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Original Research

Leucine Supplementation Does Not Improve Muscle Recovery from Resistance Exercise in Young Adults: A Randomized, Double-Blinded, Crossover Study

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ABSTRACT

International Journal of Exercise Science 14(2): 486-497, 2021. This study aimed to investigate the effects of free leucine supplementation on muscle recovery from resistance exercise (RE) in young adults. Fourteen untrained subjects (23.9 ± 3.6 years old) underwent RE sessions (leg press and hack squat: three sets of 8-12 reps at 70% 1RM) supplemented with leucine (LEU: two daily doses of 3g) or a placebo (PLA), separated by a seven-day washout period. Following each occasion, participants were evaluated in three subsequent days (24h, 48h, and 72h) for muscle recovery via a repetition-to-failure test. The following markers were assessed: repetition performance, perceived exertion, lactate, creatine kinase, muscle soreness (DOMS), testosterone, and cortisol. No significant difference was observed between LEU and PLA conditions ($p > 0.05$). Number of repetitions performed in the repetition-to-failure tests, perceived exertion, cortisol, and testosterone:cortisol ratio did not change over time ($p > 0.05$). Creatine kinase increased immediately after exercise, at 24h, and 48h, and was attenuated at 72h post-exercise, while testosterone, lactate, and DOMS increased at 24h post-exercise ($p < 0.05$) and remained elevated up to 72h. All outcomes were similar between LEU and PLA. Results indicate that a 6g daily dose of free leucine supplementation does not improve muscle recovery following lower-limb RE in untrained young adults.

KEY WORDS: Amino acids, BCAA, resistance training, muscle damage

INTRODUCTION

The provision of essential amino acids (EAAs) has been widely recognized as a potential nutritional strategy to stimulate muscle protein synthesis (MPS) during muscle recovery from resistance exercise (RE) (9, 44). Among the EAAs, the branched-chain amino acids (BCAAs), particularly leucine (Leu), have been shown to enhance MPS and reduce muscle protein breakdown by stimulating anabolic factors (7, 15, 19, 20) and reducing catabolic factors (8). This positive net protein balance induced from BCAAs have been shown to induce a greater anabolic

environment on the muscle tissue, decrease delayed onset muscle soreness (DOMS), decrease creatine kinase (CK) levels, and improve muscle function during the recovery process (18, 32, 39, 40, 46). It is important to note that attenuating the indices of muscle damage following RE is of major importance for subjects entering RE programs to keep them to the practice.

Among the plethora of data concerning the effects of EAAs and BCAAs supplementation on recovery, recent findings have shown promissory effects with the ingestion of Leu-enriched EAAs/BCAAs, or isolated Leu supplements (22, 33, 42, 45, 46). Waldron et al. (45), in a non-crossover study in young adults, recently observed that two daily doses of Leu (~ 7.5g each) increased the rate of recovery of a muscle-damaging exercise protocol (100 high-drop jumps) compared to the placebo (Pla) group. The Leu group produced higher isometric strength and jump height, and lower CK and DOMS post-exercise (45). Similarly, Kirby et al. (22) verified positive effects for Leu (two daily doses of ~19.5g of Leu) by attenuating reductions in muscle function (isometric strength and jump height) and lowering the CK activity, following a very strenuous RE protocol (100 high-drop jumps and six supramaximal leg-press sets of ten eccentric repetitionss with 120% 1RM). Although presenting sound methodologies to investigate the effects of Leu on recovery has merit, the nature of the protocols applied in these studies have limited application to real-world practice. That is, it remains to be determined whether Leu could attenuate the muscle damage-induced reduction on performance (i.e. repetitions volume) on traditional RE sessions. This is relevant to practitioners because RE volume is important to muscular adaptations (17, 25).

