

## Low Energy Availability Prevalence, Dietary Habits, and Sleep in Female Army ROTC Cadets

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### Abstract

*International Journal of Exercise Science* 18(6): 1030-1046, 2025.  
<https://doi.org/10.70252/VRDK5031>

Low energy availability (LEA) results in numerous health and performance decrements. While a high prevalence of LEA and sleep disturbance has been observed in male Army Reserve Officers' Training Corps (ROTC) cadets, no data exists in females. This study aimed to determine LEA prevalence, dietary habits, and sleep quantity and quality in female U.S. ROTC cadets. Following an overnight fast, ten cadets (22±4 yrs, 166.0±6.1 cm, 67.1±9.1 kg, 26.1±6.2% body fat, 49.2±4.8 kg fat-free mass (FFM)) had their body composition and resting metabolic rate measured. Validated questionnaires assessed LEA symptoms and sleep. Under free-living conditions, exercise energy expenditure (EEE) and sleep were quantified via accelerometers for 7-days. Concurrently, energy intake (EI) was assessed via digital food records and evaluated relative to the Military Dietary Reference Intakes (MDRIs). Cadets consumed 1983±706 kcal ·d<sup>-1</sup> with a mean EEE of 482±110 kcal ·d<sup>-1</sup>. EA was 30.6±13.2 kcal ·kg<sup>-1</sup> FFM with 40% presenting with LEA (≤ 30 kcal ·kg<sup>-1</sup> FFM) and another 50% in a suboptimal EA state (30–45 kcal ·kg<sup>-1</sup> FFM). Dietary analysis indicated 30%, 40%, 80%, and 10% of cadets met MDRIs for calories, carbohydrate, protein, and fat, respectively. Cadets slept 373±100 min ·d<sup>-1</sup>, with 20% of cadets meeting the Army recommendation for sleep. Sleep questionnaires indicated that 60% of cadets experienced poor sleep quality and 30% had poor sleep behavior. In the present study, all but one cadet was in a low or suboptimal EA state, a high prevalence of sleep disturbance was observed, and most cadets did not meet MDRIs for energy and macronutrient intakes.

**Keywords:** Military, tactical athlete, sports nutrition, relative energy deficiency in sport, nutrient intake

### Introduction

The International Olympic Committee (IOC), in their recent consensus statement, declared low energy availability (LEA) as the etiological factor of the Female Athlete Triad and Relative Energy Deficiency in Sport (REDs).<sup>1</sup> REDs, and therefore LEA, results in hormonal

dysregulation, reproductive impairments, menstrual dysfunction, metabolic rate suppression, blunted muscle protein synthesis, elevated risk of bone fractures and illness, as well as psychological disturbances.<sup>1-5</sup> In addition to the negative health consequences of the energy deficient state, physical and cognitive performance decrements have also been reported<sup>1,3,6</sup> and are purported to be caused, directly and indirectly, by the negative health consequences mentioned previously.<sup>6</sup>

LEA, calculated as energy intake (EI) minus exercise energy expenditure (EEE) divided by fat-free mass (FFM), occurs when there is a mismatch between EI and EEE such that inadequate energy is consumed for optimal health and performance.<sup>1</sup> In females, optimal physiological function is generally achieved at an energy availability of at least 45 kcal·kg<sup>-1</sup> FFM.<sup>2</sup> Conversely, at an energy availability of  $\leq 30$  kcal·kg<sup>-1</sup> FFM, perturbations of multiple bodily systems occur and has been considered the threshold for LEA.<sup>1</sup>

It is well established that female sport athletes present with a high prevalence of LEA.<sup>7,8</sup> However, the demands of sport athletes are drastically different than those of tactical athletes and thus, data in sport athletes may not translate to tactical athletes. Military members must endure high physical demands, extreme environmental conditions, and psychological stress where underperformance can have devastating consequences.<sup>3,9</sup> Tactical athletes are commonly exposed to prolonged periods of energy and sleep deprivation during training and combat operations,<sup>9</sup> both of which pose concerns for health and tactical readiness and result in LEA and REDs. Indeed, the United States Army recognizes nutrition and sleep as critical components for performance optimization and therefore, tactical readiness and mission success.<sup>10</sup> Thus, meeting the military recommendations for nutrition and sleep is critical for maintaining not only optimal performance, but also national security. Furthermore, the 4th International Congress on Soldiers' Physical Performance identified nutrition and sleep as the top 7<sup>th</sup> and 3<sup>rd</sup> priority research gaps, respectively,<sup>11</sup> making research on these topics essential.

Reserve Officer Training Corps (ROTC) cadets, a subset of the military population, simultaneously attend college while receiving military training in preparation to perform duties as a commissioned Officer in the Armed Forces. These individuals face unique challenges where they must endure the demands of military training in conjunction with balancing academic success and University life. It was recently reported that 62% of male ROTC cadets presented with LEA, with  $< 50\%$  meeting the Military Dietary Reference Intakes (MDRIs) for energy and macronutrients.<sup>12</sup> The same authors also reported sleep disturbance in 85% of male cadets. It is possible that these findings are related, as prior research suggests LEA increases the risk of sleep disturbance<sup>13</sup> and that military personnel are at increased risk for sleep disturbance compared to civilians.<sup>14</sup>

While LEA is certainly observed in males, female athletes typically present with a higher prevalence.<sup>8</sup> Interestingly, while there are plentiful data regarding LEA in female sport athletes, much of the research on the topic in military populations has investigated males. This may be attributed to the fact that females have only recently been included in combat roles or that females make up a smaller percentage of the military population.<sup>3</sup> Nonetheless, there is a lack of data in female military personnel. As female tactical athletes are at increased risk for

musculoskeletal injuries and stress fractures,<sup>4</sup> which can be exacerbated by energy deficiency,<sup>5</sup> understanding the prevalence of LEA in this population is critical. Therefore, the primary purpose of the present study was to establish the prevalence of LEA, assess the nutritional practices in comparison to the MDRI, and determine sleep quantity and quality in female ROTC cadets. To our knowledge, this is the first study to examine the prevalence of LEA in female U.S. Army ROTC cadets. We hypothesized that LEA and sleep disturbance would be detected and that the MDRI would not be met by most cadets.

