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Free leucine supplementation during an 8-week resistance training program does not increase muscle mass and strength in untrained young adult subjects

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Abstract The purpose of this study was to examine the effects of free leucine supplementation on changes in skeletal muscle mass and strength during a resistance training (RT) program in previously untrained, young subjects. In a double-blind, randomized, placebo-controlled study, 20 healthy young (22 ± 2 years) participants were assigned to two groups: a placebo-supplement group (PLA, $N = 10$) or a leucine-supplement group (LEU, $N = 10$). Both groups underwent an 8-week hypertrophic RT program (2 days/week), consuming an equivalent amount of leucine (3.0 g/day in a single post-training dose) or placebo (cornstarch). Quadriceps muscle strength, cross-sectional area (CSA) of the vastus lateralis (VL), and rectus femoris (RF), as well as the habitual dietary intake were assessed before and after the 8-week intervention period. There was a similar improvement in muscle strength (Leg press, LEU: +33% vs. PLA: +37%; $P > 0.05$, and knee extension, LEU: +31% vs. PLA: 34%; $P > 0.05$) and CSA (VL, LEU: 8.9% vs. PLA: 9.6%; $P > 0.05$, and RF, LEU: +21.6% vs. PLA: + 16.4%; $P > 0.05$) in the both groups from pre- to post-training. In addition, there was no significant ($P > 0.05$) difference in daily dietary intake between the LEU and PLA groups before and after the intervention period. Free leucine supplementation (3.0 g/day post-training) does not increase muscle strength or CSA during RT in healthy young subjects consuming adequate dietary protein intake.

Keywords Nutritional supplementation · Hypertrophy · Cross-sectional area · Ergogenic · Skeletal muscle

Introduction

Gains in skeletal muscle mass during resistance training (RT) are primarily attributed to feeding-induced increases in rates of muscle protein synthesis (MPS) as well as activation of the mammalian target of rapamycin (mTOR) signaling pathway (Wang and Proud 2006; Dickinson et al. 2011). Both MPS and mTOR signaling are highly influenced by the provision of essential amino acid (EAAs), especially the branched-chain amino-acid (BCAA) leucine (Leu) (Blomstrand et al. 2006). Indeed, Leu supplementation has been shown to increase rates of MPS rates in several conditions (Anthony et al. 2000; Koopman et al. 2006; Dreyer et al. 2008). For example, Anthony et al. (2000) showed that Leu administration (1.35 g/kg body mass) promoted greater increases in MPS and stimulation of mTOR signaling in skeletal muscle of food-deprived rats. In addition, Dreyer et al. (2008) reported a greater MPS response at 2 h postexercise in young men that consumed a beverage containing Leu-enriched EAAs compared to control group. The additional effect of Leu supplementation on rates of MPS was also observed in young (20 ± 1 years) and elderly (75 ± 1 years) men who consumed a beverage containing CHO plus protein and free Leu (CHO + Pro + Leu) compared to the ingestion of CHO only, following 30 min of physical activity (Koopman et al. 2006). Collectively, the results of these studies (Anthony et al. 2000; Koopman et al. 2006; Dreyer et al. 2008) and several others

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(Churchward-Venne et al. 2012, 2014; Luiking et al. 2014; Norton et al. 2017; Norton and Layman 2006) suggest that Leu supplementation is a key stimulator of MPS and may be efficacious towards RT-induced muscle hypertrophy.

However, previous studies that investigated the chronic effects of Leu supplementation on muscle mass gains [e.g., increased cross-sectional area (CSA) of skeletal muscle] have shown contradictory results (Coburn et al. 2006; Verhoeven et al. 2009; Leenders et al. 2011). For instance, Coburn et al. (2006) found a greater increase in the muscle strength but not hypertrophy in young men supplemented with whey protein (20 g) plus Leu (6.2 g/day), compared to placebo group (26.2 g of maltodextrin), after 8 weeks of RT. The lack of effect of Leu supplementation on muscle mass was also observed in healthy elderly men (Verhoeven et al. 2009) and elderly men with type 2 diabetes (Leenders et al. 2011) after 12 and 24 weeks of intervention (7.5 g/day), respectively. Additionally, it has been shown in previous chronic studies (4–10 weeks) that Leu supplementation combined with EAAs, creatine, whey protein, and/or CHO do not promote greater increase in muscle strength than RT alone (Antonio et al. 2000; Williams et al. 2001; Ratamess et al. 2003; Chromiak et al. 2004). Therefore, whether the increase in MPS rates induced by Leu supplementation is, in fact, translated into real gains in muscle mass and strength, principally during long-term RT programs, remains unclear.

The aim of the present study was to examine the chronic effects of free Leu supplementation combined with RT on muscle hypertrophy and strength in previously untrained young subjects. Given that Leu may increase acute MPS rates following exercise, we hypothesized that free Leu supplementation would further increase muscle CSA and strength compared to RT alone. This is the first study, to our knowledge, to investigate the chronic effects of free Leu supplementation combined with RT on skeletal muscle adaptations in untrained young subjects.

