP during LEA and show that this effect is not prevented by performing brief high-impact jumping exercise twice daily. Resting P1NP increased by ~8% at 24, 48, and 72 h following initiation of a similar twice per day high-impact jumping intervention in young men, suggesting that such interventions are capable of augmenting bone formation independent of LEA.[36] Osteoblasts are capable of generating adenosine triphosphate via numerous biochemical pathways, suggesting bone formation is energetically demanding.[37] Furthermore, osteoblast activity may play a role in the regulation of energy balance for the entire organism.[38] Bone formation rates may remain suppressed during LEA despite the application of external load because there is insufficient energy available to fully restore osteoblast activity and adequately fuel other physiological functions more vital for survival.[39]

P1NP also decreases during both BAL conditions (only significant in study arm 1), which could have occurred due to differences between habitual and experimental energy availability, macronutrient intakes, or exercise training. Glucose significantly decreased during BAL conditions in both study arms (albeit by a small amount), suggesting

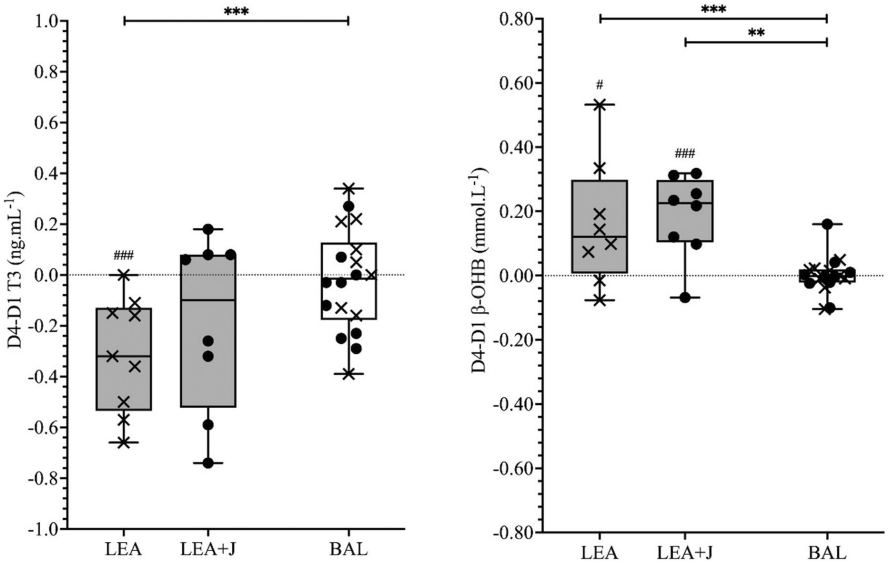


FIGURE 3 Change in T3 and β-OHB concentrations from D1 to D4 in low energy availability (LEA), low energy availability plus jumping (LEA+J) and balanced (BAL) conditions. Plots present median, lower and upper quartiles, and minimum and maximum values. BAL data from study arms 1 and 2 are combined, and individual data points are shown as crosses (study arm 1) or spots (study arm 2). Significant time (D1 vs. D4) by condition interactions (**$p < 0.01$, ***$p < 0.001$) and significant Bonferroni-adjusted D1 to D4 post hoc comparisons from GEE models are shown for LEA and LEA+J ($^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$).

that the prescribed diets may have provided less carbohydrate than the participants' habitual diet and short-term carbohydrate restriction has been shown to supress P1NP and increase β-CTx despite an energy availability ≥45 kcals kgFFM^{-1} d^{-1}.[40,41] Furthermore, participants were habitually physically active and cessation of exercise for the duration of each condition may have modulated bone turnover. Nevertheless, the current study was powered to detect a significant effect of LEA on P1NP which, when participants acted as their own controls, decreased in both LEA and LEA+J compared to BAL, supporting our conclusion that high-impact jumping failed to mitigate the effect of short-term LEA on bone formation.

Participants in study arm 2 were significantly younger than in study arm 1, which may have contributed to greater pre-intervention P1NP concentrations given the tendency for P1NP to decline with age until approximately 50 years.[42] Nevertheless, the difference in age between groups is small, age was not correlated to change in P1NP (or β-CTx) in LEA and LEA+J conditions, and, to our knowledge, there is little reason to believe that marginally higher basal rates of bone formation influenced the effects of our interventions. However, we were unable to power for an effect of high-impact jumping during LEA per se as previous data were not available. Mean percentage change in P1NP (−9.3% vs. −15.9%) and corresponding effect sizes (d = 1.66 vs. 2.36) were lower in LEA+J than LEA. Based on a partial eta squared of 0.068 from our data, it is estimated that future studies utilizing a similar mixed design would need 54 participants to be appropriately powered to detect a significant effect of high-impact jumping during short-term LEA on P1NP. Future research with greater statistical power is needed to reconcile whether high-impact jumping has any mitigating effect on the acute change in P1NP observed during short-term LEA.