A recent study by Waskiw-Ford et al. (46) observed attenuations in function impairment, muscle damage, DOMS, and CK levels in recreationally active males who consumed three daily doses Leu-enriched EAAs drink (1.6g). In contrast, Stock et al. (42) examined the effects of consuming a carbohydrate beverage with or without ~ 4g of Leu following a RE session (six sets of squat at 75% 1RM to failure) in young adults and verified no significant effect on DOMS, CK, and repetitions performed on a subsequent RE session. It is worthy to note that all these investigations were not crossover designs, that is, subjects carried out Leu or Pla conditions only (22, 33, 42, 45, 46). This does not take into account individual responsiveness and variability, which may affect the interpretation of the results (42). Due to this and the conflicting results in the literature, further studies are needed to offer stronger conclusions on whether Leu supplementation can improve muscle recovery and markers of muscle damage from RE (22, 33, 42, 45, 46).

Therefore, the present study aimed to examine whether free Leu supplementation enhances the muscle recovery process following RE-induced muscle damage in untrained young adults. Given that Leu has been shown to be a key regulator of MPS in several conditions, it was hypothesized that Leu supplementation would present benefits (5, 11, 15, 23). More specifically, we hypothesized that Leu would improve the analyzed outcomes: number of repetitions, perceived exertion, DOMS, CK levels, lactate, and testosterone:cortisol ratio.

METHODS

Participants

Recruitment was made through social media and home delivery of flyers in the university area. The inclusion criteria were: male or female adults, 18-30 years-old, eutrophic (i.e. body mass index range: 18 to 25 kg/m²), non-tobacco users, not taking any medication that could affect muscle recovery and performance, not using any ergogenic supplement, not performing RE regularly within six months before the start of the study, with no physiological (e.g. cardiorespiratory and metabolic diseases) or physical limitation (e.g. orthopedic diseases, muscular injury, or musculoskeletal pain) that could affect the ability to perform the physical test. All volunteers were screened with the Physical Activity Readiness Questionnaire and released to participate in the study.

Sample size analysis was conducted using G*Power (v. 3.1.9.2) for an F test (within-between interaction effects) based on a statistical power (1 – β) of 0.80, a moderate effect size (0.5), and an overall level of significance of 0.05. Sample size was calculated using CK levels post-exercise as the primary study outcome and a minimum of eight participants was required for this study (22 ,33, 42, 45, 46). Seventeen recreationally-active healthy individuals were recruited. Three withdrew for personal reasons, while fourteen of participants completed the study and were included for final analyzes (eleven men and three women, age = 23.9 ± 3.6 years, body mass = 75.4 ± 8.7 kg, stature = 170.0 ± 8.0 cm, body mass index = 26.1 ± 2.8 kg/m², total cholesterol = 164.9 ± 32.4 mg/dL, glucose = 80.1 ± 8.8 mg/dL, insulin = 16.6 ± 10.8 µg/dL). All participants were informed of the procedures of the investigation and signed an informed consent document approved by the Institutional Review Board of the University (protocol no: 1.667.005).

Protocol

A randomized, double-blinded, crossover trial was performed to examine the effects of Leu supplementation on the time course of muscle damage after a single session of RE in young adults (Figure 1). Participants underwent two counterbalanced RE session (T1 and T2) with 1 of 2 treatments [leucine (LEU) or placebo (PLA)], separated by a seven-day washout period. Before T1, participants completed three sessions of familiarization (leg press and hack squat, each with three sets of 8-12 repetitions, self-selected moderate load) and two sessions of one-repetition maximum (1RM) tests for both exercises. During T1 and T2, participants underwent a session of RE and were then evaluated for muscle damage recovery in three subsequent sessions of repetition-to-failure tests (24h, 48h, and 72h after), whereby the following markers of muscle damage were assessed: repetitions performed: perceived exertion, lactate, CK, DOMS, testosterone, and cortisol. Moreover, participants completed a three-day dietary intake record during the weeks of T1 and T2 to monitor any influence of diet. All sessions were performed in the same controlled laboratory, between 8 and 10 a.m. All procedures were carried out under the Declaration of Helsinki ethical standards and complied with the ethical issues of the International Journal of Exercise Science (31).

