EFFECT OF LEUCINE SUPPLEMENTATION ON LOSS OF LEAN BODY MASS DURING PROLONGED HYPOXIC EXPOSURE: A DOUBLE-BLIND RANDOMIZED STUDY

by

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STATEMENT OF THESIS APPROVAL

The following faculty members served as the supervisory committee chair and members for the thesis of Dana Carla Gershenoff.

Dates at right indicate the members' approval of the thesis.

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ABSTRACT

Trekkers exposed to prolonged hypobaric hypoxia commonly experience weight loss, especially loss of lean body mass (LBM). Evidence indicates that protein supplementation, specifically leucine, potentially attenuates loss of LBM in a catabolic state. This study investigated if leucine supplementation would prevent the loss of LBM during prolonged hypoxia. 18 trekkers (M=10 and F=8; age: 47.2 ± 11.5; range: 28-70y), completed a 13-day trek in Nepal from Lukla (2180m) to Everest Base Camp (5364m) with a mean altitude of 4140m. Participants consumed a 7.0g leucine supplement or an isocaloric, isonitrogenous placebo twice daily prior to meals. Body composition, body weight, and circumferences of bicep, thigh, and calf were taken pre- and post-trek. The participants from both treatments experienced significant loss of LBM and weight loss after 13 days at altitudes above 2810m ($P < 0.05$). However, there was no difference in loss of LBM (leucine -1.2 ± 1.6%; placebo -2.1 ± 1.5%), body weight (leucine -2.2 ± 1.5%; placebo -2.3 ± 2.0%), or circumferences between the groups. Overall, our results indicate that under the conditions of this study, leucine did not significantly reduce LBM loss during 13 days of altitude-induced hypoxia. This study was funded by Glanbia Nutritionals.

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INTRODUCTION

Healthy individuals exposed to prolonged hypobaric hypoxia (e.g., high altitude) commonly experience weight loss (1-3). The majority of this weight loss is comprised of lean body mass (LBM). For example, Rose et al. reported a 67% loss of fat-free mass compared to a 33% reduction from fat mass (1). Researchers have also observed loss of LBM in the lower limb area and a reduction in the calf muscle cross-sectional area (2, 3). The negative consequences of LBM loss are decreased physical performance and strength, increased risk of injury or edema, and impaired brain function (4). This loss of LBM has been attributed primarily to a negative energy balance.

As oxygen availability decreases with ascent to altitude, many factors contribute to a negative energy balance including acute mountain sickness (AMS), decreased appetite, and increased energy needs (1, 2, 5-12). Acute mountain sickness negatively affects energy intake with symptoms of nausea, vomiting, headache, dehydration, and loss of appetite (6, 7). Diminished energy intake (43-54%) at high altitudes is a major contributor to altitude-induced weight loss (1, 8). The decrease in appetite may be an effect of AMS or an increase in leptin (11, 12). The effects of diminished energy intake are compounded with increasing energy expenditure as seen with a raise in basal metabolic rate with ascent to altitude (9). All these factors taken singularly or collectively contribute to weight loss, especially loss of LBM. Maintaining LBM is critical for peak performance at high altitudes. As dietary protein is a major contributor to maintaining

nitrogen balance, protein supplementation in a negative energy balance may attenuate the loss of LBM.

It has been well documented that dietary protein is a requirement for maintenance of muscle mass, with essential amino acids (EAA) acting as a substrate and cell-signaling molecules for stimulation of muscle protein synthesis (MPS) (13, 14). Several factors affect MPS, including amount of dietary protein, timing of protein ingestion, and source of protein intake. Muscle protein synthesis is affected by overall energy balance, amount, and quality of dietary protein. Negative energy intake has been shown to suppress MPS by decreasing intracellular signaling proteins (15) whereas a higher protein diet consumed during a caloric deficit attenuates this reduction in LBM (16). Although consumption of protein postexercise is a potent stimulator of MPS, supplemental protein consumed steadily throughout the day and during exercise also has merit (16, 17). Protein source also affects the rate of MPS. Whey protein appears to be a superior source of protein to stimulate MPS (18) since it digests rapidly, increases blood availability of amino acids, and has a high concentration of leucine (19). It is well accepted that the branch chain amino acids (BCAA) stimulate MPS (20-22) and that leucine has the most influential role (23). Leucine is not only a substrate for protein synthesis but also has a unique ability to activate the mammalian target of rapamycin (mTOR) signaling pathway, a central regulator of MPS (24-29). Numerous factors influence the activation and inhibition of mTOR including energy status, amino acid availability, and oxygen status (30, 31). Additionally, leucine has been shown to inhibit protein degradation (28, 32).

Numerous studies have examined the effect of protein and leucine supplementation on MPS and LBM with varying results. Study conditions include

negative energy balance, hypoxia, and exercise (16, 26, 33, 34). In a negative energy balance, a high protein diet (2.3g/kg vs 1.0g/kg) may prevent loss of LBM in a healthy, active, male population (16). Similarly, another study compared the effects of the BCAA on MPS with rodents in a negative energy balance and found that leucine had the greatest increase on MPS (26). During a 21-day trek at a mean altitude of 3255 meters (m), loss of LBM was attenuated in a group consuming a BCAA supplement (5.76g leucine, 2.88g isoleucine, 2.88g valine) (33). However, the results of Schena et al. have been disputed due to lack of control of food intake and that the diets were not isonitrogenous (35). The control supplement in this study was defined as an inert substance possibly not matching the protein content in the BCAA supplement. Another study combined an exercise program and a leucine-enriched protein supplement (total 39.4g protein / 12.4g leucine) for an eight-week period and observed a slight increase in LBM (34).

Although the potential of attenuating loss of LBM with protein supplementation has been well investigated at sea level, little research has been done investigating the effects of protein supplementation at high altitude. Several studies demonstrate the loss of LBM in a state of hypoxia. One study examined leucine as part of a BCAA supplement at high altitudes. Other studies have examined the effects of leucine at sea level in a negative energy balance and during exercise. There are no studies that have investigated the effect of an isolated leucine supplement on a healthy, active population at a mean altitude above 4000m for an extended period. Therefore, the purpose of this investigation was to determine if leucine supplementation in healthy adult volunteers would prevent or attenuate the loss of LBM composition, body weight, and change in bicep, thigh, and calf circumferences during a 13-day trek to Everest base camp.

METHODS

Participants

Twenty-eight healthy, physically fit volunteers (15 males, 13 females) were recruited for the current study. Participants were identified via email from the Wilderness Medical Society (WMS) Mount Everest base camp trek. Participants were drawn from two separate treks that were similar in all aspects with exception of the start date, with the second trek starting 12 days after the first trek. Participants completed a background survey indicating their age, gender, level of physical fitness, prior altitude exposure, and symptoms related to altitudes above 2400m. The exclusion criteria included those individuals who were excluded from the WMS trek secondary to medical or physical limitations identified by a medical professional. The University of Utah Institute Review Board for Human Participants approved this study.

Study Design

The present study assessed the effect of a leucine (LEU) supplement versus a control (CON) supplement on LBM during a 13-day trek to Everest base camp. In this double-blind randomized study, participants consumed a LEU or CON supplement twice a day, one prior to breakfast and one prior to lunch. Participants were randomized by age and gender to receive either the LEU or CON supplement. Anthropometric (height, body weight, and circumferences) and body composition (ultrasound) measurements were

taken pre- and post-trek in Kathmandu, Nepal. During the trek, energy intake was recorded in food booklets and energy expenditure was measured using motion detectors.