## Methods

### *Participants*

This was an observational study consisting of one laboratory visit followed by 7 continuous days of assessing EI and EEE under free-living conditions. As our primary aim was to assess the current LEA prevalence, dietary habits, and sleep quantity and quality in female cadets, and due to the observational and free-living nature of the study design, no control group was utilized. Participants were asked not to alter their normal behaviors (eating, activity, sleeping, etc.). Further, due to the observational nature of the present study, no *a priori* power analysis was performed. However, the final sample size is aligned with previous work examining LEA and dietary habits in male Army ROTC cadets.<sup>12</sup> A convenience sample of ten female U.S. Army ROTC cadets ( $22 \pm 4$  yrs,  $166.0 \pm 6.1$  cm,  $67.1 \pm 9.1$  kg) from the local ROTC program were recruited to participate in the study during the Spring 2024 academic semester. Participant characteristics can be found in Table 1. To be included in the study, participants needed to be a female cadet currently participating in the local Army ROTC program. A minimum of 3 recorded days were required for inclusion in final analyses for food logs and actigraphy. All cadets recorded the minimum number of food records for inclusion. Mean wear times for the hip- and wrist-worn monitors were 91.8% and 90.6%, respectively. After having the study explained, each participant provided their oral and written informed consent. This study was approved by the Institutional Review Board (Pro00131888) and was therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. This research was carried out fully in accordance with the ethical standards of the *International Journal of Exercise Science*.<sup>15</sup>

### *Protocol*

Participants were asked to arrive at the laboratory in the morning between 0600 and 0900 h following an overnight fast, and having abstained from caffeine (12 h), alcohol (24 h), and strenuous exercise (24 h). Pre-testing conditions were verbally confirmed with participants. After having a chance to void their bladder and bowel, participants were asked to wear minimal clothing (i.e., compression athletic clothing) and remove their socks and shoes. Height was then measured to the nearest 0.1 cm using a stadiometer (SECA, CA). Next, weight was recorded to the nearest 0.01 kg (Bod Pod, Cosmed, IL) and body composition was assessed via Air Displacement Plethysmography (Bod Pod, Cosmed, IL). Previous work reported a coefficient of variation of 4.7% and 1.4% for body fat percentage and FFM, respectively (*ICC* = 0.973 and 0.992, respectively).<sup>16</sup> Then, in comfortable clothing, with participants lying supine for 20 minutes in a

dark, quiet, thermoneutral room, gas exchange was measured for resting metabolic rate (RMR) assessment (TrueOne 2400, ParvoMedics, USA). The average of the final 10 minutes, extrapolated to 24 hours, was utilized in the final analysis. To assess RMR suppression, measured RMR from indirect calorimetry was divided by predicted RMR calculated using the Cunningham equation.<sup>17</sup>

**Table 1.** Participant Characteristics

Characteristic	Total (N = 10)	LEA (N = 4)	No LEA (N = 6)	<i>p</i> -value	Cohen's <i>d</i>
Age (yrs)	22 ± 4	20 ± 1	23 ± 5	0.352 <sup>‡</sup>	0.61
Height (cm)	166.0 ± 6.1	162.4 ± 2.0	168.4 ± 6.9	0.171 <sup>‡</sup>	1.08
Weight (kg)	67.1 ± 9.1	71.9 ± 8.5	64.0 ± 8.7	0.190	0.93
Body Fat %	26.1 ± 6.2	30.2 ± 3.4	23.4 ± 6.3	0.085	1.27
FFM (kg)	49.2 ± 4.8	50.0 ± 5.0	48.7 ± 5.1	0.693	0.26
FM (kg)	17.9 ± 6.1	21.9 ± 4.3	15.3 ± 5.9	0.171 <sup>‡</sup>	1.24
BMI (kg/m <sup>2</sup> )	24.4 ± 3.5	27.3 ± 3.7	22.5 ± 1.7*	0.022	1.83

Data are presented as mean ± SD. FFM = fat-free mass; FM = fat mass; BMI = body mass index; <sup>‡</sup> indicates nonparametric test. \* Indicates significant difference from LEA (*p* < 0.05).

Following RMR, participants completed the 25-item Low Energy Availability in Females Questionnaire (LEAF-Q; ICC = 0.79), which has been validated<sup>18</sup> in athletic females to assess LEA risk based on associated symptoms of LEA (i.e., injuries, gastrointestinal function, menstrual function). At risk for LEA is indicated by a total score ≥ 8. Next, participants completed the 19-item Pittsburgh Sleep Quality Index (PSQI; PCC = 0.82) questionnaire<sup>19</sup> and 18-item Athlete Sleep Behavior Questionnaire (ASBQ; ICC = 0.87)<sup>20</sup> to assess sleep quality and sleep behaviors, respectively. For the PSQI, a global score ≥ 5 indicates sleep disturbance. For the ASBQ, a total score of ≥ 42 indicates impaired sleep behaviors.

EI was assessed for 7 continuous days using the National Institute of Health Automated Self-Administered 24h Dietary Assessment Tool (ASA24), which provides visual cues to aid in portion size estimation. Participants were provided with private login credentials. Average daily dietary intake was compared to the MDRI.<sup>21</sup> Concurrently, EEE and sleep parameters were assessed by triaxial accelerometry (GT3X-BT, ActiGraph, USA) for 7 continuous days, except for bathing. Participants were fitted with both a hip- and wrist-worn monitor on their non-dominant side. Two monitors were utilized as hip-worn placement has been reported as more accurate for EEE compared to wrist-worn accelerometers and wrist-worn being more accurate for assessing sleep.<sup>22,23</sup> EEE was calculated from the hip-worn device using the Freedson VM3 Combination equation. Sleep was scored from the wrist-worn device using the Sadeh algorithm for cadets ≤ 25 years old and the Cole-Kripke algorithm in cadets over 25 years old which provided time in bed, total sleep time, wake after sleep onset, number of awakenings, average awakening time, and sleep efficiency data per day. All accelerometer data (30 Hz, 60 second epochs) was processed and scored using ActiLife v6.13 software. As this was a free-living study, participants were asked not to alter their normal behaviors (eating, activity, sleep, etc.).