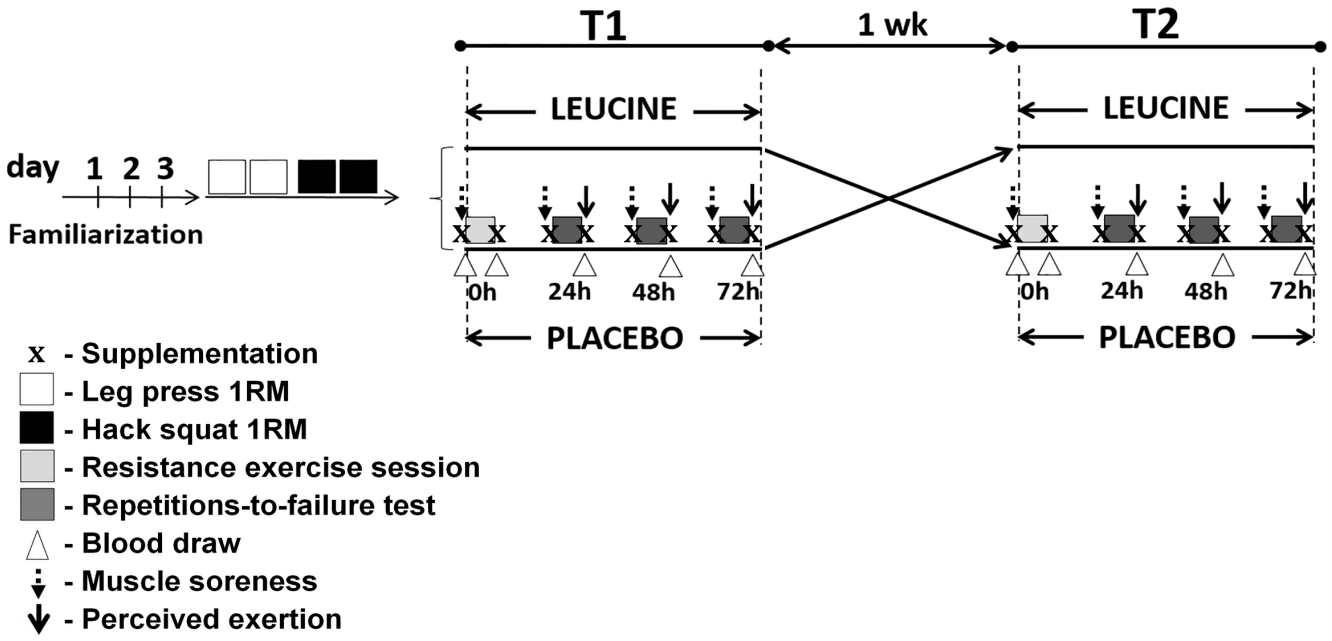

Figure 1. Experimental design.

The 1RM tests for leg press and hack squat exercises were performed at the Nakagym in São Paulo, Brazil, using a standard protocol for both days (38). A brief warm-up set (~ 15 repetitions) was completed prior to testing for each exercise. After a two-minute rest, three 1RM attempts were performed with a progressively increasing load, with a 4 – 5 minute rest between them. The exercises were standardized and continuously monitored by the same experienced rater in an attempt to assure the assessment quality and determine the 1RM within three attempts. The interclass correlation coefficients (ICC) test-retest were ≥ 0.94 for each 1RM test.

The RE session involved leg press and hack squat exercises. Both exercises were performed in three sets of 8-12 repetitions at 70% of the 1RM, in a tempo of 1:2 for concentric:eccentric muscle actions (metronome controlled), with two-minute rest between sets and exercises. This protocol was designed following recommendations of conventional RE for novice individuals (1). The RE sessions began with a general (moderate walking on the treadmill for ten minutes) and specific (one set of 8 - 12 repetitions with a self-selected moderate load) warm-up exercise regimen. Two Physical Education professionals supervised each participant individually during the workout.

The repetition-to-failure test was performed on the leg press and consisted of one set at 50% of 1RM until concentric failure. The test was performed to assess muscle function during the recovery period. Participants were verbally encouraged by an experienced evaluator blinded to treatments.