Supplement

The participants ingested either LEU or CON twice daily during the 13-day trek to Everest base camp (EBC). Each LEU supplement contained 7.0g leucine (14.5g protein) and the CON supplement contained 0.3g leucine (11.3g protein). To isolate the effects of leucine, the supplements were isonitrogenous. See Table 1 for detailed kilocalorie (kcal) and macronutrient content and BCAA content. The supplements were chocolate flavored, dry powder mixed with boiled water. Participants consumed the supplements prior to meals in order to maximize the absorption of leucine and to standardize ingestion. On a daily basis the participants recorded the number of supplements consumed in the AMS Assessment Log. Glanbia Foods, International, Twin Falls, ID manufactured, labeled and supplied the supplements. The participants and investigators were blinded to the supplement contents.

Table 1
Supplement Composition

	LEU	CON
Serving size (g)	25.1	22.6
Leucine (g)	7.0	0.3
Nitrogen	2.0	2.0
Calories (kcal)	93.0	92.9
CHO (g)	2.7	3.4
Protein (g)	14.5	11.3
Fat (g)	2.9	4.0
BCAA content		
Isoleucine (g)	0.6	0.2
Leucine (g)	7.0	0.3
Valine (g)	0.5	0.3

Anthropometrics, Body Composition, and Circumferences

Anthropometrics were measured pre- and post-trek in Kathmandu, Nepal. Height was self-reported. Body weight was obtained using a CPW-150 scale (Adam Equipment, Inc. Danbury, CT). Participants were weighed (pre- and post-trek) immediately after voiding, prior to breakfast, and in lightweight shorts and shirt.

Baseline body composition was measured in Kathmandu either 1 or 2 days prior to trek departure. All post-trek measurements were performed within 1 day post-trek in Kathmandu. Each trek had its own trained investigator that performed both the pre- and post-measurements. Body composition was determined using a handheld ultrasound device (BodyMetric Ultrasound Body Composition Tester, IntelaMetrix, Inc., Livermore, CA). The device uses ultrasound waves to measure the fat thickness located between the skin layer and the muscle layer. The body fat accuracy of the ultrasound technique (UT) compared to dual-energy X-ray absorptiometry (DEXA) measurements was found to be more accurate and reliable than other methods such as bioelectrical impedance (BIA) or air displacement plethysmography (ADP) (36). Hydration status, recent exercise, and caffeine consumption are not confounding factors with the ultrasound device. Ultrasound measurements included single point and scan data on established skinfold sites (men: chest, abdomen, thigh; women tricep, anterior suprailiac, and thigh) (37, 38). Body fat was calculated with the Jackson & Pollock (1978) equation for men and the Jackson & Pollock (1980) for women. Lean body mass was calculated by subtracting body fat (kg) from body weight (kg). As hydration levels may affect body weight, hydration status will be measured first void via urine specific gravity strips during the entire trek.

Circumferences were measured using the International Standards for Anthropometric Assessment (ISAK) (39). Circumference measurements included arm, thigh, and calf on the right side of the body. Each measurement was performed twice, nonconsecutively; if there was a difference >1% between them, a third measurement was taken. In the case of a third measurement the data set used the median value of the data.

Diet and Physical Activity

Participants were instructed to keep 3-day food record booklets twice during the trek, for a total of 6 days. Food booklets were collected to compare caloric and macronutrient intake between LEU and CON groups. Prior to the trek, the participants received the food booklets and instructions on how to complete them accurately. The food booklets included common teahouse menu items for each meal and estimation of portion sizes to assist the participant in keeping accurate records. To decrease participant burden, food intake was recorded for two 3-day periods versus the entire trek. Booklets were completed at the start of the trek for 3 days and then again for 2 days prior to arrival in base camp and 1 day at base camp. The daily energy intake and macronutrient composition was determined using Food Processor SQL (version 10.11.1 2012; ESHA Research).

Energy expenditure was measured with a motion detector (Actical, Mini Mitter, Inc., Bend, OR) provided by the United States Army Research Institute of Environmental Medicine (USARIEM). The motion detectors were worn continuously throughout the 13-day trek excluding bathing. The Actical tracked and recorded their physical activity in 1-minute intervals during the entire 13-day trek. USARIEM developed a macro that converted the data into 15-minute intervals. Energy expenditure was calculated using the

Compendium of Physical Activities equation and included pack weight with body weight, (MET x 3.5) x (body weight + pack weight) / 200. Daily energy expenditure was calculated for the entire 24 hours.

Statistical Analysis

Data are expressed as means ± SD. A power analysis indicated that a sample size of six per treatment group was necessary to detect a 2.2 kg weight difference (± 1.1) between groups with 80% power and alpha < 0.05. Participant characteristics were calculated using descriptive statistics (mean ± SD). Normality was assessed using a Kolmogorov-Smirnov test and all variables were normally distributed. Differences in LBM, body weight, body fat percentage, and circumferences were analyzed using separate 2 x 2 repeated measures analysis of variance (ANOVA) (time [pre, post] x group [LEU, CON]). 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. The level of significance for all analyses was set at $P < 0.05$. Statistical analysis was performed using SPSS, Version 20 (Chicago, IL).

RESULTS

Participants

A total of 18 participants completed the study (LEU: n=8; CON: n=10). Participant characteristics are listed in the Table 2. There were no significant differences in baseline characteristics and pre-trek measurements between the groups. Attrition rate was 36%. Participants withdrew from the study primarily due to acute mountain sickness and altitude and/or food-related gastrointestinal distress.

Supplement

Supplements were consumed twice daily for 13 days of the trek. Complete supplement data was available for 13 of the participants (LEU n=6 and CON n=7).

Table 2
Baseline Participant Characteristics[1]

	LEU (n=8)	CON (n=10)	ALL (N=18)
Female	4	4	8
Male	4	6	10
Age (year)	44.5 ± 10.4	49.4 ± 12.3	47.2 ± 11.5
Body weight (kg)	76.9 ± 15.2	76.7 ± 13.7	76.8 ± 14.0
Height (cm)	172.4 ± 9.7	175.0 ± 9.7	173.9 ± 9.5
BMI (kg/m^2)	25.7 ± 3.6	24.9 ± 2.5	25.2 ± 3.0
Lean mass (kg)	58.1 ± 11.2	59.4 ± 11.1	58.8 ± 10.8
Body fat (kg)	18.8 ± 6.9	17.3 ± 5.3	18.0 ± 5.9

[1]All values are ± mean SD. No significant differences between groups.

Overall compliance (e.g., 2 packets per day) was 93% ± 12% with LEU at 89% ± 17% and CON at 96% ± 6%. There was no significant difference in compliance between the two groups. Average leucine consumption for LEU was significantly greater than CON (12.5 ± 2.5 g/day and 0.7 ± 0.0 g/day, respectively ($P < 0.05$).