Bone resorption marker β-CTx exhibited divergent changes between LEA and LEA+J conditions, whereby increases were observed in 3 days only in the absence of jumping exercise. The mechanisms underpinning these findings cannot be elucidated but could involve factors released from bone cells which regulate bone resorption, such as sclerostin and osteoprotegerin. Free-living endurance athletes exhibit large day-to-day variation in energy availability, including acute and transient periods of (severely) LEA.[43,44] Transient increases in the rate of bone resorption may initiate the development of bone stress injury, as per the primary remodeling hypothesis, as well as contribute to the loss of bone mass and strength.[45] Indeed, female endurance athletes exhibiting signs of long-term LEA experience a greater rate of bone stress injury and have impaired bone structure and strength compared to healthy counterparts.[16,18,46] Our data suggest that high-impact jumping exercise can mitigate a rise in the rate of bone resorption during acute and transient periods of LEA, which could help to protect bone health and reduce injury rates in active females repeatedly exposed to such periods. However, as per the bone remodeling transient hypothesis, an acute reduction in bone resorption may be succeeded by a reduction in bone formation given the two processes typically coupled to formation at individual remodeling sites, such that a measurable increase in bone mass does not necessarily occur.[47] The relationship between acute change in bone markers and longer-term structural bone changes are complex and not well understood, such that future research should investigate longer-term protective effect of high-impact jumping in individuals who experience LEA bone before it may be considered a viable intervention. It is also important to consider that, during LEA, any intervention that raises the physiological importance of one process (e.g., maintaining a more balanced bone (re)modeling activity) may lead to greater competition for available energy to the detriment of other important processes, such as reproductive function.[39] Any practitioner considering implementing high-impact jumping to protect bone during planned, transient periods of LEA could look to replicate our intervention (using the jump performance data presented) but should be aware of the potential for negative effects in other physiological systems.

In agreement with previous research in a similar population,[14] we show no significant interaction when comparing change in β-CTx concentration over 3 days at 15 (LEA) and 45 kcals kgFFM^{-1} d^{-1} (BAL). There was a non-significant 10% increase in β-CTx during BAL in study arm 1 which may have limited statistical power to detect a significant interaction compared to LEA independent of energy availability—possibly due to differences in habitual and experimental carbohydrate intake, as previously described for the decrease in P1NP in the same condition. Nevertheless, type I procollagen carboxyterminal propeptide (PICP, a marker of bone formation) reduced following 5 days at 20 and 10 kcals kg lean body mass^{-1} d^{-1}, while urinary N-telopeptide (NTX, a marker of bone resorption) only increased following 10 kcals·kg lean body mass^{-1} d^{-1} in young exercising women.[48] It is not fully clear whether LEA alone increased bone resorption in the current study; however, previous research suggests that bone resorption may be more robust to the effects of short-term LEA than bone formation such that any mitigating effects of high-impact jumping on β-CTx may only be beneficial in relatively extreme circumstances.

Bone resorption increases within days of bone unloading induced by bed rest so it could be hypothesized that the cessation of exercise during experimental conditions contributed to the increase in β-CTx during LEA,[49] particularly given β-CTx also showed average increases in

BAL conditions. In this context, our high-impact jumping intervention may have merely provided a useful substitute to prevent a rise in β-CTx while habitual exercise is restricted. However, Papageorgiou and colleagues found that β-CTx area under the curve was significantly greater over 5 days at 15 versus 45 kcals $kgFFM^{-1}$ d^{-1} despite arduous treadmill running in both conditions, and the 14.3% increase in β-CTx from pre- to post-LEA was similar to the 15.2% increase shown in our data.[13] This comparison highlights that high-impact jumping seems to have greater benefit in preventing a rise in β-CTx during LEA compared to more moderate-impact exercise such as treadmill running, at least in the absence of other exercise. Nevertheless, future research must consider integrating habitual exercise and dietary habits within the experimental design to fully elucidate the bone resorptive response to short-term LEA and the mitigating effects of high-impact jumping exercise.

T3, glucose, and β-OHB all exhibited changes of moderate or large effect size during LEA and LEA+J conditions, which are consistent with the previously reported effects of three to 5 days of LEA.[8,9,13,14,50–52] This indicates that participants were compliant with the dietary intervention and that similar severities of LEA were successfully induced in both conditions. T3 suppression is an established marker of LEA in females and decreased by a similar magnitude on average in both LEA and LEA+J conditions.[8,9] It is unclear why T3 was not suppressed in four cases during LEA+J, but it could be that LEA was less severe in these cases and that this contributed to the maintenance of β-CTX. However, this conclusion is not supported by correlations made between T3 and β-CTX or β-OHB data, which exhibited the expected response to LEA with less variability.