Muscle soreness was measured before the repetition-to-failure test, using a visual analog scale (28). The scale consists of a 10-cm line whose points were labeled with "no pain" (left) and "unbearable pain" (right). The participants were instructed to palpate the vastus lateralis muscle of the dominant leg and mark a scale point that best represented their momentary local soreness. The score was the distance (in cm) from the left side of the scale to the point marked. Palpation

was performed in a circular motion and constant pressure in a clockwise direction, with the tips of the index and middle fingers toward the deeper tissues. This was done for approximately three seconds. Participants practiced palpation prior to starting the study to reproduce the constant pressure within a low variation between trials.

The rating of perceived exertion was measured immediately after repetition-to-failure tests using the OMNI-RES scale (37). The participants were instructed to report the perceived exertion value by indicating a number on the OMNI-RES scale (0 for "no effort" and 10 for "maximal effort") that best represented their muscular effort (14, 37). Participants were adapted to the scale during the familiarization sessions.

Blood samples were collected at pre- and post-exercise, as well as immediately after muscular fatigue tests (24h, 48h, and 72h after RE session) for analyses of CK, lactate, cortisol, and testosterone levels. The blood samples were allowed to coagulate at room temperature for 60-minutes, then centrifuged at 2000 X g for fifteen minutes, and the serum was frozen at -80^{o}C until analysis. All analyses were performed in a laboratory equipped with automated systems using commercial kits for kinetic (CK and lactate) and Chemiluminescence (cortisol and testosterone) techniques.

The participants were supplemented orally with an identical looking and equivalent amount (6 g/d) of Leu (LEU) or Placebo-cornstarch (PLA) dissolved in water. The supplements were analyzed and confirmed for purity before the study in a specialized laboratory. Both were consumed in two equal doses 30 minutes before (3g) and immediately after (3g) the RE sessions and fatigue tests. Such protocol was chosen because peak concentration of plasma Leu seems to occur within 30 minutes after ingestion, 1and a dose of 6g of Leu has been reported to increase the MPS rate without inducing any adverse event (3, 10, 41, 47). To ensure the double-blinded design, an individual who was not involved in the study was responsible for placing the supplements into bags and label with the participants' names according to the randomization. Participants were requested to report any discomfort or adverse effects of supplements during the study period.

For the analysis of macronutrient dietary intake, participants completed a 3-day dietary intake record during weeks of T1 and T2. Records were made from the day which the RE session was performed, and days 1 (24h) and 2 (48h) of the recovery sessions. The macronutrient composition of the diets was calculated using software for nutritional assessment (Avanutri, v. 3.1.4. Rio de Janeiro, Brazil). Participants were asked to maintain their habitual diet.

Statistical Analysis

Data were checked for normality and homogeneity using the Shapiro-Wilk's and Levene's tests, respectively. Paired t-test was used to compare treatments for dietary intake and accumulated total repetition in the recovery. Repeated measures analysis of variance (ANOVA) was used to evaluate changes over time and between treatments. If the sphericity assumption was violated as indicated by Mauchly's test, Greenhouse-Geisser correction was used. Multiple comparisons testing was performed using Tukey's post-hoc correction to identify the differences. The

significance level was set at $p \leq 0.05$. Data were presented as mean, standard deviation, and confidence intervals. Data were analyzed were using Jamovi (v. 1.0.7. The Jamovi Project).

RESULTS

No discomfort or adverse effect of the supplementation was reported. No significant difference was observed between T1 and T2 moments for total energy intake (T1: 2037.8 ± 778.8 kcal/d; T2: 1888.8 ± 500.4 kcal/d; $p = 0.580$), protein (T1: 1.3 ± 0.4 g/kg/d; T2: 1.3 ± 0.4 g/kg/d; $p = 0.340$), carbohydrate (T1: 3.6 ± 1.5 g/kg/d; T2: 3.2 ± 1.1 g/kg/d; $p = 0.440$), and lipid (T1: 0.8 ± 0.3 g/kg/d; T2: 0.8 ± 0.2 g/kg/d; $p = 0.940$). All participants presented adequate protein (>1.2 g/kg/d) and carbohydrate (>3 g/kg/d) intake during the study period, attending the recommendations (2). The results of the main outcomes are represented in Figure 2.