Body Composition and Circumferences

Prior to the intervention, lean mass (kg), body weight (kg), and body fat (kg) did not differ between groups (Table 3). To standardize the differences in body composition between the participants, percentage change was calculated by dividing the measurement difference (post - pre) by the pre-trek measurement. The participants from both treatments experienced significant loss of LBM and body weight after 13 days at a mean altitude of 4139m ($P < 0.05$). However body fat did not change significantly (Figure 1).

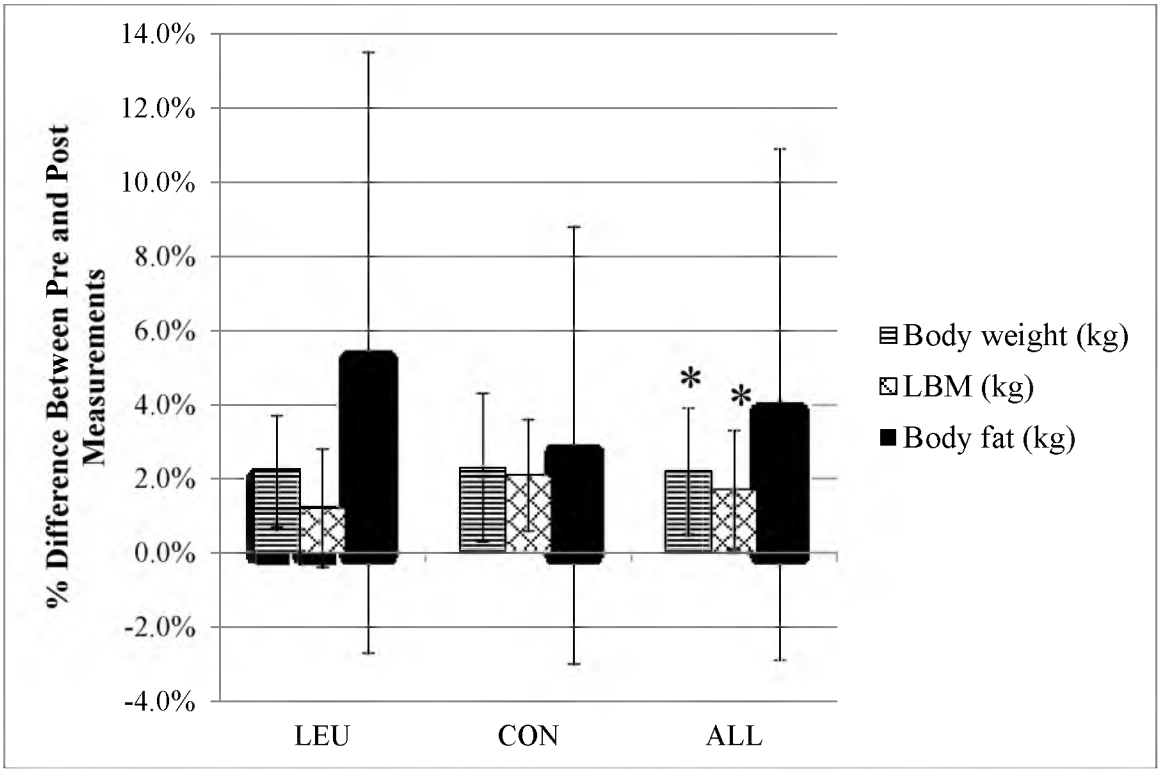


Figure 1. Body composition loss percentage calculated by dividing loss of body weight, LBM, and body fat by pre-trek measurements. *Significant loss ($P < 0.05$).

For all participants, LBM loss was 1.0 ± 0.9 kg (1.7% ± 1.6%), body weight loss was 1.9 ± 1.5 kg (2.2% ± 1.7%), and body fat loss was 0.8 kg ± 1.2 (4.0% ± 6.9%). However, there was no significant difference in LBM, body weight, or body fat between LEU and CON (Figure 1). As hydration level may affect overall body weight, hydration status was determined via urine specific gravity strips. There was no difference in hydration status between the groups. For the LEU group, LBM loss was 0.8 ± 1.0 kg (1.2% ±1.6%), body weight loss was 1.8 ± 1.4 kg (2.2% ± 1.5%), and body fat loss was 1.1 kg ± 1.4 (5.4% ± 8.1%). For the CON group, LBM loss was 1.2 ± 0.9 kg (2.1% ± 1.5%), body weight loss was 1.9 ± 1.7 kg (2.3% ± 2.0%), and body fat loss was 0.6 kg ± 1.1 (2.9% ± 5.9%). For all participants, LBM was 56% of body weight loss and body fat was 44% of body weight loss. For the LEU group, LBM and body fat was 42% and 58%, respectively, of body weight loss. For the CON group, LBM and body fat was 66% and 34%, respectively, of body weight loss. Similarly, participants from both treatments saw significant loss in bicep, thigh, and calf circumferences ($P < 0.05$), although there was no difference in circumferences between the LEU and CON groups (Table 4).

Diet and Physical Activity

Energy intake and macronutrient composition was determined for each day the participants had complete data in the food booklet. The daily average was then calculated based on the number of days completed. Completion of food records was as follows, 6 days (n=14), 5 days (n=1), and 3 days (n=3). Daily averages for energy intake and macronutrient by group and overall are listed in Table 5. There were no significant differences in energy intake and grams of carbohydrates, protein and fats between the two

Table 3

Body Composition Pre- and Postmeasurements[1]

	LEU (n=8)		CON (n=10)		ALL (N=18)	
	Pre	Post	Pre	Post	Pre	Post
Body weight (kg)	76.9 ± 15.2	75.1 ± 14.3	76.7 ± 13.7	74.8 ± 12.6	76.8 ± 14.0	75.0 ± 12.9*
Lean mass (kg)	58.1 ± 11.2	57.3 ± 10.8	59.4 ± 11.1	58.2 ± 10.9	58.8 ± 10.8	57.8 ± 10.6*
Body fat (kg)	18.8 ± 6.9	17.8 ± 6.7	17.3 ± 5.3	16.7 ± 4.6	18.0 ± 5.9	17.2 ± 5.5

[1]All values mean ± SD. *Significant decrease ($P < 0.05$)

Table 4

Anthropometrics Pre- and Postmeasurements[1]

	LEU (n=8)			CON (n=10)			ALL (N=18)		
	Pre	Post	% Difference	Pre	Post	% Difference	Pre	Post	% Difference
Bicep (cm)	32.0 ± 2.8	31.2 ± 2.6	-2.4 ± 1.8	34.1 ± 6.1	33.5 ± 6.4	-2.0 ± 1.9	33.2 ± 4.9	32.5 ± 5.1	-2.2 ± 1.8*
Thigh (cm)	53.2 ± 3.8	52.9 ± 3.4	-0.6 ± 1.2	50.5 ± 6.3	49.8 ± 5.6	-1.2 ± 2.0	51.7 ± 5.4	51.2 ± 4.9	-0.9 ± 1.7*
Calf (cm)	38.9 ± 1.9	38.6 ± 2.1	-0.9 ± 1.3	37.5 ± 3.9	37.1 ± 3.8	-1.0 ± 1.2	38.1 ± 3.2	37.7 ± 3.1	-1.0 ± 1.2*

[1]All values mean ± SD. *Significant decrease ($P < 0.05$)

groups. Additionally there was no significant difference in macronutrient distribution between the two groups (Table 6). Based on pre-trek participant weight average, daily energy intake per kg for all participants was 25.2 ± 6.0 kcal/kg, carbohydrate was 3.1 ± 0.8 g/kg, and protein was 1.1 ± 0.2 g/kg. The LEU group was 25.8 ± 7.3 kcal/kg, carbohydrate was 3.2 ± 0.9 g/kg, and 1.2 ± 0.3 g/kg protein and CON group was 24.7 ± 5.0 kcal/kg, carbohydrate was 3.1 ± 0.7 g/kg, and 1.0 ± 0.2 g/kg protein. There were no significant differences in kcal/kg, carbohydrate g/kg, and protein g/kg between the two groups. Without the supplement the amount of protein per body weight was significantly lower with all participants (N=18) at 0.8 ± 0.2 g/kg protein, the LEU group at 0.8 ± 0.2 g/kg protein, and the CON group at 0.7 ± 0.1 g/kg protein.