EA was categorized as optimal ( $\geq 45$  kcal  $\cdot$  kg<sup>-1</sup> FFM), suboptimal (30 – 45 kcal  $\cdot$  kg<sup>-1</sup> FFM), or low (LEA;  $\leq 30$  kcal  $\cdot$  kg<sup>-1</sup> FFM). EA was calculated by subtracting EEE from EI, relative to FFM using the following equation <sup>1</sup>:

$$\text{Energy Availability} = \frac{(\text{Energy Intake (kcal)} - \text{Exercise Energy Expenditure (kcal)})}{\text{Fat Free Mass (kg)}}$$

### Statistical Analysis

Data were analyzed using SPSS Statistics software package (version 29, IBM, New York). To address the primary aim of the present study, participant characteristics, EA status, dietary habits, and sleep parameters are presented using descriptive statistics. As an exploratory analysis, two-tailed independent samples t-tests were used to detect differences between cadets with and without LEA. Normality was assessed using Shapiro-Wilk tests. If normality was violated, Mann-Whitney U tests were utilized. To better interpret group comparisons, Cohen's *d* effect sizes were utilized. Effects were defined as small ( $0.2 < d < 0.5$ ), medium ( $0.5 < d < 0.8$ ), and large ( $d \geq 0.8$ ). Exploratory analyses were also performed utilizing Pearson correlation coefficients to determine the relationships between EA and body composition, dietary habits, EEE, and sleep. Correlations were defined as small ( $r < 0.3$ ), moderate ( $r = 0.3 - 0.5$ ), and strong ( $r > 0.5$ ). Linearity was assessed by visual inspection and normality was tested via Shapiro-Wilk test. If normality was violated, Spearman's correlations were utilized. For all tests, if outliers were present, data were included in final analyses as these were true measures. Significance was set at  $p < 0.05$  with a trend considered  $0.05 < p < 0.10$ . Data are presented as mean  $\pm$  standard deviation.

## Results

### Participants

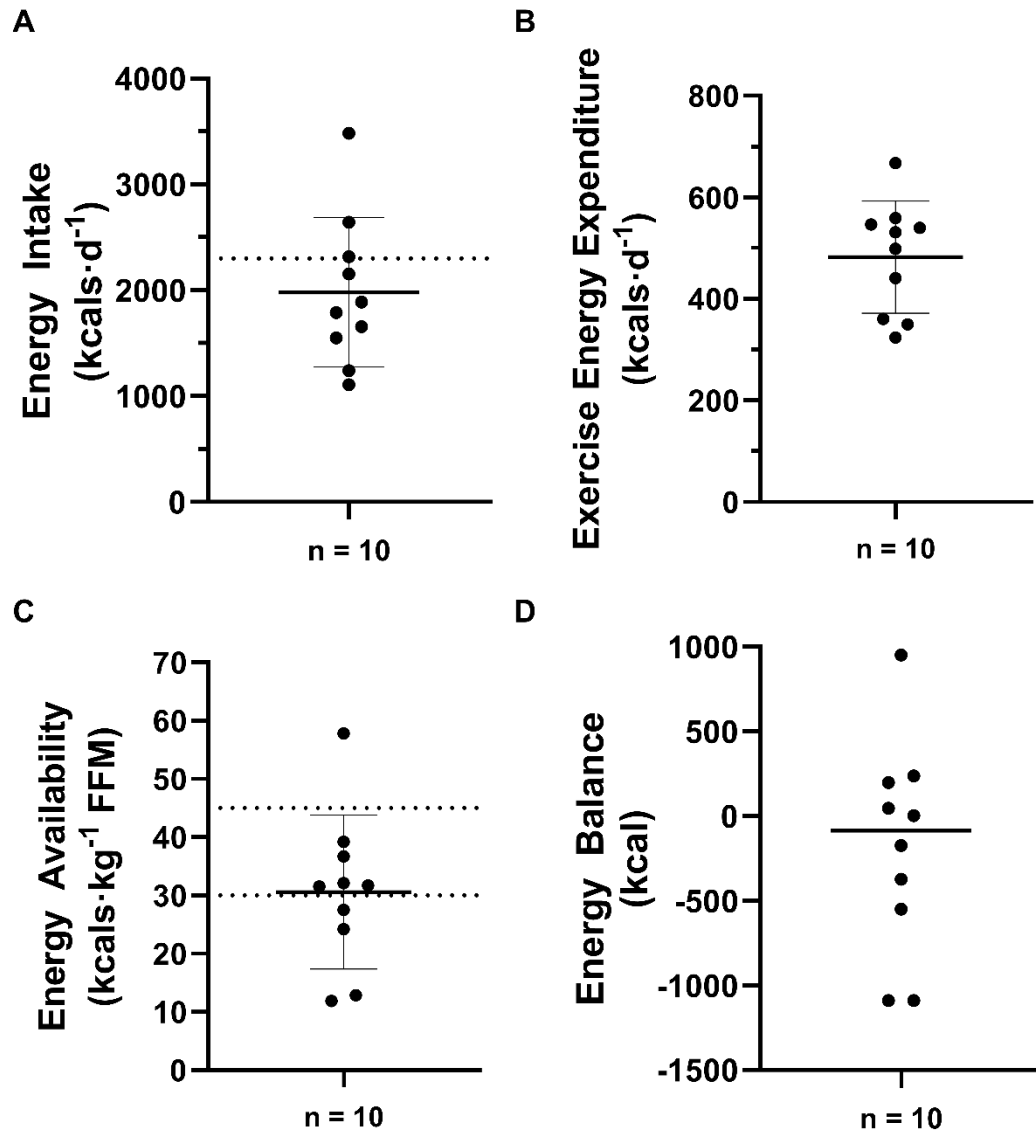
Participant characteristics can be found in Table 1. BMI was greater ( $p = 0.022$ ,  $d = 1.83$ ) in cadets with LEA compared to those without. There was also a trend for greater body fat ( $p = 0.085$ ,  $d = 1.27$ ) in cadets with LEA compared to those without.