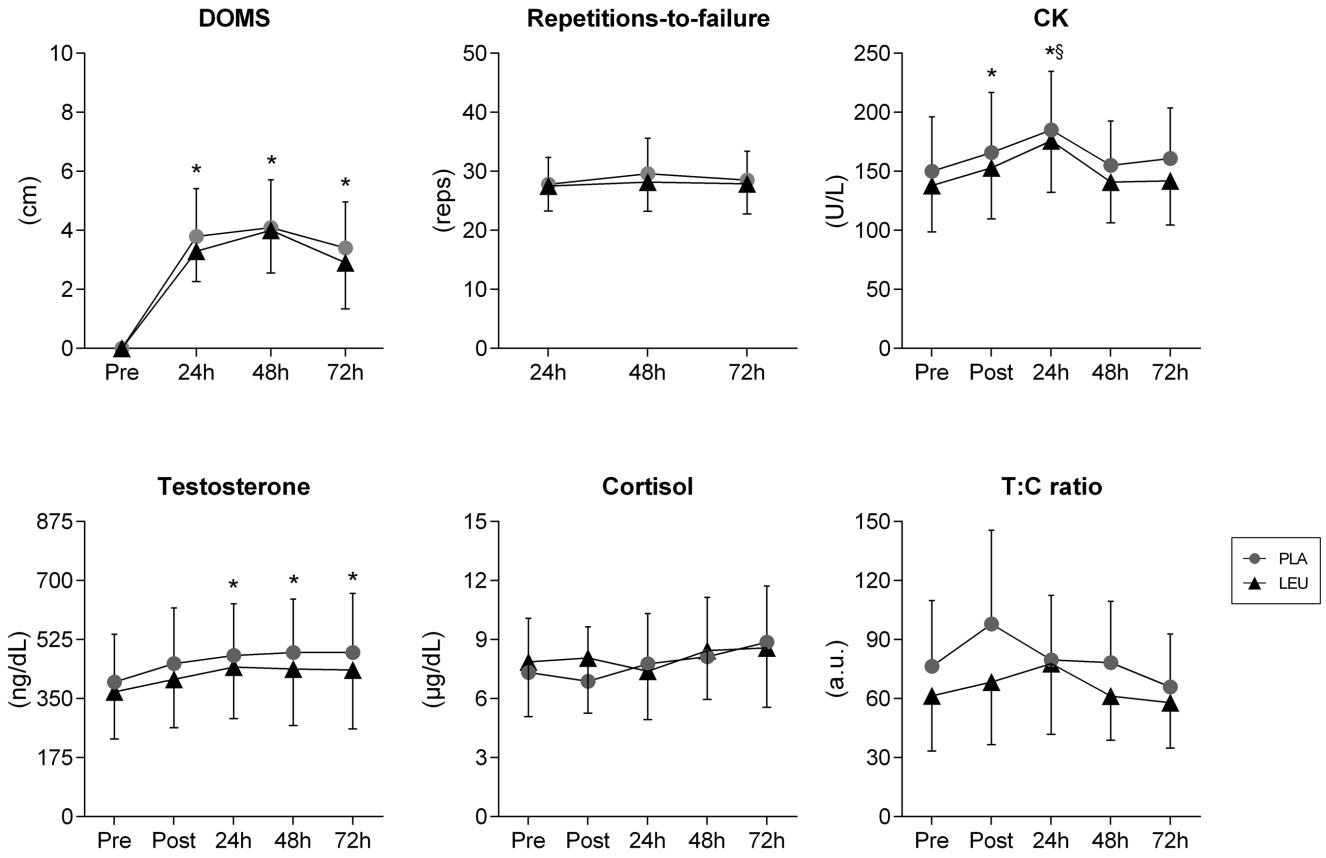

Figure 2. Responses immediately after (Post) and throughout the 3-d recovery period (24h, 48h, and 72h post-exercise) for Leucine (LEU) and Placebo (PLA) conditions ($n = 14$). DOMS = delayed onset muscle soreness. CK = creatine kinase. T:C = testosterone:cortisol. Data is presented as mean and 95% confidence intervals. Only time effects were observed (*$p < 0.05$ vs. Pre; §$p < 0.05$ vs. Post).