Table 5
Energy Intake by Kcal and Macronutrient Composition[1]

	Daily Average		
	LEU (n=8)	CON (n=10)	ALL (N=18)
kcal	1895.5 ± 378.3	1879.3 ± 489.9	1886.5 ± 431.4
CHO (g)	236.0 ± 49.9	235.4 ± 67.7	235.7 ± 58.8
FAT (g)	68.3 ± 16.1	71.1 ± 22.5	69.8 ± 19.4
PRO (g)	86.0 ± 14.7	76.9 ± 10.2	80.9 ± 12.9

[1]All values are mean SD. Participants completed two 3-day food records during the 13-day trek, days 1-3 and days 8-10. No significant differences between groups.

Table 6
Energy Intake by Macronutrient Distribution[1]

	Daily Average		
	LEU (n=8)	CON (n=10)	ALL (N=18)
CHO	50%	50%	50%
FAT	32%	34%	33%
PRO	18%	16%	17%

[1]Participants completed two 3-day food records during the 13-day trek, days 1-3 and days 8-10. No significant differences between the groups.

Mean energy expenditure did not differ between the groups. Overall daily energy expenditure (N=16) was 3737 ± 686 kcal/day. LEU (n=7) mean energy expenditure was 3653 ± 641 kcal/day and CON (n=9) was 3803 ± 750 kcal/day. Twelve participants completed the full 6-day food record and had full energy expenditure results. Figure 2 illustrates the negative energy balance between energy expenditure and energy intake for these 12 participants. Average daily deficiency (N=12) was 1575 ± 651 kcal/day for the 6 days that food records were kept.

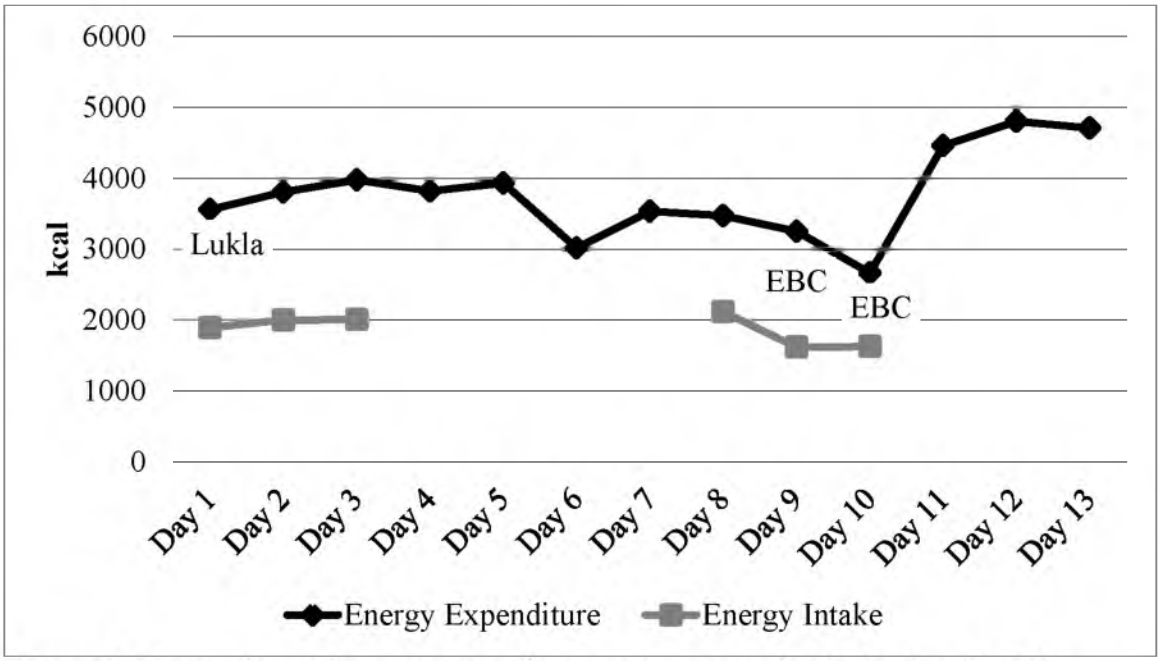


Figure 2. Mean values of energy expenditure versus energy intake by day (N=12). Participants excluded if they did not complete the full 6-day food record or did not have a complete energy expenditure value.

DISCUSSION

The current study investigated the effects of leucine supplementation on LBM during an extended trek at a mean altitude of 4139m. Supplementation with 7g of leucine twice daily (daily total=14g) during the 13-day trek did not attenuate the loss of LBM or body weight in the LEU group when compared to the CON group. Additionally, leucine supplementation did not attenuate the decrement in bicep, thigh, and calf circumferences. However, it is of interest that in as little as 13 days at high altitude, participants saw a significant loss of LBM, body weight, but not body fat and significant decreases in bicep, thigh, and calf circumferences.

Current evidence proposes that protein and/or leucine supplementation can attenuate the loss of LBM under conditions such as hypoxia and negative energy balance; conditions noted in the present study. Our data are in contrast to Schena et al. (33) 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 3255m. One possible difference between the studies was that our experiment was conducted at a higher altitude (+914 m). Hypoxia may negatively affect mTOR function, subsequently impairing MPS. Vigano et al. (31) investigated the effect of hypoxia on the mTOR signaling pathway in humans traveling from sea level to 4559m. After only 6 days, the study found a significant decrease in the expression level of mTOR after long-term hypoxic exposure. Therefore, it is possible that MPS may have been suppressed to a greater extent due to the higher altitude (e.g., hypoxia) in our study than Schena et al. (33).

Additionally, our data suggest a negative energy balance. Walker et al. (34) found a slight increase of LBM with an 8-week leucine supplementation in a population that maintained their overall energy balance. This is in contrast to our study, which indicated a significant caloric deficit. In a negative energy balance with low carbohydrate availability, dietary protein or existing protein tissue may be catabolized for energy purposes. BCAA, and in particular leucine, is a primary fuel source for skeletal muscle for stimulating MPS and maintaining LBM (20-22). If dietary protein is being utilized for energy, this may explain a lack of effect of leucine supplementation on attenuating loss of LBM.

