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Effect of leucine supplementation on fat free mass with prolonged hypoxic exposure during a 13-day trek to Everest Base Camp: A double-blind randomized study

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Abstract

Loss of body weight and fat free mass (FFM) are commonly noted with prolonged exposure to hypobaric hypoxia. Recent evidence suggests protein supplementation, specifically leucine, may potentially attenuate loss of FFM in subcaloric conditions during normoxia. The purpose of this study was to determine if leucine supplementation would prevent the loss of FFM in subcaloric conditions during prolonged hypoxia. Eighteen physically active male (n=10) and female (n=8) trekkers, completed a 13-day trek in Nepal to Everest Base Camp with a mean altitude of 4140 m (range 2810-5364 m). In this double-blind study, participants were randomized to ingest either leucine (LEU) (7 g leucine, 93 kcal, 14.5g whey-based protein) or an isocaloric isonitrogenous control (CON) (0.3 g LEU, 93 kcal, 11.3 g collagen protein) twice daily prior to meals. Body weight, body composition, and circumferences of bicep, thigh, and calf were measured pre and post trek. There was a significant time effect for body weight (-2.2%±1.7%), FFM (-1.7%±1.5%), fat mass (-4.0% ± 6.9%), and circumferences (p< 0.05). However, there was no treatment effect on body weight (CON -2.3 ± 2.0%; LEU -2.2 ± 1.5%), FFM (CON -2.1 ± 1.5%; LEU -1.2 ± 1.6%), fat mass (CON -2.9% ± 5.9%; LEU -5.4% ± 8.1%), or circumferences. Although a significant loss of body weight, FFM, and fat mass was noted in 13 days of high altitude exposure, FFM loss was not attenuated by leucine. Future studies are needed to determine if leucine attenuates loss of FFM with longer duration high altitude exposure.

Keywords: high altitude, weight loss, protein supplementation, body composition

Introduction

It is generally accepted that weight maintenance is difficult at elevations above 5000 m. Although weight loss of 5-10% is common, some individuals may lose as much as 20% of their body weight secondary to negative energy balance associated with altitude-induced anorexia (Pulfrey and Jones 1996; Butterfield et al. 1992; Rose et al. 1988). The result is a loss of fat but also the undesired effect of decreased lean body mass (LBM) (Westerterp et al. 1992; Kayser et al. 1994).

Several studies have reported substantial losses of LBM with high altitude exposure. Most report approximately 60-70% of body weight loss as LBM with altitude exposure ≥ 4 300 m (Fulco et al. 2002; Armelini et al. 1997). Additionally, studies examining muscle cross-sectional area reported a 17% reduction in the thigh muscle following 40 days in a hypobaric chamber simulating 8000 m (Rose et al. 1988) and a 20% reduction in the *vastus lateralis* after 8 weeks above 5000 m (Hoppler et al. 1990). The significant loss in lean tissue results in decreased muscle oxidative capacity (Hoppeler et al. 1990), muscle strength (Sergi et al. 2010), physical performance (Fulco et al. 1998), and increased infection and poor wound healing (Murdoch et al. 1995).

Altitude-induced loss of LBM is poorly understood. Energy balance alone does not appear to explain this phenomenon. Macdonald et al. (2009) reported loss of LBM despite increased energy intake and high initial fat mass. Previous studies have suggested that loss of LBM is secondary to increased utilization of protein for energy and decreased exercise intensity (muscle disuse) (Imoberdorf et al. 2006), but few have examined the potential effect of altitude directly on protein turnover. In rats exposed to hypobaric hypoxia (7620 m) for 14 days, protein degradation increased to a much greater extent than protein synthesis (five-fold vs. 1.5-fold)

compared to normoxia (Chaudhary et al. 2012). Further, caloric restriction (Pasiakos et al. 2010) or other related high altitude factors (i.e. acid-base balance) (Preedy et al. 1985) may influence protein synthesis. Data suggests that after only 7-9 days of exposure to approximately 4500 m, the mammalian target of rapamycin, a key regulator of protein synthesis, is reduced (Vigano et al. 2008).

Leucine is a substrate for protein synthesis, inhibitor of protein degradation, and an important cell signaling nutrient (Balage and Dardevet 2010). Recent data suggests that loss of LBM from caloric restriction at sea level may be attenuated by leucine (Donato et al. 2006; Jitomir and Willoughby 2008; Balage and Dardevet 2010). Whether or not leucine is beneficial for LBM preservation with caloric restriction at altitude is unclear. Only one published study has examined the effects of branched chain amino acids on LBM at high altitude (Schena et al. 1992). Although BCAA were found to significantly attenuate the loss of LBM and strength during a high altitude trek, it is unknown if the BCAA, total kilocalories, or total protein were responsible for the attenuation. Therefore, the main objective of this study was to determine the effects of supplemental leucine compared to a low-leucine supplement on fat free mass (FFM) of individuals participating in a 13-day trek to Mount Everest base camp. It was hypothesized that FFM loss would be attenuated in those individuals who consumed the higher-content leucine supplement.