### Energy Availability

Mean EA was  $30.6 \pm 13.2$  kcal  $\cdot$  kg<sup>-1</sup> FFM, with 4 of 10 (40%) cadets experiencing LEA. An additional 5 of 10 (50%) cadets were not experiencing LEA but were in a suboptimal EA state. Cadets consumed  $1,983 \pm 706$  kcal  $\cdot$  d<sup>-1</sup> with an EEE of  $482 \pm 110$  kcal  $\cdot$  d<sup>-1</sup>. Mean LEAF-Q scores were  $8.2 \pm 4.2$ , with 6 of 10 cadets at risk for LEA. LEAF-Q responses indicated that one cadet used a hormonal implant contraceptive, one used a copper IUD, three used oral contraceptives, and five indicated no hormonal contraceptives were used. Two cadets reported menstrual irregularities. Regarding typical bleeding length, one cadet reported 7-8 days, four cadets reported 5-6 days, and four reported 3-4 days. No cadet reported that menstruation stopped for  $\geq 3$  consecutive months. Measured RMR was  $1,488 \pm 149$  kcal, with predicted RMR being  $1,605 \pm 106$  kcal. RMR ratio (measured/predicted RMR) was  $0.93 \pm 0.07$ . There were no differences in



either measured or predicted RMR in cadets with LEA compared to those without. However, RMR ratio was greater in those with, compared to those without, LEA ( $0.97 \pm 0.01$  vs  $0.89 \pm 0.07$ ;  $p = 0.036$ ,  $d = 1.45$ ). In total, 3 of 10 cadets (30%) presented with RMR suppression. Interestingly, none of the cadets who presented with RMR suppression were currently experiencing LEA, though all were in a suboptimal EA state (mean EA in those with RMR suppression =  $33.3 \text{ kcal} \cdot \text{kg}^{-1} \text{ FFM}$ ). Refer to Figure 1 for EA, EI, EEE, and energy balance information and Table 2 for EA and energy balance parameters by EA status.



**Figure 1.** Energy intake (A), exercise energy expenditure (B), energy availability (C), and energy balance (D) in female ROTC cadets. Data are presented as mean  $\pm$  SD. Dashed lines represent (A) Military Dietary Reference Intake for energy intake and (C) optimal ( $\geq 45 \text{ kcal} \cdot \text{kg}^{-1} \text{ FFM}$ ), suboptimal ( $30 - 45 \text{ kcal} \cdot \text{kg}^{-1} \text{ FFM}$ ), and low ( $\leq 30 \text{ kcal} \cdot \text{kg}^{-1} \text{ FFM}$ ) energy availability.

**Table 2.** Energy Availability, Energy Balance, and Macronutrient Intakes in Female ROTC Cadets

	Total (N = 10)	LEA (N = 4)	No LEA (N = 6)	<i>p</i> -value	Cohen's <i>d</i>
Energy availability (kcal·kg <sup>-1</sup> FFM)	30.6 ± 13.2	19.1 ± 7.9	38.2 ± 10.1*	0.013	2.04
Energy intake (kcal·d <sup>-1</sup> )	1983 ± 706	1422 ± 307	2337 ± 639*	0.029	1.71
Relative energy intake (kcal·kg <sup>-1</sup> ·d <sup>-1</sup> )	29.9 ± 10.1	20.1 ± 5.3	36.5 ± 6.0*	0.002	2.84
CHO intake (g·d <sup>-1</sup> )	214 ± 62	151 ± 22	254 ± 26**	<0.001	3.99
Relative CHO intake (g·kg <sup>-1</sup> )	3.3 ± 1.2	2.1 ± 0.6	4.1 ± 0.6**	<0.001	3.27
PRO intake (g·d <sup>-1</sup> )	78 ± 25	71 ± 33	83 ± 23	0.601	0.35
Relative PRO intake (g·kg <sup>-1</sup> )	1.2 ± 0.4	1.0 ± 0.5	1.3 ± 0.3	0.311	0.70
Fat intake (g·d <sup>-1</sup> )	77 ± 25	60 ± 24	90 ± 24	0.088	1.25
Relative fat intake (g·kg <sup>-1</sup> )	1.2 ± 0.4	0.9 ± 0.3	1.4 ± 0.3*	0.024	1.8
RMR (kcal·d <sup>-1</sup> )	1488 ± 149	1581 ± 109	1425 ± 145	0.108	1.17
EEE (kcal·d <sup>-1</sup> )	482 ± 110	475.2 ± 103	486 ± 124	0.887	0.09
TEF (kcal·d <sup>-1</sup> )	198 ± 71	142 ± 31	236 ± 65*	0.029	1.71
Total energy expenditure (kcal·d <sup>-1</sup> )	2168 ± 257	2198 ± 194	2145 ± 305	0.779	0.19
Energy Balance	-184 ± 625	-776.1 ± 369	210 ± 392*	0.004	2.57

Data are presented as mean ± SD. CHO = carbohydrate; PRO = protein; RMR = resting metabolic rate; EEE = exercise energy expenditure; TEF = thermic effect of food (estimated as 10% of energy intake). \* Indicates significant difference from LEA ( $p < 0.05$ ); \*\* Indicates significant difference from LEA ( $p < 0.001$ ).