Our data also found a low amount of carbohydrate intake during the trek. During high levels of physical activity, as evidenced in our study with a mean energy expenditure of 3737 ± 686 kcal/day, recommended levels of carbohydrates are 7-10 g/kg of body weight to maintain glycogen stores (40). The mean carbohydrate intake during the trek was only 3.1 ± 0.8 g/kg of body weight. A diet with a carbohydrate intake of less than 5 g/kg results in decreased muscle glycogen stores (41). It is likely that at this level of inadequate carbohydrate consumption, dietary protein, particularly leucine and BCAA, was being metabolized for energy rather than being used for generating muscle tissue. Howarth et al. (42) induced a glycogen-depleted state with a low carbohydrate diet and found that during endurance exercise, whole body leucine oxidation was higher in the low carbohydrate diet than the high carbohydrate diet. In the current study, the participants were most likely in a glycogen-depleted state and it is probable that dietary protein, and specifically leucine, was catabolized for energy rather than for synthesizing muscle tissue.

Additionally, the overall intake of dietary protein may have been insufficient to stimulate MPS in a negative energy balance. In Mettler et al. (16), the control group received 1.0 g/kg of protein while the treatment group received 2.3 g/kg of protein in a 60% caloric deficiency with the treatment group indicating attenuation of LBM after four weeks of supplementation. In the current study, the overall protein intake for all participants was 1.1 ± 0.2 g/kg protein of body weight. The reduced level of protein intake combined with the observed caloric deficit may have lessened the impact of leucine supplementation on LBM. General guidelines for endurance athletes recommend a higher intake of protein, 1.2-1.4 g/kg of body weight to maintain adequate nitrogen balance (43). Furthermore, weight loss studies indicate that a diet high in protein, 1.6 g/kg, provides a more efficient loss of fat mass and preservation of LBM than high carbohydrate diets (44). Another point to consider is that both supplements in our study provided an additional 29.0 g protein (LEU) and 22.6 g protein (CON) per day. Without either supplement, average daily protein intake decreased significantly (1.1 ± 0.2 g/kg to 0.8 ± 0.2 g/kg protein per body weight) and may have prevented even greater loss of LBM than if no supplement was consumed. This is another difference between Schena et al. (33) and our study. In Schena et al., the control supplement was defined as an inert substance and the protein amount may not have been matched between the groups. Thus, the attenuation of LBM may be attributable to the difference in overall protein intake versus the influence of solely the BCAAs.

The current study identified a significant loss of LBM, body weight, and decreases in arm, thigh, and calf circumferences in as little as 13 days at a mean altitude of 4139 m ($p<0.05$). Loss of LBM (1.7 ± 1.6%) and body weight in the current study is

similar to trends found in other high altitude studies (1, 2, 8, 9, 33). Our results for all participants (56% from fat-free mass (FFM) and 44% from fat mass (FM)) indicate that LBM represented a majority of the body mass loss similar to Rose et al. (67% from FFM and 33% from fat mass FM). Fulco et al. (45) investigated the effect of a 1500 kcal per day deficit for 21 days and also saw a significant loss of body weight with 69% from LBM. This in contrast to two other studies that indicated a majority of FM loss, with 67% FM and 33% FFM (2) and 90% FM and 10% FFM in the placebo group (33). Although not statistically significant, the LEU group (42% from FFM and 58% FM) had less FFM loss than the CON group (66% from FFM and 34% from FM). Of importance in the current study is the timeframe of the weight loss and the mean altitude of the trek. Several of the high altitude weight losses occurred at higher altitudes and for longer periods (21-40 days). Our study indicated loss of significant body weight within only 13 days and at a mean altitude of 4139m.

A likely contributor to loss of LBM and body weight at high altitude was a negative energy balance with our results matching and exceeding those of previous studies. In the current study, a mean caloric deficit of 1575 ± 651 kcal/day was observed for the 6 days that food records were kept. This was approximately 43% of measured total daily energy expenditure. Although the caloric deficit observed in the 6-day food booklet is a snapshot of the entire trek, it likely represents the remaining 7 days of the trek. Previous studies have noted similar caloric deficits. Westerterp et al. (2) found a deficit of 1385 ± 430 kcal/day and Westerterp-Plantenga et al. (8) a deficit of 955 ± 358 kcal/day. Rose et al. (1) also saw a similar trend. Again, this caloric deficit together with

low carbohydrate availability, may have attenuated the effect of the leucine supplementation on MPS.

Our study also supports previous research on loss of LBM primarily in the limbs (1-3, 33). Bicep, thigh, and calf circumferences decreased significantly in 13 days ($P < 0.05$). Several studies have observed similar results. Rose et al. (1) reported decreases in upper arm, thigh, and calf circumferences and Westerterp et al. (2) observed a 6% loss in lower limb anthropometry. Schena et al. (33) found a significant decrease in cross-sectional area of the arm and thigh while Edwards et al. (3) specifically saw a significant decrease in the calf cross-sectional area. In both the present data and past data, significant muscle atrophy in the limbs was observed signifying loss of LBM and catabolism of muscle protein during prolonged periods to high altitude.

Strengths and Limitations

Strengths of the current study include that it was as a field study versus in a hypobaric chamber, allowing for free-living conditions rather than a restricted environment. Additionally, the study took place in Nepal with a mean altitude of 4139m, which provided higher altitudes than those available in the United States. Of those participants that completed the study, compliance was strong for both completion of food booklets and supplement consumption. Lastly, the use of the ultrasound for measurement of LBM provides an accurate measurement without concern for hydration status.

There were several limitations to the present study. Unlike in a laboratory setting, external factors were difficult to control. The extreme altitude environment resulted in several AMS, HAPE (high altitude pulmonary edema), and gastrointestinal problems that contributed to attrition. The international location was also a limiting factor. Due to the

cost, remoteness, and length of the trek, the sample size was restricted. Although the food booklets were quite detailed, it is generally noted that participants frequently under report energy intake when completing food records. The sample size was small, not allowing for a third group that would have received an isocaloric supplement with no protein content. Additionally, a post hoc power analysis indicated that with current LBM loss and standard deviation, each group needs a sample size of 90 participants. An alternative would be to decrease confounding factors by limiting participants by age, gender, BMI, or body fat. This would diminish the variability amongst the participants and minimize the standard deviation, which in turn decreases the sample size to detect a 2.2 kg difference between the groups. Lastly, leucine supplement dose was not based on kg of body weight. Consequently, some participants were receiving much g/kg higher doses than others. Due to the nature of this study (e.g., field vs. laboratory) it was not feasible to package the leucine supplements in varying amounts. Future studies may consider prescribing separate standardized doses to males and females or per kg of body weight.

Conclusion

In conclusion, our data did not indicate an attenuation of LBM at high altitudes with 7.0 g leucine supplement consumed twice daily compared to the placebo group. It is possible that the negative energy balance and suboptimal carbohydrate and protein intake may have negatively affected the potential impact of leucine supplementation on MPS. Of importance is the loss of LBM within such a short duration of 13 days at mean altitude of 4319m. Further research is warranted to investigate a leucine supplement in a negative energy balance with a higher level of overall protein intake and under hypoxic conditions combined with measures of MPS.

APPENDIX A

BACKGROUND SURVEY

ALTITUDE AND ACTIVITY BACKGROUND SURVEY FORM

INSTRUCTIONS: Please complete all items to the best of your knowledge. If you have any questions please ask the investigators for clarification. Thank you for your cooperation.