Materials and Methods

Participants

Twenty-eight healthy, recreationally active volunteers (15 males, 13 females) were recruited from two separate but identical Wilderness Medical Society (WMS) Mount Everest base camp treks. All participants were exposed to the same barometric pressure, daily

temperature, relative humidity, and solar load. To participate in the treks, volunteers were required to be in good health and fitness. Physical activity records indicate participants were of similar training status with pre-trek exercise regimens consisting of resistance training 2 to 4 times per week for 30 to 60 minutes and aerobic training for 2 to 4 times per week for 30 to 60 minutes at moderate to high intensity. Following approval from the University of Utah institutional review board, participants were informed of the study purpose, procedures, and potential risks and benefits prior to providing written consent.

Ten participants (5 male, 5 female) withdrew from the study immediately prior to beginning the trek (n=2) or during the trek (n=8). Prior to the trek, participants withdrew secondary to family medical reasons (n=1) and self-reported chocolate intolerance (n=1). During the trek participants withdrew secondary to food-associated gastrointestinal illness resolved with antibiotics (n=2; day 2), loss of appetite (n=1; day 2), acute mountain sickness (n=3; days 5-8), high altitude pulmonary edema (n=1; day 8), and fall-related injury (n=1; day 2). Participants who withdrew did not differ from those that completed the study in age, body mass index (BMI), prior physical activity, baseline body weight, or baseline body composition. Withdrawn participants were not included in the final analyses. Participant characteristics (N=18) are summarized in Table 1. There were no differences in baseline measurements between treatment groups.

Study Design

The present study evaluated the effect of a leucine (LEU) supplement versus a control (CON) supplement on FFM during a 13-day trek to Everest base camp. In double-blind fashion, participants were randomized by age and gender to receive either LEU or CON twice daily (pre-breakfast, pre-lunch) for 13 days. Anthropometric (height, body weight, and circumferences)

and body composition were measured pre- and post-trek in Kathmandu, Nepal. During the trek, 6 days of energy intake and daily energy expenditure, hydration, and symptoms of acute mountain sickness (AMS) were recorded.

Nutritional Supplements

The nutritional composition of LEU and CON is shown in Table 2. The isocaloric (93 kcal) isonitrogenous supplements (7 g leucine, 14.5 g whey-based protein LEU; 0.3 g leucine, 11.3 g collagen protein CON) were chocolate-flavored dry powder mixed with approximately 240 ml of boiled water (Glanbia Nutritionals, Twin Falls, ID). Supplements were manufactured from leucine peptides isolated from whey protein (PepForm™) (LEU) and collagen (CON) with limited amounts of the branched chain amino acids valine and isoleucine to isolate the effects of leucine yet still support protein synthesis. To account for a heterogenous study population (i.e., gender and age) and the impracticality of creating a supplement on a per kg body weight basis, a highly enriched supplement of 7 g LEU was designed to maximize the anabolic response of feeding and exercise. This dose has been observed in other reports as anabolically maximal and tolerable in both young (7g; Dreyer et al. 2008) and older adults (6 g; Burd et al. 2012) with no adverse effects noted. Additionally, the LEU daily study dose of 14 g is below the Upper Tolerable Level of 500 $mg \cdot kg^{-1} \cdot d^{-1}$ (Pencharz et al. 2012). Because breakfast and lunch were communal and were similar for all participants, participants ingested LEU or CON immediately prior to these meals. Standardized timing allowed investigators to personally monitor and encourage supplement consumption. Additionally, because muscle anabolic sensitivity to protein ingestion has been shown to persist at least 24 hours following exercise (Burd et al. 2011), ingestion with meals captured the beneficial additive anabolic effects of prior exercise.

To document compliance, participants also recorded the number of supplement packets they ingested daily.

Anthropometric Measurements

All anthropometric measurements were conducted in Kathmandu, Nepal. Body weight, self-reported height, circumferences, and body composition were measured 1-2 days prior to trek departure and again within 24 hours of trek completion. Participants were weighed (pre- and post-trek) on a digital portable scale (CPW-150, Adam Equipment, Inc. Danbury, CT) to the nearest 0.01 kg following an overnight fast and morning void in lightweight shorts and shirt. One trained investigator per trek performed all measurements.

Body composition was estimated from skinfold thicknesses measured with a handheld ultrasound device (BodyMetric Ultrasound Body Composition Tester, IntelaMetrix, Inc., Livermore, CA) on established skinfold sites (men: chest, abdomen, thigh; women tricep, anterior suprailiac, and thigh) (Jackson & Pollock 1978; Jackson et al. 1980). Unlike other field methods of body composition assessment, ultra sound is not influenced by hydration status, recent exercise, and caffeine consumption and has been shown to be a more accurate measure of body composition compared to skinfolds measured with calipers (Selkow et al. 2011). Ultrasound also has good agreement with Dual-Energy X-Ray Absorptiometry (DEXA) (Pineau et al. 2007) and hydrostatic weighing (Utter and Hager 2008). Body fat percent was calculated with the equation of Jackson & Pollock (1978) for men and Jackson et al. (1980) for women. Fat free mass was calculated by subtracting body fat (kg) from body weight (kg). To standardize the differences in body composition between the participants, percentage change was also calculated by dividing the measurement difference (post - pre) by the pre-trek measurement and multiplying by 100.