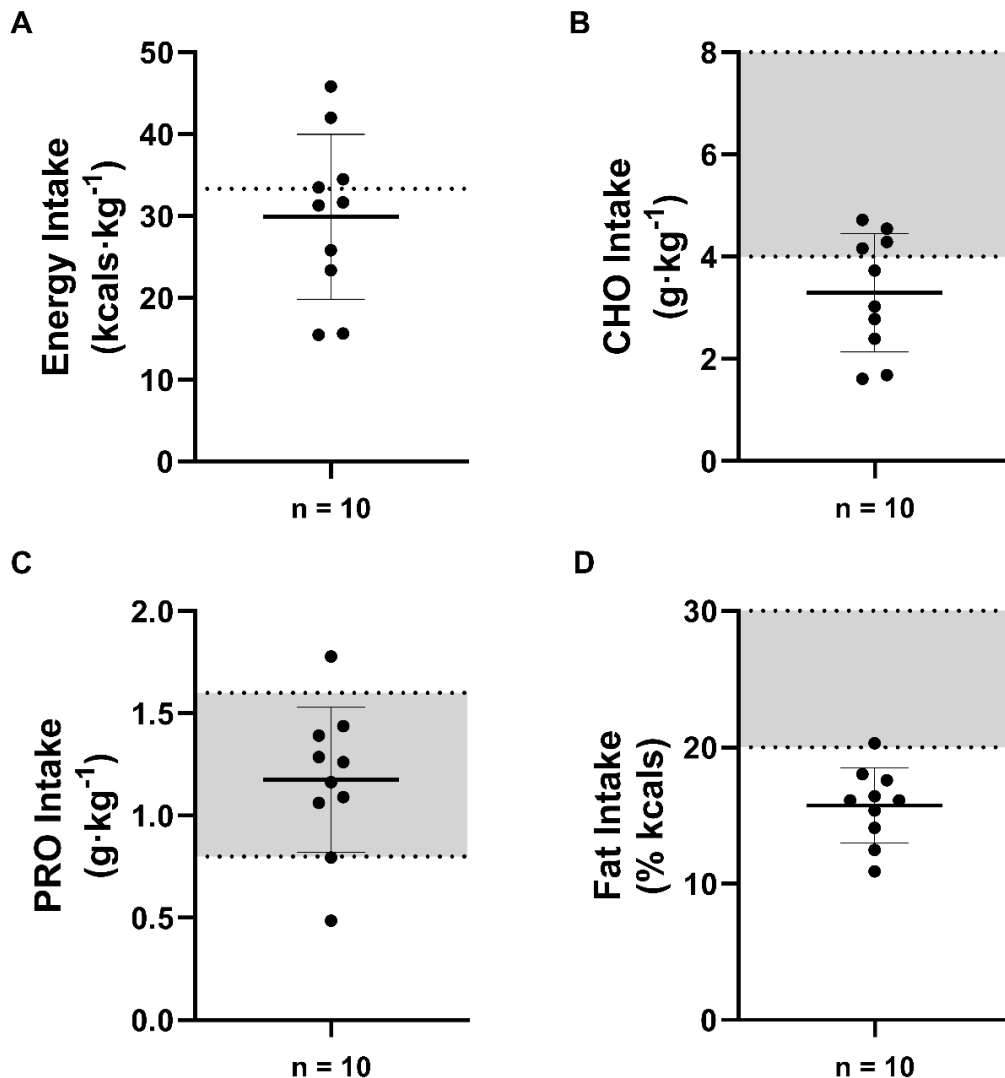
### Dietary Habits

On average, cadets consumed  $29.9 \pm 10.1$  kcal·kg<sup>-1</sup> calories,  $3.3 \pm 1.2$  g·kg<sup>-1</sup> CHO,  $1.2 \pm 0.4$  g·kg<sup>-1</sup> PRO, and  $1.2 \pm 0.4$  g·kg<sup>-1</sup> fat. Relative to the MDRI, 30% of female cadets met intake requirements for calories (33.33 kcal·kg<sup>-1</sup>), 40% met intake requirements for CHO (4-8 g·kg<sup>-1</sup>), 80% met intake requirements for PRO (0.8-1.6 g·kg<sup>-1</sup>), and 10% met intake requirements for fat (20-30% kcal). Those with LEA consumed significantly fewer calories (absolute and relative,  $p < 0.05$ ), fewer CHO (absolute and relative;  $p < 0.001$ ), and less fat (relative;  $p = 0.024$ ) compared to those without. No differences were observed for absolute or relative PRO. Refer to Figure 2 for relative energy and macronutrient intakes and Table 2 for dietary intakes by EA status.

For micronutrient intakes, cadets consumed  $13.8 \pm 4.5$  g·d<sup>-1</sup> of fiber,  $12.8 \pm 3.7$  mg·d<sup>-1</sup> of iron,  $766.2 \pm 281.0$  mg·d<sup>-1</sup> of calcium, and  $0.5 \pm 1.4$  mcg·d<sup>-1</sup> of vitamin D. Relative to the MDRI, none of the cadets met intake requirements for fiber (28 g·d<sup>-1</sup>), iron (18 mg·d<sup>-1</sup>), or vitamin D (15 mcg·d<sup>-1</sup>) while 20% met intake requirements for calcium (1000 mg·d<sup>-1</sup>). Those with LEA consumed less iron compared to those without ( $10.0 \pm 2.7$  vs.  $14.6 \pm 3.1$  mg·d<sup>-1</sup>;  $p = 0.042$ ). No differences were observed for any other analyzed micronutrients.

Meal consumption patterns can be found in Table 3. Of the potential opportunities for breakfast, female cadets skipped this meal on 12 (17.6%) occasions. Female cadets skipped lunch on 16 (23.5%) occasions and dinner on 7 (10.3%) occasions. Three cadets reported consuming a snack on all days. In total, snacks were consumed on 47.1% of possible eating occasions, with 3 cadets not taking advantage of this opportunity for energy and macronutrient intake. There were no differences in meal consumption patterns for main meals in those with compared to those without LEA. However, there was a trend ( $p = 0.055$ ) where those with LEA consumed snacks less frequently.

For breakfast, cadets consumed  $494 \pm 208$  kcal,  $20 \pm 14$  g PRO,  $21 \pm 14$  g fat, and  $58 \pm 13$  g CHO. For lunch, cadets consumed  $710 \pm 251$  kcal,  $35 \pm 14$  g PRO,  $30 \pm 11$  g fat, and  $77 \pm 35$  g CHO. For dinner, cadets consumed  $726 \pm 253$  kcal,  $40 \pm 23$  g PRO,  $32 \pm 14$  g fat, and  $68 \pm 35$  g CHO. For snacks, cadets consumed  $436 \pm 232$  kcal,  $12 \pm 7$  g PRO,  $21 \pm 12$  g fat,  $51 \pm 31$  g CHO. Cadets consumed similar caloric intakes for breakfast and snacks. Similarly, cadets consumed similar caloric intakes for lunch and dinner. Lunch and dinner caloric intakes were greater than breakfast and snack intakes ( $p < 0.05$ ). CHO intake at lunch was significantly lower ( $50 \pm 12$  vs  $99 \pm 32$  g;  $p = 0.026$ ) with a trend for lower caloric intake at lunch ( $554 \pm 114$  vs.  $835 \pm 269$  kcal;  $p = 0.094$ ) in those with LEA compared to those without, respectively.



**Figure 2.** Relative energy and macronutrient intakes in female ROTC cadets. Data are presented as mean  $\pm$  SD. The dashed line (A) represents the Military Dietary Reference Intake (MDRI) in moderately active females for relative energy intake ( $33.3$  kcal·kg<sup>-1</sup>·d<sup>-1</sup>). The shaded areas (B-D) represent the MDRI for carbohydrate (CHO,  $4$ - $8$  g·kg<sup>-1</sup>), protein (PRO,  $0.8$ - $1.6$  g·kg<sup>-1</sup>), and fat ( $20$ - $30$ % kcal).