Estrogen inhibits osteoclast activity to regulate bone resorption and can become chronically suppressed during longer-term LEA.[53,54] Recent evidence suggests that hormonal fluctuations during the menstrual cycle do not cause predictable variations in bone marker concentrations,[55,56] but, nonetheless, all conditions in the current study were completed within the early follicular phase of the menstrual cycle to minimize estrogen fluctuations. 17β-estradiol concentrations remained stable during all conditions, suggesting changes in β-CTx (or P1NP) during 3 days of LEA are not greatly influenced by estrogen. Calcium, phosphorus, and magnesium were measured as key nutrients for bone health that are influence by diet.[57] Provision of a multimineral supplement may have caused the moderate and small increases in magnesium in LEA and LEA+J; however, it is unlikely this impacted our conclusions regarding the effects of high-impact jumping given that no measured micronutrient was differentially affected in LEA versus LEA+J.

Seven participants who registered a positive ovulation test result following the BAL condition did not following the corresponding LEA condition. A link between LEA and anovulation has been established previously and may explain these findings.[54,58] Alternatively, ovulation could have been delayed beyond the 8-day testing window, as a short luteal phase seems to be the most commonly observed menstrual disturbance during energy deficiency.[58] It is important to note that six participants did not register a positive result throughout the study. Data regarding habitual dietary practices, LEAF-Q score, and stable hormones and metabolites in BAL conditions suggest this was not likely due to pre-existing energy deficiency. Participants took the ovulation test kits home following each condition and may not all have used them as instructed.

This study has several limitations. Circulating bone marker concentrations are inherently limited in several ways, including that site-specific bone (re)modeling cannot be determined and collagen-borne markers (including P1NP and β-CTx) may arise from activity in collagen containing tissue other than bone.[59] There is potential for high interindividual variability in basal bone marker concentration, which limits conclusions made based on between-groups comparisons.[60] However, conclusions made on between-groups comparisons were largely supported by within-participant comparisons made within each study arm. Plasma volume changes were small and non-significant, so blood marker concentrations were not adjusted for change in plasma volume to avoid unnecessarily introducing an additional source of error. Previous research has adjusted bone marker concentration for much larger changes of >11.9% and has not adjusted for a similar 1.3% change.[61,62] The study was originally powered to detect a significant effect of LEA on P1NP and not β-CTx. However, the partial eta squared used for a priori power calculation is comparable to that calculated for β-CTx using the current data (0.33), suggesting that analyses were not considerably underpowered for either marker. Use of supplements known to impact bone health was not measured, and the bone-loading stimulus provided by the evening sessions may have been sub-optimal (e.g., unreported missed sessions, less than maximal effort, and not always performed barefoot). These factors may have influenced our findings; however, a similar twice daily high-impact jumping intervention did not have a different effect on P1NP and β-CTx compared to once daily in males, nor were the effects impacted by concomitant collagen supplementation.[36] Findings are not generalizable to males or other female populations, such as oral contraceptive users and older women who may be more resilient to short-term LEA.[52] Also, participants were not highly trained and were instructed to avoid structured exercise during trials such that findings may not be generalized to female athletes, arguably the population most at risk of LEA.[1]

In conclusion, regularly menstruating young women should avoid periods of severe dietary restriction lasting 3 days or longer to maintain a normal balance of bone formation and resorption. If planned bouts of dietary restriction are unavoidable, such as during planned weight loss for athletic performance or health, brief bouts of high-impact jumping, performed twice-daily in the morning and evening, may help to mitigate a rise in bone resorption and reduce bone loss within the first 3 days.

4.1 | Perspective

LEA is prevalent in endurance athletes and recreational exercisers.[63,64] Long-term LEA has been associated with impaired bone structure, osteoporosis, and increased rates of bone injury in females.[16,21] It is unavoidable that athletes will experience at least transient periods of LEA, which can increase and decrease rates of bone resorption and formation, respectively, within three to 5 days.[13,14] We have shown that a very brief bout of high-impact jumping performed morning and evening during LEA can mitigate the rise in bone resorption otherwise observed. It is plausible that reduced bone resorption during transient periods of LEA will help to minimize bone loss and protect long-term bone health. These data provide evidence that high-impact jumping should be investigated as a potential therapeutic intervention to prevent osteoporosis and bone injury in athletes and exercisers at risk of LEA.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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