Arm, thigh, and calf circumferences were measured using the International Standards for Anthropometric Assessment for anatomical landmark assessment (International Society for the Advancement of Kinanthropometry 2001). Measurements were obtained on the right side of the body using a nonstretch tape measure while participants were standing. Two nonconsecutive measurements were made for all sites. A third measurement was taken if there was >1% difference between the first two measurements. If two measurements were taken, the mean value was used for data analysis. If three measurements were taken, the median value was used for data analysis.

Diet and Activity

To ensure there were no treatment group differences in energy and macronutrient intake, following instruction on food items and portion sizes, participants completed 3-day food record booklets twice during the trek (days 1-3 and 8-10) for a total of 6 days. To decrease participant burden, food booklets were not recorded the entire trek. Booklets included common teahouse menu items for each meal and estimation of portion sizes to improve accuracy of food records. Daily energy and macronutrient intake was determined using Food Processor SQL (version 10.11.1 2012; ESHA Research).

Energy expenditure was measured continuously (excluding bathing) in 1-minute intervals throughout the 13-day trek using an omni-directional accelerometer-based activity monitor (Actical, Mini Mitter, Inc., Bend, OR) worn on the ankle. Data were converted to 15-minute intervals by the United States Army Research Institute of Environmental Medicine (USARIEM). Energy expenditure was calculated by USARIEM using the following Compendium of Physical Activities equation [(MET)(3.5)(body weight+pack weight)/200]15. Daily energy expenditure was calculated by summing all energy expenditure data within a 24-hour period.

Hydration

Because body weight is influenced by hydration status, urine specific gravity (USG) was measured daily throughout the study with urine strips (HydraTrend™, UriDynamics, Inc., USA). Participants were provided with urine strips and following his/her first morning void, returned urine strips to the investigator for measurement and recording.

Acute Mountain Sickness

To rule out AMS as a potential confounding variable, a survey and assessment of AMS was conducted twice daily (morning, evening). The incidence and severity of AMS was determined from information gathered using a subset of the Environmental Symptoms Questionnaire (ESQ) and the Lake Louise AMS Scoring System (LLS). The ESQ is a self-reported, 68-question inventory used to document symptoms induced by altitude and other stressful environments (Sampson et al. 1983). In this study, participants were asked to answer 16 of the 68 questions. The LLS consists of a six question (questions 21-26) self-reported assessment of AMS symptoms (Roach et al. 1993). To determine the prevalence of AMS, a weighted average of scores from 11 symptoms designated AMS-C was calculated. AMS-C scores >0.7 were defined as indicating the presence of AMS. LLS scores ≥3 with presence of headache were diagnostic of AMS.

Statistical Analysis

Data are expressed as means ± SD for all variables. Normality was assessed using the Kolmogorov-Smirnov test. All variables were normally distributed. Differences in body weight, FFM, fat mass, and circumferences were analyzed using separate 2 x 2 repeated measures analysis of variance (ANOVA) (time [pre, post] x group [CON, LEU]). There were no violations of the sphericity assumption indicated by the Mauchley test of sphericity. Participant

characteristics, energy intake, energy expenditure, and supplement compliance and consumption were analyzed using independent *t*-tests. For all analyses, statistical significance was set at $p < 0.05$. Statistical analysis was performed using SPSS, Version 20 (Chicago, IL).

Results

A total of 18 participants completed the study (*n*=10 CON; *n*=8 LEU). Supplement compliance data was retrieved for 13 participants with 96% ± 6% CON and 89% ± 17% LEU consuming the prescribed two packets per day. There was no significant difference in compliance between the two groups. Average leucine consumption was significantly greater for LEU than CON (12.5 ± 2.5 g·day^{-1}; 16.8 ± .05 g·kg^{-1} and 0.7 ± 0.0 g·day^{-1}; .01± .00 g·kg^{-1}, respectively) ($p < 0.05$). No side effects were reported.

Anthropometric measurements

There was a significant main time effect for body weight [-1.9 ± 1.5 kg (-2.2% ± 1.7%); *p*=.000], FFM [1.0 ± 0.9 kg (-1.7%±1.5%); *p* =.000)], and fat mass [-0.8 ± 1.2 kg (-4.0% ± 6.9%); *p* =.010.]. There were no differences between groups for body weight (-2.3% ± 2.0% CON; 2.2% ± 1.5% LEU), FFM (-2.1% ± 1.5% CON; -1.2% ±1.6% LEU), or fat mass (-2.9% ± 5.9% CON; -5.4% ± 8.1% LEU) (Fig.1). The majority of weight loss for CON was FFM (66%) and for LEU fat mass (58%). There was also a significant time effect for loss of bicep, thigh, and calf circumferences ($p < 0.05$), with no differences between groups (Fig. 2).