**Table 3.** Meal Consumption Patterns in Female ROTC Cadets

Meal	Reported	Kcals
Breakfast (%)	82.4	494 ± 208 <sup>a</sup>
Skipped Breakfast (%)	17.6	
Lunch (%)	76.5	710 ± 251 <sup>b</sup>
Skipped Lunch (%)	23.5	
Dinner (%)	89.7	726 ± 253 <sup>b</sup>
Skipped Dinner (%)	10.3	
Snack (%)	47.1	436 ± 232 <sup>a</sup>
Skipped Snack (%)	52.9	

Data are presented as a percentage of all 68 possible eating opportunities. Dissimilar letters indicate significant differences in caloric intake ( $p < 0.05$ ).

### Sleep

Mean daily total time in bed was  $398 \pm 103$  min, total sleep time was  $373 \pm 100$  min, wake after sleep onset was  $25 \pm 10$  min, number of awakenings was  $12 \pm 3$ , awakening time was  $2 \pm 1$  min, and sleep efficiency was  $93.3 \pm 2.6\%$ . Only two cadets achieved the Army recommended 7–8 h of sleep<sup>10</sup> over the 7-day observation window. PSQI scores were  $6.2 \pm 3.1$ , with 6 of 10 (60%) cadets experiencing poor sleep quality as indicated by a global score  $\geq 5$ . ASBQ scores were  $38.1 \pm 5.7$ , with 3 of 10 cadets (30%) experiencing poor sleep behavior as indicated by a total score  $\geq 42$ . In those with LEA, 75% experienced sleep disturbance as determined by PSQI score compared to 50% without LEA. However, no statistical differences were observed in those with compared to those without LEA for any sleep metric. Refer to Table 4 for sleep metrics by EA status.

**Table 4.** Sleep metrics in female ROTC cadets

	Total (N = 10)	LEA (N = 4)	No LEA (N = 6)	p-value	Cohen's d
Time in bed (min)	398 ± 103	354 ± 116	428 ± 91	0.293	0.73
Total sleep time (min)	373 ± 100	329 ± 114	402 ± 87	0.279	0.75
WASO (min)	25 ± 10	25 ± 16	26 ± 6	0.983	0.02
Awakenings (#)	12 ± 3	19 ± 4	12 ± 2	0.610 <sup>†</sup>	0.04
Awakenings (min)	2 ± 1	2 ± 1	2 ± 1	0.705	0.25
Sleep efficiency (%)	93.3 ± 2.6	92.3 ± 4.1	94.0 ± 0.5	0.349	0.64
PSQI (global score)	6.2 ± 3.1	6.8 ± 3.6	5.8 ± 3.1	0.476 <sup>†</sup>	0.28
ASBQ (total score)	38.1 ± 5.7	36.5 ± 6.0	39.2 ± 5.8	0.503	0.45

Data are presented as mean ± SD. WASO = Wake after sleep onset; PSQI = Pittsburgh Sleep Quality Index; ASBQ = Athlete Sleep Behavior Questionnaire; <sup>†</sup> indicates nonparametric test. There were no differences between those with compared to those without LEA.

### Correlations

There were no significant relationships between EA and body composition variables, EEE, or sleep measures. There was a significant strong, positive relationship between EA and absolute ( $r = 0.946$ ;  $p < 0.001$ ) and relative ( $r = 0.949$ ;  $p < 0.001$ ) EI, as well as absolute ( $r = 0.694$ ;  $p = 0.026$ )

and relative ( $r = 0.679$ ;  $p = 0.031$ ) fat intake. Strong, positive trends for the relationship between EA and absolute CHO ( $r = 0.608$ ;  $p = 0.062$ ), absolute PRO ( $r = 0.559$ ;  $p = 0.093$ ), and relative PRO ( $r = 0.597$ ;  $p = 0.069$ ) were observed.

## Discussion

The present study supports our hypothesis that LEA and sleep disturbance would be detected and that the MDRI would not be met by most cadets. To our knowledge this was the first study to examine LEA prevalence in this population.

In the present study, we observed a mean EA of  $30.6 \pm 13.2$  kcal·kg<sup>-1</sup> FFM, with 40% prevalence of LEA and 50% prevalence of suboptimal EA. We observed a slightly lower prevalence of LEA in female cadets compared to other reports in Army cadets.<sup>12,24</sup> In a recent investigation, it was shown that LEA prevalence in male cadets (mean EA:  $28.3 \pm 8.2$  kcal·kg<sup>-1</sup> FFM) was 62% with the remaining 38% in a suboptimal EA state with no participants in an optimal EA state.<sup>12</sup> Though, it should be noted that there is debate regarding the use of 30 kcal·kg<sup>-1</sup> FFM as a cutoff for LEA in males as males may be able to withstand greater energy deficits without noticing adverse outcomes.<sup>1</sup> Similar results were observed in female British Army cadets in camp conditions. Edwards et al. utilized doubly labeled water for EE assessment and reported a 57.1% LEA prevalence (mean EA:  $25 \pm 10$  kcal·kg<sup>-1</sup> FFM) with the remaining 42.9% in a suboptimal state and no participants in an optimal state.<sup>24</sup> The present study reported one cadet in an optimal EA state. However, discrepancies in EE determined by doubly labeled water compared to accelerometry have been reported, resulting in lower EA values in females during military training with doubly labeled water.<sup>25</sup> As such, it is possible that discrepant findings are due to methodological differences between studies. Notwithstanding, in the one cadet with optimal EA in the present study, mean EI was largely influenced by one day where intake was ~5x greater than the average EI of all other days. If this data point was removed, EA in this cadet drops from 57.8 to 27.2 kcal·kg<sup>-1</sup> FFM. In this case, we would have observed a 50% prevalence of LEA with no cadets achieving an optimal state and an overall mean EA of  $27.5 \pm 9.1$  kcal·kg<sup>-1</sup> FFM, which is more closely aligned with other reports in ROTC cadets. Interestingly, this cadet also reported the highest LEAF-Q score, suggesting a high prevalence of symptoms associated with LEA. Given the known inaccuracies of self-reported dietary records,<sup>26</sup> it is possible that erroneous recording of EI influenced our result. However, EI is typically underreported as opposed to overreported.<sup>26,27</sup> Given the high prevalence of symptoms associated with LEA in this cadet, it is more likely that they are chronically in a LEA state and that this single day of consumption does not accurately represent normal consumption patterns. This claim is also supported by the average EI during the other days of observation in this cadet and highlights the need for additional research examining LEA across longer durations in female tactical athletes.