SUBJECT ID# (to be filled out by investigator):_____ TODAY'S DATE: _____
day month year

PART 1: BASIC INFORMATION

1. Age: _____ Date of Birth: _____

2. Gender: _____ Male _____ Female

3. Racial Identity *(check the best category that applies to you):*
   _____ American Indian / Alaska Native
   _____ Asian
   _____ Black / African American
   _____ Native Hawaiian / Pacific Islander
   _____ White

4. Ethnicity *(check the best category that applies to you):*
   _____ Not Hispanic or Latino
   _____ Hispanic or Latino

5. Height: _____ (e.g. without shoes) (ft./in.)

6. Weight: _____ (e.g. without shoes) (ft./in.)

7. Your Place of Birth: _____ _____ _____
   (Town) (State) (Nation)

8. Current Place of Residence: _____ _____ _____
   (Town) (State) (Nation)

9. Do you smoke? _____ Yes; if yes, list # of cigarettes per day: _____
   _____ No

10. Do you chew tobacco? _____ Yes
    _____ No

11. Do you have any food allergies?_____ Yes, if yes, food item(s): _____
_____ No

12. Do you use any dietary supplements (including protein powders)? _____ Yes; if yes, list product/daily dosage: _____
_____ No

13. Are you taking any medications? If so, please list medication.
_____

PART 2: ALTITUDE EXPERIENCE

This section related to your experience at altitudes of **8,000 feet or more above sea level.** If you have spent at least **8 or more hours** at an altitude of **8,000 feet or above,** please answer questions 11 – 15. (Note the following examples of 8,000 and greater elevations: Grand Canyon, Yellowstone, and Yosemite National Parks; Buena Vista, CO; Crested Butte, CO; Vail, CO; Breckenridge, CO).

13. Have you ever been at an altitude of 8,000 feet or above for a day or more?
_____ Yes
_____ No **(if no, go directly to question #18.)**

14. How long were you at an altitude of 8,000 feet or above?
_____ 0 – 8 hours
_____ Greater than 8 hours

15. Have you ever slept one or more days at an altitude of 8,000 feet or above?
_____ Yes
_____ No

16. How many day have you spent at an altitude of 8,000 feet or above in the past 60 days?
_____ Less than 2 weeks (< 14 days)
_____ The last 2 weeks (~ 14 days)
_____ The last 4 weeks (~ 30 days)
_____ The last 8 weeks (~ 60 days)

17. When you were at an altitude of 8,000 feet or above, at anytime did you ever experience any of the following symptoms? *(Check as many answers as appropriate.)*

_____	not affect at all	_____	nausea	_____	loss of appetite
_____	headache	_____	weakness	_____	irritable
_____	urinate more	_____	urinate less	_____	dizziness
_____	tired	_____	could not sleep		

_____ N/A *(not applicable)

PART 3: ACTIVITY HISTORY

18. How many times per week do you currently take part in **strength conditioning** (ex., weight lifting and resistance training) or **calisthenics** (example: sit-ups and push-ups)? *(Check only one answer.)*
    _____ 1 day per week
    _____ 2 – 4 days per week
    _____ More than 4 days per week
    _____ Do not participate **(skip to question 21)**

19. For each **strength conditioning or calisthenics session,** how long do you participate? *(List each activity).*
    _____ Less than 30 minutes: _____
    _____ 30 – 60 minutes: _____
    _____ Great than 60 minutes: _____

20. Please check the overall degree of intensity for the above-mentioned **strength conditioning or calisthenics session.** *(Check only one answer).*
    _____ Light
    _____ Moderate
    _____ Intense
    _____ Very Intense

21. How many times per week do you currently take part in **aerobic conditioning** (example: running, walking, cycling, playing basketball, and rowing)? *(Check only one answer.)*
    _____ 1 day per week
    _____ 2 – 4 days per week
    _____ More than 4 days per week
    _____ Do not participate **(skip to question 23)**

22. For each **aerobic conditioning session**, how long do you participate? *(List each activity.)*
    _____ Less than 30 minutes: _____
    _____ 30 – 60 minutes: _____
    _____ Great than 60 minutes: _____

23. Please check the overall degree of intensity for the above-mentioned **aerobic conditioning session.** *(Check only one answer.)*
    _____ Light
    _____ Moderate
    _____ Intense
    _____ Very Intense

For military personnel only:

24. For your appropriate military service (e.g., Army, Navy, Marines or Air Force), estimate your most recent run time: *(List only one answer.)*
    _____ Time (min./sec.) to run 2 miles (Army)
    _____ Time (min./sec.) to run 1.5 miles (Navy)
    _____ Time (min./sec.) to run 3 miles (Air Force)

End of Survey. Thank you for your participation.

APPENDIX B

FOOD BOOKLET

Leucine Study Food Record

NAME: _____ SID#: _____

*Please write down **everything** you eat for three days. Include the name of the food, description if necessary, and the amount. In order to compare treatment groups, we need as accurate of record as you can complete. You will be interviewed at the end of each day of recorded dietary intake.*

Note: daily beverages will be tracked in AMS log book on hydration page.

Food records will be completed for 3 days at 2 time points:

FIRST TIME PERIOD

1) *Fly to Lukla Trek to Phakding*
2) *Namche*
3) *Namche – Hike to Khumjung, Syangboche*

SECOND TIME PERIOD

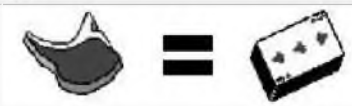
1) *Gorakshep-Kalapatthar-Gorakshep*
2) *Everest Base Camp*
3) *Everest Base Camp*

FOOD SIZES

Seven Ways to Size Up Your Servings

Measure food portions so you know exactly how much food you're eating.
When a food scale or measuring cups aren't handy, you can still estimate your portion.

Remember:

1	3 ounces of meat is about the size and thickness of a deck of playing cards or an audiotape cassette.	
2	A medium apple or peach is about the size of a tennis ball.	
3	1 ounce of cheese is about the size of 4 stacked dice.	

BREAKFAST Day:_____ Date: _____ Location: _____

Check	Breakfast item – circle specific	Amounts – circle one
	Fried egg	Eggs: 1 2 3 4
	Egg omelet	Eggs: 1 2 3 OR Cups: 1/2 1
	Scrambled eggs	Eggs: 1 2 3 OR Cups: 1/2 1
	Fried potato w/eggs	Cups: 1/4 1/2 3/4 1 1½ 2
	Porridge – plain or cinnamon	Cups: 1/4 1/2 3/4 1 1½ 2
	Muesli - plain or w/hot milk	Cups: 1/4 1/2 3/4 1 1½ 2
	Cornflakes	Cups: 1/4 1/2 3/4 1 1½ 2
	Rice pudding	Cups: 1/4 1/2 3/4 1 1½ 2
	French toast	Slices: 1 2 3 4
	Tibetan Bread or toast w/jam	Slices: 1 2 3 4
	Other – specify	Amount (write in):
	Other – specify	Amount (write in):
	Other – specify	Amount (write in):

Note: beverage consumption and supplements are tracked in the daily fluid log (AMS log book).