Diet and Physical Activity

Diets of participants during the trek consisted primarily of common teahouse foods such as eggs, toast, muesli, chapati, potatoes, noodles, fried rice, lentils, vegetable soup, pizza, and tea. Very few participants brought their own sport foods (i.e. bars and drinks). Snacks purchased along the trek consisted of potato chips, candy bars, and soda. Daily averages for energy and

macronutrient intake are listed in Table 3. There were no significant differences in absolute (kcal·d^{-1}; g) or relative (kcal·kg^{-1}; g·kg^{-1}) energy, carbohydrate, protein, or fat intake between groups.

Daily physical activity consisted of seven to eight hours of moderate to vigorous hiking with backpacks (mean backpack weight 7.8 kg ± 2.4 kg) over uneven, hilly terrain with an approximately 60 to 90 minute lunch mid-day. Following dinner at the evening's lodging, participants then completed an additional 30 to 60 minute acclimatization hike before retiring for the evening. There were no significant differences in average total daily energy expenditure between groups (3803 ± 750 kcal·day^{-1} CON; 3653 ± 641 kcal·day^{-1} LEU). Average caloric deficit was 1575 ± 651 kcal·day^{-1} for the 6 days that food records were recorded. Energy balance (energy intake and expenditure) is depicted in Fig. 3.

Hydration

Participants were dehydrated (USG ≥1.020) an average of 4.1 ± 3.3 days of the 13 day trek with no differences between groups (3.7 ± 3.4 CON; 4.5 ± 3.3 LEU). Mean USG was 1.018 for both groups.

Acute Mountain Sickness

Acute Mountain Sickness (AMS) was highly prevalent with approximately 65% experiencing at least 1 day of AMS during the 13 day trek with an average of 2 days with AMS. Mean Lake Louise score was 1.06 CON and 1.01 LEU and ESQ .12 CON and .07 LEU. There were no significant differences in AMS prevalence (AMS ≥ 1 day) between groups (60% CON; 71.5% LEU).

Discussion

The current study investigated the effects of leucine supplementation on FFM during an

extended trek to Everest base camp at a mean altitude of 4139 m. Supplementation with 7g of leucine twice daily during the 13-day trek did not attenuate the loss of body weight, FFM, or circumferences compared with an isocaloric isonitrogenous control. However, after only 13 days at a high altitude, participants saw a significant loss of body weight, FFM, fat mass, and bicep, thigh, and calf circumferences. Further studies are needed to evaluate if leucine supplementation is effective for preserving FFM following longer duration hypobaric hypoxic exposure.

Our study suggests that a similar loss in FFM noted in studies of longer duration (21-40 days) and higher elevations can be observed in a much shorter duration (13 days) and lower elevation. In the present study, the average weight loss for CON was 1.7% with the majority of weight loss (66%) attributed to FFM. Rose et al. (1988) reported a loss of 67% FFM following a 40 day simulated ascent of Mt. Everest and Fulco et al. (2002) reported a loss of 69% FFM following a 1500 kcal per day deficit for 21 days at 4300 m. Conversely, in the present study, the majority of weight loss in LEU was fat mass, not FFM (58% fat mass, 42% FFM).

Although a significant time effect was noted in body weight, FFM, fat mass, and circumferences there was no treatment effect. The present findings are in contrast to Schena et al. (1992) who found that BCAA (5.76 leucine, 2.88g isoleucine, 2.88g valine) attenuated the loss of LBM during a 21-day trek at a mean altitude of 3255 m. This may partially be explained by the shorter duration (13 days vs. 21 days) and higher mean altitude (4139 vs. 3255 m) in the present study. The higher altitude would induce greater hypoxia and potentially greater suppression of protein synthesis and/or increased protein degradation since hypoxia may negatively affect anabolic cellular signaling (i.e., mTOR) and impair protein synthesis (Vigano et al. 2008) and increase protein degradation (Chaudhary et al. 2012). Further, dietary intake is unknown so it is difficult to determine if participants were in negative energy balance or if the

total kilocalorie intake, protein intake, or the BCAAs were responsible for the attenuation of LBM loss noted by Schena et al. (1992).

A likely contributor to the loss of FFM and body weight and lack of treatment effect may be the considerable caloric deficit, and in particular, low carbohydrate and protein intake, noted in the present study. A mean caloric deficit of 1575 ± 651 kcal·day^{-1} (43% of average total daily energy expenditure) was observed for the 6 days of recorded food intake. Previous high altitude studies have noted similar caloric deficits (Westerterp et al. 1992; Westerterp-Plantenga et al. 1999; Rose et al. 1988). Pasiakos et al. (2010) demonstrated that a 19% reduction in energy intake resulted in a 19% decrease in protein synthesis and downregulation of intracellular signaling proteins responsible for stimulating protein synthesis. Further, during caloric deficit with low carbohydrate availability, dietary protein or existing protein tissue may be catabolized for energy purposes. The mean carbohydrate intake for the present study was only 3.1 ± 0.8 g·kg^{-1} compared to the recommended 7-10 g·kg^{-1} per physical activity level (Burke et al. 2001).