The LEAF-Q is a validated questionnaire for assessing LEA risk in female athletes.<sup>18</sup> In the present study, mean LEAF-Q scores for female cadets were  $8.2 \pm 4.2$ , indicating cadets were at risk of LEA and REDs. This suggests that female cadets experience a high prevalence of symptoms associated with LEA. Ultimately, six cadets were at risk for LEA and were experiencing a high prevalence of symptoms according to their LEAF-Q scores. However, only

two of these cadets were in a LEA state. In the remaining cadets identified as at risk, three were in a suboptimal state and one was in an optimal EA state. The LEAF-Q also failed to identify two cadets who were experiencing LEA but were below the reported threshold for LEA risk. LEA can occur unintentionally due to increases in training demands but can also occur intentionally, such as in the case of purposeful body recomposition. Detriments of LEA are also a product of the magnitude and duration of the LEA state, whereby short-term LEA resulting from this purposeful body recomposition may not result in adverse health outcomes<sup>1</sup> and may instead serve to enhance athletic performance.<sup>6</sup> Thus, it is also possible that the individuals experiencing LEA in the present study who did not meet the threshold for LEA risk may have been in a state of adaptable LEA and were not experiencing adverse health outcomes due to this.<sup>1</sup>

RMR suppression is quantified as the ratio between measured RMR and predicted RMR, with a ratio of  $< 0.9$  indicating maladaptive responses to energy deficiency in active females.<sup>17</sup> As this is, to our knowledge, the first study to examine RMR suppression in ROTC cadets, it is not possible to make direct comparisons to existing literature. However, in the current study, 30% of cadets presented with RMR suppression. Interestingly, none of the cadets with RMR suppression were characterized as having LEA. Rather, all cadets with RMR suppression were in a suboptimal EA state. First, it must be considered that an absolute threshold of 30 kcal  $\cdot$  kg<sup>-1</sup> FFM has been questioned due to likely variation in individual response,<sup>28</sup> which could explain our findings. The IOC recently proposed the term “adaptable LEA” to describe those experiencing short-term and often purposeful energy deficiency but with minimal or no detrimental health or performance consequences.<sup>1</sup> Thus, it is possible that those with LEA but not RMR suppression were in a state of adaptable LEA, especially considering the greater BMI and trends for greater body fat percentage ( $d = 1.27$ ) in those with LEA. In the opposite case, where cadets were experiencing RMR suppression but not LEA, it is possible that these cadets have been in an energy deficient state for an extended period of time, resulting in adverse outcomes. A LEA load model has been proposed to account for both the magnitude and duration of exposure to suboptimal EI.<sup>29</sup> In this model, greater LEA load (i.e., greater magnitude and/or duration of LEA) is theorized to result in greater detriment to physiological function. Though this model has not been validated, it is possible that cadets with RMR suppression but with suboptimal rather than low EA, are experiencing large LEA loads and therefore, greater detriments to physiological function.

The Army recognizes nutrition as a critical component for performance optimization and therefore, tactical readiness and mission success.<sup>10</sup> However, dietary analysis indicated that female cadets failed to meet most of the macronutrient recommendations established in the MDRI.<sup>21</sup> Mean EI was  $1984 \pm 706$  kcal  $\cdot$  d<sup>-1</sup>, far below the recommended 2300 kcal  $\cdot$  d<sup>-1</sup> for a 69 kg female performing moderate activity, the Army reference for female MDRI. When examined relative to body mass, only 30% of female cadets achieved the recommended 33.33 kcal  $\cdot$  kg<sup>-1</sup>  $\cdot$  d<sup>-1</sup>. Most cadets met the MDRI for protein, with 80% consuming the recommended 0.8 – 1.6 g  $\cdot$  kg<sup>-1</sup>. In contrast, only 10% and 40% of female cadets consumed adequate fat (20-30% EI) and CHO (4 – 8 g  $\cdot$  kg<sup>-1</sup>), respectively. These findings partially agree with intake data in male cadets, where adequate PRO was consumed but intakes were insufficient for both calories and CHO.<sup>12</sup>

However, the same authors reported that in males, all but one cadet met or exceeded the recommended intake for fat while the present study observed 90% consuming less than the MDRI. Taken together, the present study highlights the need for greater caloric intake in female cadets, primarily through increased CHO and fat. Considering nutrition education has been effective for improving energy and macronutrient intakes as well as EA status and LEAF-Q scores in female athletes,<sup>30</sup> incorporating nutrition education sessions may be one such way to do so.

In line with the recent IOC consensus statement,<sup>1</sup> we<sup>31</sup> and others,<sup>12,32</sup> have observed reduced energy and CHO intake in those with LEA. This reduced CHO intake appears consistently and has been observed in both males<sup>12,32</sup> and females<sup>31,32</sup> and in both sport<sup>31,32</sup> and tactical athletes.<sup>12</sup> Thus, CHO supplementation likely represents an effective strategy to improve EA status in various athletes, including female cadets. In addition to reduced energy and CHO intakes, we also observed reduced fat intake in female cadets with LEA compared to those without. Similar findings were reported for male cadets where less energy, CHO, and fat was consumed in those with LEA with no differences in PRO intake.<sup>12</sup> The findings of the present study offer valuable insight as suboptimal energy and macronutrient intakes result in decrements in health, psychological state, and physical and cognitive performance in military populations.<sup>3</sup> Thus, although not measured in the present study, it is likely that these cadets are performing sub-optimally. Additional work is needed investigating the impact of LEA on female tactical athlete performance.