LUNCH Day:_____ Date: _____ Location: _____

Check	Lunch item – circle specific	Amounts – circle one
	Soup – tomato / chicken / garlic	Cups: 1/4 1/2 3/4 1 1½ 2
	Fried Noodles – plain or mixed	Cups: 1/4 1/2 3/4 1 1½ 2
	Curry – rice or chicken	Cups: 1/4 1/2 3/4 1 1½ 2
	Sizzlers – chicken or yak	Cups: 1/4 1/2 3/4 1 1½ 2
	Tuna sandwich	Sandwiches: 1/2 1 1½ 2
	Cheese pizza – plain / mushroom	Slices: 1 2 3 4
	Spaghetti	Cups: 1/4 1/2 3/4 1 1½ 2
	Fried potatoes w/eggs	Cups: 1/4 1/2 3/4 1 1½ 2
	Potato Momo (dumplings)	Dumplings: 1 2 3 4
	Mashed potatoes	Cups: 1/4 1/2 3/4 1 1½ 2
	Dal Bhat (lentils + rice)	Cups: 1/4 1/2 3/4 1 1½ 2
	Plain rice	Cups: 1/4 1/2 3/4 1 1½ 2
	French fries or Potato chips	Cups: 1/4 1/2 3/4 1 1½ 2
	Tibetan bread or Chapati	Slices: 1 2 3 4
	Other – specify	Amount (write in):
	Other – specify	Amount (write in):

Note: beverage consumption and supplements are tracked in the daily fluid log (AMS log book).

SNACKS Day:_____ Date: _____ Location: _____

Check	Snack item – circle specific	Amounts – circle one
	Potato chips	Bag size: 1 oz 2 oz 3 oz 4 oz
	Energy bar - specify	Bar size: 1 oz 2 oz 3 oz 4 oz
	Energy bar - specify	Bar size: 1 oz 2 oz 3 oz 4 oz
	Candy bar - specify	Bar size: 1 oz 2 oz 3 oz 4 oz
	Candy bar - specify	Bar size: 1 oz 2 oz 3 oz 4 oz
	Other – specify	Amount (write in):
	Other – specify	Amount (write in):
	Other – specify	Amount (write in):
	Other – specify	Amount (write in):

Note: beverage consumption and supplements are tracked in the daily fluid log (AMS log book).

DINNER Day:_____ Date: _____ Location: _____

Check	DINNER item – circle specific	Amounts – circle one
	Soup – tomato / chicken / garlic	Cups: 1/4 1/2 3/4 1 1½ 2
	Fried Noodles – plain or mixed	Cups: 1/4 1/2 3/4 1 1½ 2
	Curry – rice or chicken	Cups: 1/4 1/2 3/4 1 1½ 2
	Sizzlers – chicken or yak	Cups: 1/4 1/2 3/4 1 1½ 2
	Tuna sandwich	Sandwiches: 1/2 1 1½ 2
	Cheese pizza – plain / mushroom	Slices: 1 2 3 4
	Spaghetti	Cups: 1/4 1/2 3/4 1 1½ 2
	Fried potatoes w/eggs	Cups: 1/4 1/2 3/4 1 1½ 2
	Potato Momo (dumplings)	Dumplings: 1 2 3 4
	Mashed potatoes	Cups: 1/4 1/2 3/4 1 1½ 2
	Dal Bhat (lentils + rice)	Cups: 1/4 1/2 3/4 1 1½ 2
	Plain rice	Cups: 1/4 1/2 3/4 1 1½ 2
	French fries or Potato chips	Cups: 1/4 1/2 3/4 1 1½ 2
	Tibetan bread or Chapati	Slices: 1 2 3 4
	Other – specify	Amount (write in):
	Other – specify	Amount (write in):

Note: beverage consumption and supplements are tracked in the daily fluid log (AMS log book).

APPENDIX C

AMS ASSESSMENT LOG

AMS Assessment Log Book

NAME: _____

SID#: _____

Please carry your AMS log book in your trail pack and complete every day.

Directions for booklet usage:

- *AMS LOG (2 pages) – before breakfast*
- *Supplement packets and daily hydration (1 page) – throughout the day*
- *Energy Bars, medications, and vitamins/supplements (1 page) – throughout the day*
- *AMS (2 pages) – before bed*

Medications include any type of medicine, vitamin, or supplement. Please include brand names (if available), dosage and timing.

Again, thank you for your participation.

ALL **DAY & DATE:** _____ TIME: _____ LOCATION: _____ ALT: _____

Check	Study Packets	Amounts – circle one			
	Study Packets	Amount:	0	1	2
		Times:		|	|

Check	Fluid	Amounts – circle one						
	Water	Ounces:	16	32	64	96	128	Other:
	Sports drink (specify)	Ounces:	8	16	24	32	64	Other:
	Soda (specify)	Ounces:	8	16	24	32	64	Other:
	Tea	Ounces:	4	6	8	12	16	Other:
	Coffee	Ounces:	4	6	8	12	16	Other:
	Hot Cocoa	Ounces:	4	6	8	12	16	Other:
	Juice (specify)	Ounces:	4	6	8	12	16	Other:
	Milk	Ounces:	4	6	8	12	16	Other:
	Beer (specify)	Ounces:	4	6	8	12	16	Other:
	Wine (specify)	Ounces:	4	6	8	12	16	Other:
	Other – specify	Amount (write in):						
	Other – specify	Amount (write in):						

Notes:

REFERENCES

1. Rose MS, Houston CS, Fulco CS, Coates G, Sutton JR, Cymerman A. Operation Everest. II: Nutrition and body composition. J Appl Physiol 1988;65(6):2545-51.

2. Westerterp KR, Kayser B, Brouns F, Herry JP, Saris WH. Energy expenditure climbing Mt. Everest. J Appl Physiol 1992;73(5):1815-9.

3. Edwards LM, Murray AJ, Tyler DJ, et al. The effect of high-altitude on human skeletal muscle energetics: P-MRS results from the Caudwell Xtreme Everest expedition. PloS one 2010;5(5):e10681. doi: 10.1371/journal.pone.0010681.

4. Carbone JW, McClung JP, Pasiakos SM. Skeletal muscle responses to negative energy balance: effects of dietary protein. Adv Nutr 2012;3(2):119-26. doi: 10.3945/an.111.001792.

5. Evans WJ. Skeletal muscle loss: cachexia, sarcopenia, and inactivity. Amer J Clin Nutr 2010;91(4):1123S-7S. doi: 10.3945/ajcn.2010.28608A.

6. Imray C, Wright A, Subudhi A, Roach R. Acute mountain sickness: pathophysiology, prevention, and treatment. Prog Cardiovasc Dis 2010;52(6):467-84. doi: 10.1016/j.pcad.2010.02.003.

7. Hamad N, Travis SP. Weight loss at high altitude: pathophysiology and practical implications. Eur J Gastroenterol Hepatol 2006;18(1):5-10.

8. Westerterp-Plantenga MS, Westerterp KR, Rubbens M, Verwegen CR, Richelet JP, Gardette B. Appetite at "high altitude" [Operation Everest III (Comex-'97)]: a simulated ascent of Mount Everest. J Appl Physiol 1999;87(1):391-9.

9. Butterfield GE, Gates J, Fleming S, Brooks GA, Sutton JR, Reeves JT. Increased energy intake minimizes weight loss in men at high altitude. J Appl Physiol 1992;72(5):1741-8.

10. Reynolds RD, Lickteig JA, Deuster PA, et al. Energy metabolism increases and regional body fat decreases while regional muscle mass is spared in humans climbing Mt. Everest. J Nutr 1999;129(7):1307-14.