Additionally, the total daily dietary intake of protein may have been insufficient to stimulate protein synthesis or reduce protein degradation during negative energy balance. Weight loss studies suggest that a diet high in protein (1.6 g·kg^{-1}) is necessary to attenuate LBM loss (Layman et al. 2009). In a recent study 2.3 g·kg^{-1} of daily protein was needed to preserve LBM during two weeks of a 40% reduction in habitual caloric intake (Mettler et al. 2010). In the current study, the average protein intake for all participants was 1.1 ± 0.2 g·kg^{-1}protein. The reduced level of protein intake combined with the observed caloric and carbohydrate deficit may have attenuated the impact of leucine supplementation on FFM.

There were limitations to the present study. Field studies present numerous challenges. The extreme environment resulted in several altitude-related illnesses that contributed to study

attrition. No side effects from the high dose leucine were reported so it is unlikely the leucine was responsible for the attrition. Additionally, there were no differences in baseline measurements between those who completed the study and those who did not to suggest study selection bias. Due to the monetary expense, remoteness, and length of the trek, the sample size was limited as was the duration of altitude exposure and elevation attained. Because weight and LBM loss is a function of the duration at altitude and elevation, a treatment effect would be more likely to be noted in those climbing Everest or residing at Everest base camp. Based on the current data, a sample size of 63 per group would have been necessary to achieve statistical significance (medium effect, 80% power, α=0.05). However, with longer duration high altitude exposure as noted in Operation Everest III (40 day simulated ascent of Everest) (Rose et al. 1988), a 5.05±1.35 kg loss of FFM with a mean treatment difference of 1.65 kg would require a sample size of 9 per group (medium effect, 80% power, α=0.05). Unsuccessful attempts were made to recruit individuals summiting Everest and/or residing at base camp. Additionally, the small sample size prevented the inclusion of a third group that would have received an isocaloric supplement with no protein content.

In conclusion, 7.0 g leucine supplement consumed twice daily did not attenuate loss of FFM compared to an isocaloric control. Of importance is the loss of LBM within a short duration of 13 days at mean altitude of 4319 m. Further research is warranted to investigate the effects of leucine supplementation on LBM under longer duration hypobaric, hypoxic conditions.

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Table 1. Characteristics of participants in 2 groups consuming 1 of 2 protein drinks: control (CON) or leucine (LEU).

	CON	LEU	*p*-value
n (males/females)	10 (6/4)	8 (4/4)	
Age (y)	49.4 ± 12.3	44.5 ± 10.4	.653
Height (cm)	175.0 ± 9.7	172.4 ± 9.7	.847
BMI ($kg \cdot m^{-2}$)	24.9 ± 2.5	25.7 ± 3.6	.490
Body weight (kg)	76.7 ± 13.7	76.9 ± 15.2	.629
Fat free mass (kg)	59.4 ± 11.1	58.1 ± 11.2	.494
Body fat (%)	22.5 ± 5.0	24.2 ± 6.0	.353

Note: BMI, body mass index. All values are means ± SD. Level of significance $p \leq 0.05$. There were no significant differences between treatment groups.

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Table 2. Nutritional composition of supplement beverages per serving.

	CON	LEU
Serving size (g)	22.6	25.1
Calories (kcal)	92.9	93.0
Carbohydrate (g)	3.4	2.7
Fat (g)	4.0	2.9
Protein (g)	11.3	14.5
Leucine (g)	0.3	7.0
Isoleucine (g)	0.2	0.6
Valine (g)	0.3	0.5

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Table 3. Daily energy and macronutrient intake of participants in the control (CON) and leucine (LEU) supplementation groups.

	CON (*n*=10)	LEU (*n*=8)	*p*-value
Energy, kcal·d (kcal·kg^{-1})	1879.3 ± 489.9 (24.7 ± 5.0)	1895.5 ± 378.3 (25.8 ± 7.3)	.522
Carbohydrate g (g·kg^{-1})	235.4 ± 67.7 (3.1 ± 0.7)	236.0 ± 49.9 (3.2 ± 0.9)	.410
Protein g (g·kg^{-1})	76.9 ± 10.2 (1.0 ± 0.2)	86.0 ± 14.7 (1.2 ± 0.3)	.116
Fat g	71.1 ± 22.5	68.3 ± 16.1	.497

Note: Data calculated from six days of food records collected on days 1-3 and 8-10. All values are reported as mean ± SD. Level of significance $p \leq 0.05$. There were no significant differences between groups.