LEA occurs when there is a mismatch between EI and EE such that insufficient energy is available for normal physiological function.<sup>1</sup> While we observed differences in EI between cadets with and without LEA, we observed no differences in EEE ( $475 \pm 103$  and  $486 \pm 124$  kcal, respectively). In agreement with our findings, no differences were observed for EEE in male cadets with LEA compared to those without ( $489 \pm 325$  and  $456 \pm 350$  kcal, respectively).<sup>12</sup> This appears to suggest that, at least in garrison conditions, LEA is primarily a function of reduced and insufficient EI as opposed to excess EEE in ROTC cadets, highlighting the importance of nutrition education in this population. In support of this claim, significant correlations were observed between EA and absolute ( $r = 0.946$ ,  $p < 0.001$ ) and relative ( $r = 0.949$ ,  $p < 0.001$ ) EI but not EEE ( $r = 0.042$ ,  $p = 0.907$ ). During field training exercises in British ROTC cadets, EI was reduced and EE increased,<sup>24</sup> creating a greater mismatch between EI and EE and potentially shifting the contributing factors to LEA during field training compared to garrison conditions. As the present study only investigated EA status during garrison conditions, additional work investigating LEA during military specific training is needed, especially in female tactical athletes.

The Army also recognizes sleep as a critical component of tactical athlete performance.<sup>10</sup> Yet, sleep disturbance is a common issue in military personnel, with tactical athletes experiencing greater sleep disturbance compared to civilians.<sup>14,33</sup> In male cadets, 85% presented with sleep disturbance as determined by a global score  $\geq 5$  on the PSQI.<sup>12</sup> As it has been suggested that sleep disturbance presents differently between sexes<sup>34</sup> and as fewer data exist on sleep disturbance in female military personnel,<sup>35</sup> we sought to assess sleep quantity and quality in

female cadets. In the present study, we observed sleep disturbance in 60% of cadets via a global score  $\geq 5$  on the PSQI. Using validated wrist-worn accelerometers,<sup>23</sup> we were also able to quantitatively assess sleep in these cadets. The Army recommends 7-8 h of nightly sleep.<sup>10</sup> Yet, in the present study, only 20% of cadets achieved this recommendation over the 7-day observation window. Others investigating sleep quantity in ROTC cadets reported that only 7.4% averaged  $\geq 7$  h of sleep per night<sup>33</sup> which agrees with our findings. Taken together, sleep disturbance is highly prevalent in this population with most cadets failing to meet the Army recommendation. It also appears that female cadets sleep less than other college students<sup>36</sup> and student athletes.<sup>37</sup> Given that impaired sleep has been shown to increase injury risk<sup>38</sup> and reduce motivation and tactical performance,<sup>33</sup> efforts to improve sleep in cadets should be prioritized. It has been previously suggested the LEA exacerbates sleep disturbance<sup>13</sup> though we observed no statistical differences in those with LEA for any sleep metric. The non-significant finding for sleep time in those with LEA compared to those without ( $329 \pm 114$  and  $402 \pm 87$  min, respectively), despite a medium to large effect size ( $d = 0.75$ ), is likely due to the sample size, highlighting the need for additional work investigating sleep differences between those with and without LEA in female cadets utilizing larger sample sizes.

The primary strength of this study is that it is the first to characterize the prevalence of LEA in female U.S. ROTC cadets. Given the dearth of information currently available on LEA in female tactical athletes, additional work is required in this area and the present study sought to address this gap in the literature. Furthermore, the free-living conditions of the present study also represent a strength as these conditions allow for ecological validity, whereby a more accurate representation of true EA status, dietary habits, and sleep are presented. Additionally, we build upon existing literature on LEA in ROTC cadets by quantitatively assessing sleep using non-invasive wearable technology as opposed to questionnaires only. Similarly, to our knowledge, we are the first to assess RMR suppression in this population. Lastly, we quantitatively measured EEE using validated non-invasive wearable technology rather than estimating from self-reported records. Considering that EEE is utilized to determine LEA and that individuals typically overrepresent their physical activity,<sup>39</sup> this is an important strength in the current study.

However, there are limitations which must be addressed. There are known inaccuracies with self-reported food logs which could have impacted our findings.<sup>26</sup> Similarly, there is some suggestion that the accelerometers utilized in the present study may overestimate EE at lower exercise intensities and underestimate EE during higher intensity or sport-specific type exercise<sup>40</sup> which could have impacted our findings. Additionally, the present study investigated LEA prevalence during a single 7-day period in garrison conditions in a relatively small sample of female ROTC cadets. The present study collected data from a single ROTC program which, given that females make up a smaller proportion of the military compared to males,<sup>3</sup> likely impacted our sample size. As such, exploratory comparisons in cadets with and without LEA in the present study may be underpowered to draw definitive conclusions and should be interpreted with caution. Future work should investigate dietary habits and sleep in female cadets with and without LEA utilizing larger sample sizes or multi-site designs. Our results may not represent long-term LEA or LEA during field operations and may not translate to other



military populations. Thus, future work should investigate LEA prevalence in female cadets and other female tactical athletes at multiple time points and during various military specific scenarios.

LEA and impaired sleep result in a plethora of adverse health and performance outcomes. The present study demonstrates a high prevalence of LEA and sleep disturbance as well as inadequate dietary intake in female cadets. The Army recognizes both nutrition and sleep as critical components of tactical athlete performance, and therefore tactical readiness and mission success. Indeed, the 4th International Congress on Soldiers' Physical Performance identified nutrition and sleep as the top 7<sup>th</sup> and 3<sup>rd</sup> priority research gaps, respectively.<sup>11</sup> Accordingly, efforts to improve tactical athlete dietary and sleep habits should be prioritized. Data from the present study can be utilized by Command personnel and practitioners to enhance decision-making and education related to nutrition and sleep in female cadets. Given that the majority of cadets did not consume adequate calories under garrison conditions, primarily through low CHO and fat intakes, cadets would likely benefit from supplemental CHO or energy bars to increase intakes of these key macronutrients. Providing these extra calories as a snack may be optimal considering the trend for lower snack frequency in those with compared to those without LEA. Future work should investigate the utility of an additional snack on habitual dietary intakes of ROTC cadets. Further, as all cadets in the present study were experiencing symptoms associated with LEA and/or presented with insufficient or disturbed sleep, cadets would likely benefit from additional nutrition and sleep education.

### Acknowledgements

The authors would like to thank Lieutenant Colonel Nicole Dallochio for allowing us to complete this study. We would also like to thank the cadets who participated in this study for their time and effort. This research was funded by the University of South Carolina Upstate Office of Sponsored Awards and Research Support and by a RISE grant from the Office of the Vice President for Research at the University of South Carolina.

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