The DOMS increased at 24h, 48h, and 72h post-exercise compared to Pre (time: $p < 0.001$), but in similar magnitudes between LEU and PLA (treatment x time: $p = 0.942$). The number of repetitions in the repetitions-to-failure test did not differ between LEU and PLA conditions

(treatment x time: $p = 0.874$), and remained similar over time (time: $p = 0.494$). Total repetitions accumulated over the 3 days of recovery was not different between LEU and PLA conditions (LEU: 84 ± 23 vs. PLA: 86 ± 25 reps; treatment: $p = 0.804$). All participants rated maximum exertion (score 10) for all tests in both conditions (treatment x time: $p = 1.000$; time: $p = 1.000$). The lactate levels increased immediately post-exercise (vs. Pre: $p < 0.001$) and following the repetitions-to-failure tests increased at 24h (vs. Pre: $p < 0.001$), 48h (vs. Pre: $p < 0.001$), and 72h (vs. Pre: $p < 0.001$) post-exercise compared to Pre (time: $p < 0.001$), but in similar magnitudes between LEU and PLA (treatment x time: $p = 0.418$). The serum CK levels increased immediately post-exercise (vs. Pre: $p = 0.008$) and further increased following the repetitions-to-failure tests at 24h (vs. Pre: $p < 0.001$; vs. Post: $p < 0.001$), then attenuated after 48h (vs. Pre: $p = 0.947$), and 72h (vs. Pre: $p = 0.513$) post-exercise, but in similar magnitudes between LEU and PLA (time: $p < 0.001$; treatment x time: $p = 0.785$). Cortisol levels did not differ over time (time: $p = 0.083$), neither between LEU and PLA (treatment x time: $p = 0.491$). The testosterone levels did not alter immediately post-exercise (vs. Pre: $p = 0.122$), but increased following the repetitions-to-failure tests at 24h (vs. Pre: $p = 0.001$), 48h (vs. Pre: $p < 0.001$), and 72h (vs. Pre: $p = 0.001$) post-exercise, but in similar magnitudes between LEU and PLA (time: $p = 0.003$; treatment x time: $p = 0.887$). Testosterone:cortisol ratio did not differ over time (time: $p = 0.051$), neither between LEU and PLA (treatment x time: $p = 0.355$).

DISCUSSION

The finding of the current investigation was that Leu supplementation did not improve recovery from RE in untrained young adult individuals. Given that Leu has been shown to be a key regulator of MPS in several conditions, the initial hypothesis was that Leu supplementation would enhance recovery process after RE (11, 15, 16, 23). However, it was not confirmed.

It has been established that adequate protein intake is important to obtain major RE adaptations, as well as to increase MPS to support the recovery process (11, 27, 29, 30, 36). Moreover, some authors suggested that Leu intake may be the main determinant to maximizing MPS, then muscle recovery after exercise (10), and this effect appears to be obtained by achieving a Leu "threshold" of ~3 g of Leu per meal (10, 29). For example, Churchward-Venne et al. (10) showed that 6.25g of whey protein was as effective as 25g of whey protein and whey + BCAA to stimulate MPS during recovery from RE when Leu amount (5g) was equated. However, it seems that Leu may be dependent on the co-ingestion of other nutrients to improve MPS (10, 15, 23) and recovery (45, 46). Several previous studies have shown increased MPS after consumption of a nutrients mixture (e.g. EAAs, whey protein, or CHO) containing Leu, but not Leu alone (3, 4, 11, 15, 23, 26, 43). For example, Dreyer et al. (15) investigated the effects of a beverage containing Leu-enriched EAAs. Koopman et al. (23) used a beverage containing CHO plus protein and free Leu (CHO + Pro + Leu). While adding important pieces to the literature, the experimental design of these studies precludes the ability to discern the isolated effects of Leu supplementation. Moreover, following traditional RE sessions, Leu presented no effect on recovery, while Leu + EAAs did, when compared to CHO-only Pla (42, 46). Also, one study that observed major effects for Leu alone (40 - 60g per day) suggested a benefit for very high doses (22). However, Leu was compared to non-caloric drinks and was not energy-matched to Pla. This hampers conclusive