11. Shukla V, Singh SN, Vats P, Singh VK, Singh SB, Banerjee PK. Ghrelin and leptin levels of sojourners and acclimatized lowlanders at high altitude. Nutr Neurosci 2005;8(3):161-5.

12. Tschop M, Strasburger CJ, Hartmann G, Biollaz J, Bartsch P. Raised leptin concentrations at high altitude associated with loss of appetite. Lancet 1998;352(9134):1119-20. doi: 10.1016/S0140-6736(05)79760-9.

13. Phillips SM, Van Loon LJ. Dietary protein for athletes: from requirements to optimum adaptation. J Sports Sci 2011;29 Suppl 1:S29-38. doi: 10.1080/02640414.2011.619204.

14. Drummond MJ, Glynn EL, Fry CS, Timmerman KL, Volpi E, Rasmussen BB. An increase in essential amino acid availability upregulates amino acid transporter expression in human skeletal muscle. Am J Physiol Endocrinol and Metab 2010;298(5):E1011-8. doi: 10.1152/ajpendo.00690.2009.

15. Pasiakos SM, Vislocky LM, Carbone JW, et al. Acute energy deprivation affects skeletal muscle protein synthesis and associated intracellular signaling proteins in physically active adults. J Nutr 2010;140(4):745-51. doi: 10.3945/jn.109.118372.

16. Mettler S, Mitchell N, Tipton KD. Increased protein intake reduces lean body mass loss during weight loss in athletes. Med Sci Sports Exerc 2010;42(2):326-37. doi: 10.1249/MSS.0b013e3181b2ef8e.

17. Phillips SM. The science of muscle hypertrophy: making dietary protein count. Proc Nutr Soc 2011;70(1):100-3. doi: 10.1017/S002966511000399X.

18. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol 2009;107(3):987-92. doi: 10.1152/japplphysiol.00076.2009.

19. Hulmi JJ, Lockwood CM, Stout JR. Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: A case for whey protein. Nutr Metab (Lond) 2010;7:51. doi: 10.1186/1743-7075-7-51.

20. Garlick PJ, Grant I. Amino acid infusion increases the sensitivity of muscle protein synthesis in vivo to insulin. Effect of branched-chain amino acids. Biochem J 1988;254(2):579-84.

21. Wolfe RR. Regulation of muscle protein by amino acids. J Nutr 2002;132(10):3219S-24S.

22. Cuthbertson D, Smith K, Babraj J, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB J 2005;19(3):422-4. doi: 10.1096/fj.04-2640fje.

23. Garlick PJ. The role of leucine in the regulation of protein metabolism. J Nutr 2005;135(6 Suppl):1553S-6S.

24. Stipanuk MH. Leucine and protein synthesis: mTOR and beyond. Nutr Rev 2007;65(3):122-9.

25. Pasiakos SM, McClung JP. Supplemental dietary leucine and the skeletal muscle anabolic response to essential amino acids. Nutr Rev 2011;69(9):550-7. doi: 10.1111/j.1753-4887.2011.00420.x.

26. Crozier SJ, Kimball SR, Emmert SW, Anthony JC, Jefferson LS. Oral leucine administration stimulates protein synthesis in rat skeletal muscle. J Nutr 2005;135(3):376-82.

27. Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS, Kimball SR. Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. J Nutr 2000;130(10):2413-9.

28. Dodd KM, Tee AR. Leucine and mTORC1: a complex relationship. Am J Physiol Endocrinol and Metab 2012;302(11):E1329-42. doi: 10.1152/ajpendo.00525.2011.

29. Drummond MJ, Rasmussen BB. Leucine-enriched nutrients and the regulation of mammalian target of rapamycin signalling and human skeletal muscle protein synthesis. Curr Opin Clin Nutr Metab Care 2008;11(3):222-6. doi: 10.1097/MCO.0b013e3282fa17fb.

30. Laplante M, Sabatini DM. mTOR Signaling. Cold Spring Harb Perspect Biol 2012;4(2). doi: 10.1101/cshperspect.a011593.

31. Vigano A, Ripamonti M, De Palma S, et al. Proteins modulation in human skeletal muscle in the early phase of adaptation to hypobaric hypoxia. Proteomics 2008;8(22):4668-79. doi: 10.1002/pmic.200800232.

32. Baptista IL, Leal ML, Artioli GG, et al. Leucine attenuates skeletal muscle wasting via inhibition of ubiquitin ligases. Muscle Nerve 2010;41(6):800-8. doi: 10.1002/mus.21578.

33. Schena F, Guerrini F, Tregnaghi P, Kayser B. Branched-chain amino acid supplementation during trekking at high altitude. The effects on loss of body mass, body composition, and muscle power. Eur J Appl Physiol Occup Physiol 1992;65(5):394-8.

34. Walker TB, Smith J, Herrera M, Lebegue B, Pinchak A, Fischer J. The influence of 8 weeks of whey-protein and leucine supplementation on physical and cognitive performance. Int J Sport Nutr Exerc Metab 2010;20(5):409-17.

35. Wagenmakers AJ. Branched-chain amino acid supplementation during trekking at high altitude. The effects on loss of body mass, body composition and muscle power. Eur J Appl Physiol Occup Physiol 1993;67(1):92-5.

36. Pineau JC, Guihard-Costa AM, Bocquet M. Validation of ultrasound techniques applied to body fat measurement. A comparison between ultrasound techniques, air displacement plethysmography and bioelectrical impedance vs. dual-energy X-ray absorptiometry. Ann Nutr Metab 2007;51(5):421-7. doi: 10.1159/000111161.

37. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. Br J Nutr 1978;40(3):497-504.

38. Jackson AS, Pollock ML, Ward A. Generalized equations for predicting body density of women. Med Sci Sports Exerc 1980;12(3):175-81.

39. Kinanthropometry ISfhAo. International Standards for Anthropometric Assessment. Potchefstroom, South Africa: International Society for the Advancement of Kinanthropometry,, 2001.

40. Burke LM, Cox GR, Culmmings NK, Desbrow B. Guidelines for daily carbohydrate intake: do athletes achieve them? Sports Med 2001;31(4):267-99.

41. Sherman WM, Doyle JA, Lamb DR, Strauss RH. Dietary carbohydrate, muscle glycogen, and exercise performance during 7 d of training. Amer J Clin Nutr 1993;57(1):27-31.

42. Howarth KR, Phillips SM, MacDonald MJ, Richards D, Moreau NA, Gibala MJ. Effect of glycogen availability on human skeletal muscle protein turnover during exercise and recovery. J Appl Physiol 2010;109(2):431-8. doi: 10.1152/japplphysiol.00108.2009.

43. Rodriguez NR, DiMarco NM, Langley S, et al. Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. J Acad Nutr Diet 2009;109(3):509-27.

44. Layman DK, Evans EM, Erickson D, et al. A moderate-protein diet produces sustained weight loss and long-term changes in body composition and blood lipids in obese adults. J Nutr 2009;139(3):514-21. doi: 10.3945/jn.108.099440.

45. Fulco CS, Friedlander AL, Muza SR, et al. Energy intake deficit and physical performance at altitude. Aviat Space Environ Med 2002;73(8):758-65.