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Fig. 1. Mean change in body weight, fat free mass, and fat mass from baseline to post-trek for control and leucine groups. Error bars indicate standard deviation (SD). Asterick (*) indicates difference from baseline values ($p<0.05$). There were no significant differences between groups.

Fig. 2. Changes in circumferences from baseline to post-trek for Control (A) and Leucine (B). Error bars indicate standard deviation (SD). All circumferences decreased significantly from baseline ($p<0.05$). There were no significant differences between groups.

Fig. 3. Mean daily energy expenditure and energy intake for leucine (LEU) and control (CON) groups and mean daily elevation. Error bars indicate standard deviation (SD). Average caloric deficit for both groups = 1 575 ± 651 kcal·day^{-1}. There were no significant differences between groups. Level of significance $p<0.05$

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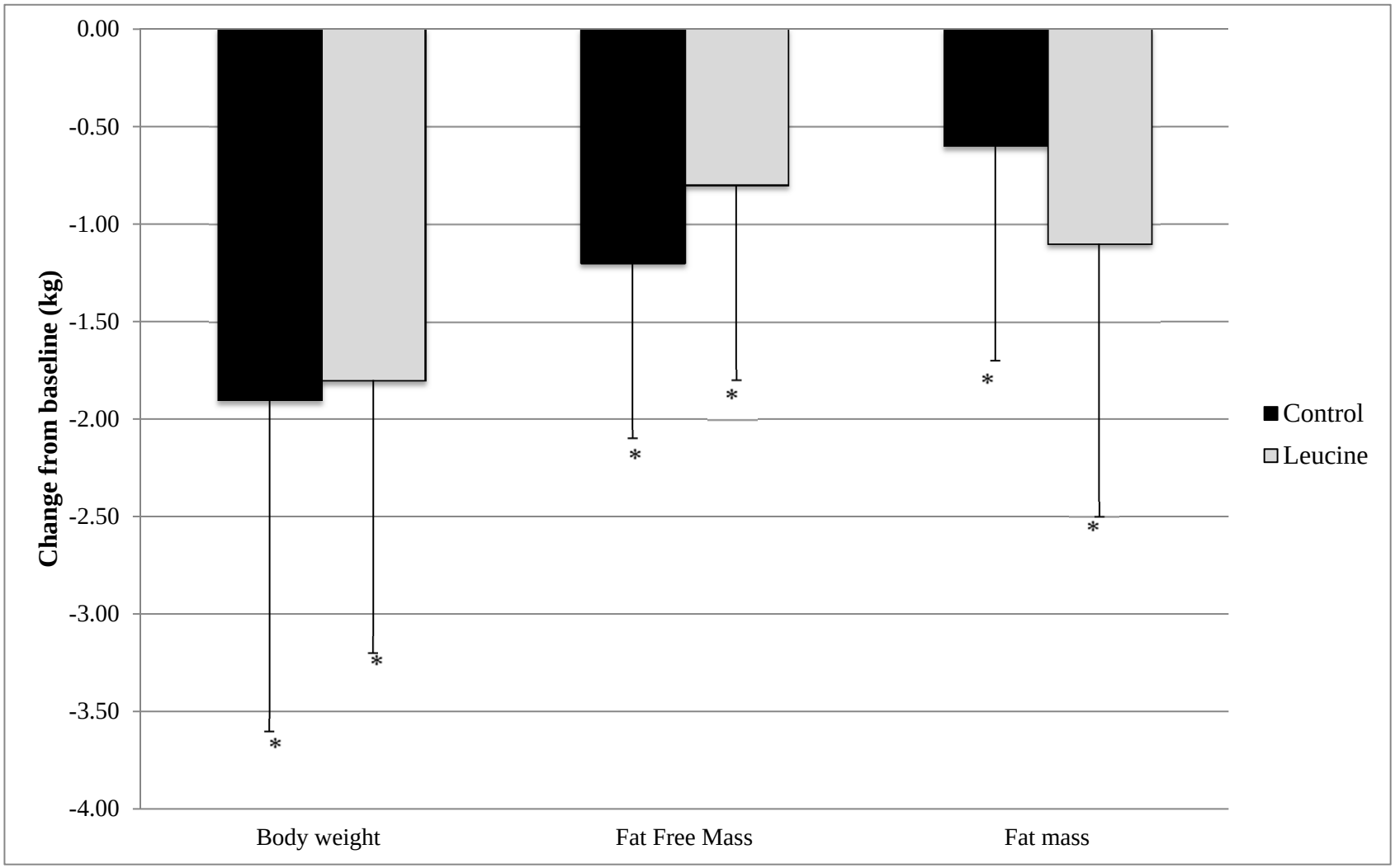
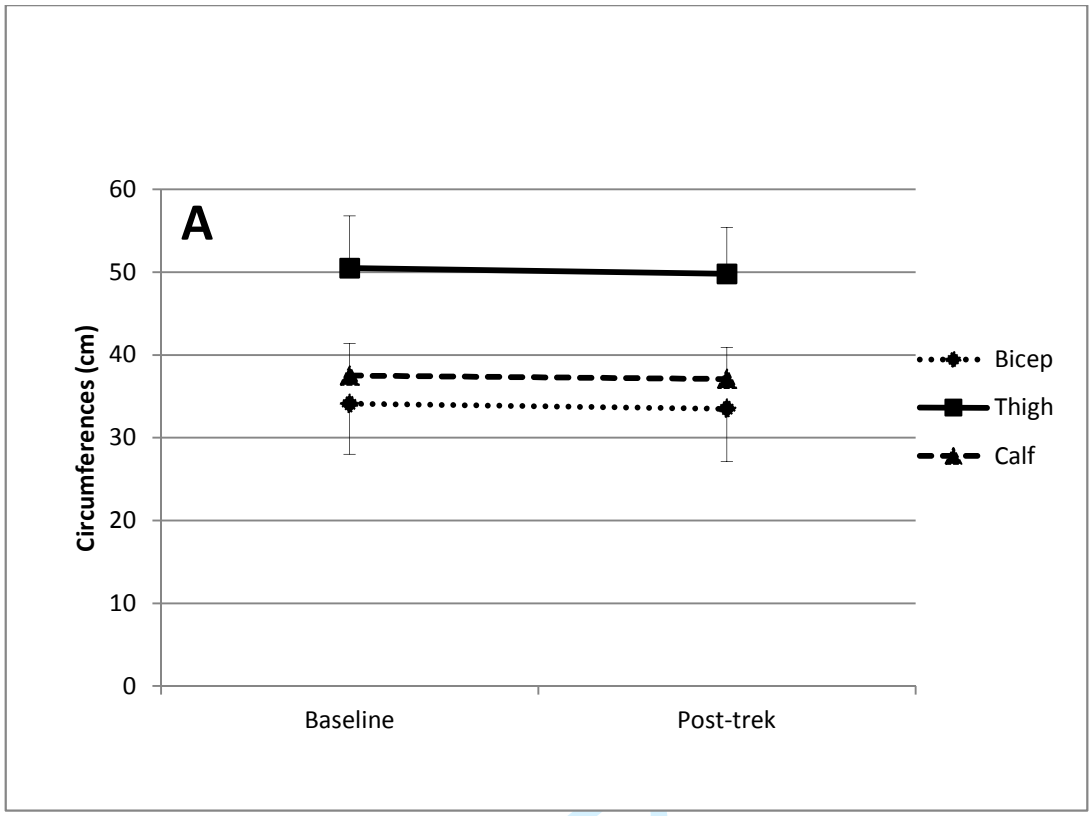
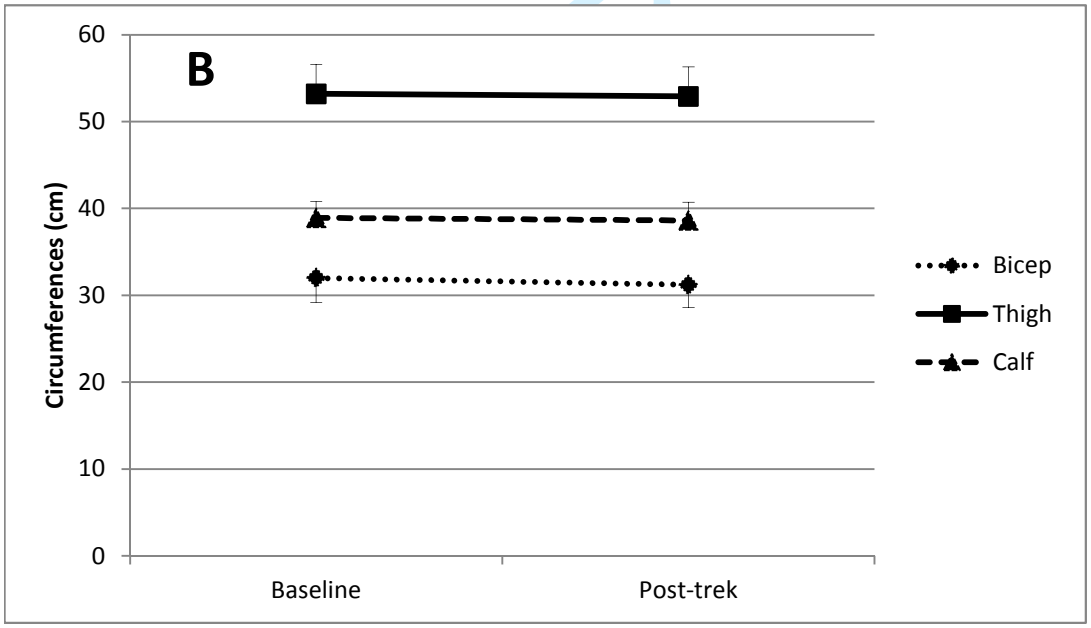
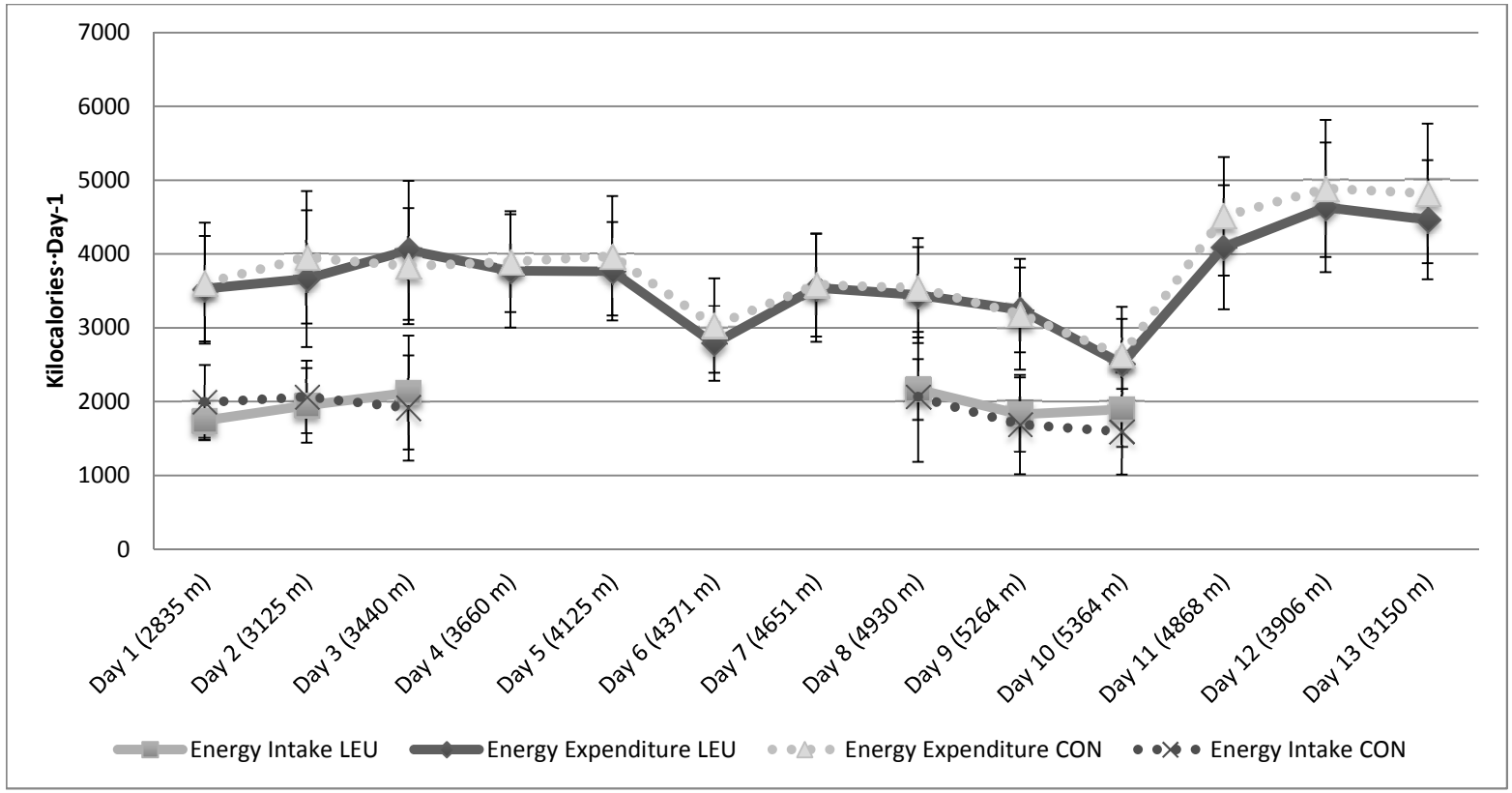

0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
-3.50
-4.00
Change from baseline (kg)
Body weight
Fat Free Mass
Fat mass
Control
Leucine
*


A
Circumferences (cm)
60
50
40
30
20
10
0
Baseline
Post-trek
Bicep
Thigh
Calf


B
Circumferences (cm)
60
50
40
30
20
10
0
Baseline
Post-trek
Bicep
Thigh
Calf


7000
6000
5000
4000
3000
2000
1000
0
Kilocalories··Day-1
Day 1 (2835 m)
Day 2 (3125 m)
Day 3 (3440 m)
Day 4 (3660 m)
Day 5 (4125 m)
Day 6 (4371 m)
Day 7 (4651 m)
Day 8 (4930 m)
Day 9 (5264 m)
Day 10 (5364 m)
Day 11 (4868 m)
Day 12 (3906 m)
Day 13 (3150 m)
Energy Intake LEU
Energy Expenditure LEU
Energy Expenditure CON
Energy Intake CON