results and makes it possible to speculate that Leu also fulfilled energy functions beyond anabolic systems. The above-mentioned circumstances help to explain, at least in parts, the lack of beneficial effects of free Leu supplementation on markers of muscular function, anabolism, and damage during recovery from RE in our study. Our findings expand the suggested evidence that all EAAs may be required for muscle repairing and that free Leu supplementation has no further effect on post-exercise muscle recovery when EAAs are provided (48).

Another possible explanation for the lack of effect of Leu supplementation in our study may be the adequate daily consumption of proteins (~1.3 g/kg/d) observed for the participants. Previous works that showed an additional effect of Leu supplementation on MPS have investigated subjects with a dietary restriction or reduced capacity of MPS, like in food-deprived (5) or cancer cachexia conditions (13, 21, 34). For example, Anthony et al. (5) showed that a high dose of Leu administration promoted an important increase in MPS rate and stimulation of mTOR signaling pathway in the skeletal muscle of food-deprived rats. Moreover, it has been shown that acute Leu supplementation attenuates muscle wasting in mice with cancer cachexia (34) and restores the postprandial stimulation of MPS in old rats (13). Katsanos et al. (21) reported a maximization in rates of MPS in elderly subjects, but not young, after ingestion of Leu-enriched EAAs (41% Leu), suggesting that anabolic effects of Leu supplementation may depend on background protein synthesis capacity – given that elderlies present a blunted MPS response (i.e., anabolic resistance) to food intake (6, 12, 35). Taken together, these results suggest that Leu intake may be a favorable strategy to increase MPS in conditions in which there is a protein deficit. Moreover, free Leu supplementation failed to increase the hypertrophic response during a long-term RE program in healthy young subjects consuming an adequate amount (>1.2 g/kg/d) of dietary protein (3, 4). Our findings are in line with previous studies showing no additional effect of Leu supplementation on markers of recovery from RE (e.g., CK, muscle soreness, and repetitions to failure) under conditions where adequate amounts of protein are ingested (24, 42).

A few issues regarding the present study must be mentioned. First, we did not analyze plasma Leu concentration to confirm the absorption of the supplement. However, previous studies that used a lower dose of Leu (i.e. up to 3.4g) showed an increase in plasma Leu concentrations, which suggests that the dose used in our study (6g per day) was sufficient to elevate Leu levels (21, 47). Second, we did not assess direct markers of muscle tissue regeneration and damage (e.g. histological changes). Third, we collected dietary records of the participants in three days; assessing more days could better characterize their dietary habits. Finally, we used an exercise protocol limited to a lower-body extremity, and therefore we cannot rule out the possibility that in exercise protocols involving whole-body muscles the increase in demand for ingested protein to stimulate MPS could evidence the ergogenic effects of free Leu supplementation. Moreover, our sample included only untrained young men and women. Further studies are warranted to address the effectiveness of free Leu supplementation on muscle recovery in other populations (e.g. elderly), with different training status (e.g. recreational practitioners and/or athletes).

In conclusion, 3g doses of Leu 30 minutes before and immediately post-exercise (6g total per day) does not improve muscle recovery following RE-induced muscle damage in untrained

young adults consuming an adequate amount of dietary protein. With the conflicting results in the literature, it is premature to recommend Leu supplementation (in the doses tested to date) as an ergogenic aid to improve muscle recovery from RE in this population.

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