Methods

Experimental design

A randomized, double-blind, repeated-measures design was conducted to examine the chronic effects of free Leu supplementation on muscle mass and strength during a 8-week RT program in previously untrained young subjects (Fig. 1). All subjects were monitored for the gains in training load (as indicator of muscle strength), nutrient intake, and performed Doppler ultrasound examination on 2 separate moments [before (M1) and after (M2) an 8-week high-intensity RT program] following a 1-week familiarization period (Fig. 1). After baseline testing (M1), the subjects

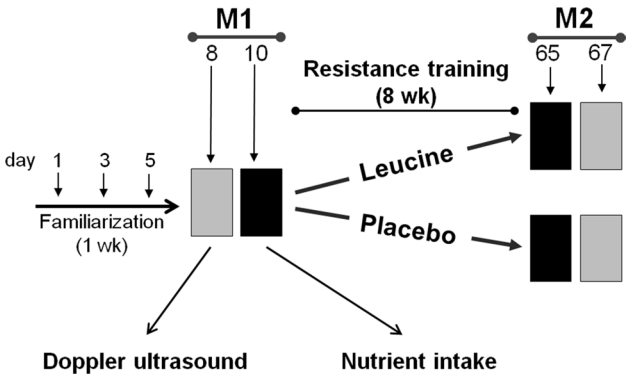

Fig. 1 Experimental design

were matched according to sex and strength, and then randomly assigned in a double-blind fashion to a leucine (LEU, $N = 10$) or placebo group (PLA, $N = 10$). Two days after the 8-week RT program, the subjects completed again the Doppler ultrasound (M2) to examine possible group-by-time interactions (Fig. 1).

Subjects

Healthy young subjects aged 18–30 years were recruited via advertisements posted on the University campus to participate in this study. An a priori power analysis was conducted (G*Power v. 3.0.1) for an F test (repeated measures, within-between interaction factors for three time points) to assess the required number of participants in each group. On the basis of a statistical power ($1 - \beta$) of 0.80, a moderately large effect size (0.5), and an overall level of significance of 0.05, least 10 subjects were required for this study. Participants were excluded if they: (1) were vegetarian, (2) had ingested any ergogenic supplement or anabolic steroids for the 6 months prior to the start of study, (3) were taking any medication that could affect muscle growth or the ability to train intensely, (4) had participated in a RT program for at least 6 months prior to the start of study, (5) were unable to provide a detailed description of their lifestyle and daily food intake, and (6) did not have medical approval to perform physical exercise. Twenty subjects who met these criteria were included in the study. The physical characteristics of the LEU and PLA groups (five men and five women in each group) at baseline are presented in Table 1. All subjects were carefully informed of the purpose, procedures, benefits, risks, and discomfort of the investigation prior to providing signed, informed consent. The study procedures were approved by the Institutional Review Board of the University (Protocol No: 44487715.6.0000.0108—CAAE). All procedures were performed according to the principles outlined in the latest version of Declaration of Helsinki.

Table 1 Baseline characteristics of the placebo (PLA) and leucine (LEU) groups

	PLA ($N = 10$)	LEU ($N = 10$)	P value
Age (years)	22.2 ± 2.3	22.0 ± 2.2	0.83
Body mass (kg)	67.6 ± 7.4	66.1 ± 9.6	0.71
Height (cm)	172.6 ± 6.4	171.7 ± 8.4	0.80
BMI (kg/m^2)	22.6 ± 1.3	22.3 ± 2.2	0.73

Values are mean ± SD. *BMI* body mass index. There were no differences between the groups

Nutrient intake

Participants completed a 3-day dietary intake record (including 1 weekend day) before (M1) and after (M2) the 8-week RT program. The macronutrient composition of the diets was calculated using software for nutritional assessment (Avanutri, version 3.1.4, Rio de Janeiro, RJ, Brazil). Participants were instructed to maintain their habitual daily diet throughout study and water intake was ad libitum. The participants were also instructed to report any adverse events from the supplements.

Familiarization protocol

All participants completed a 1-week orientation program before randomization (LEU or PLA) to ensure familiarization with the exercises (bilateral knee extension and leg press). The protocol consisted of 3 sets of 8–12 repetitions, with 1-min rest between the sets and exercises. Qualified personnel individually supervised each participant during the familiarization period. All familiarization sessions and physical tests were performed at the same location, between 6 and 9 p.m.

Resistance training

All participants trained under the same protocol (2 days/week; 3 sets of 8–12 repetitions, with 1-min rest between sets and exercises) during the 8-week RT program (American College of Sports Medicine 2009). The training program focused on quadriceps muscles [e.g., rectus femoris (RF) and vastus lateralis (VL)] using two commercial exercise machines (Nakagym equipment, São Paulo, Brazil) in the following order: (1) bilateral leg press and (2) knee extension exercises. For both exercises, the repetition cadence was 1-s concentric: 2-s eccentric according to metronome. Each training session began with a warm-up that consisted of moderate walking on treadmill for 10 min and then 1 set of 12 repetitions with a self-selected load. Qualified personnel supervised individually each participant during every workout. Each subject received a training logbook in which the researchers recorded the weekly training load for each exercise. The training load was adjusted every 15 days according to number of repetitions performed at the end of the third set of each exercise. Specifically, 2 kg was added every one repetition that exceeded the 12 repetitions of third set of each exercise. The total time of one training session for each participant was approximately 30 min. The sessions were performed between 6 and 9 pm.

Supplementation

The LEU group orally ingested 3 g/day (single dose after the training session) encapsulated leucine (Probiótica®) dissolved in 200 mL of water, whereas the PLA group ingested an identical looking and equivalent amount of placebo (cornstarch). The leucine and placebo drinks were analyzed for purity prior to the study. An individual who was not involved in the study was responsible for placing the supplements into bags and labeling the capsules with the subjects' names according to the randomization list. We chose to provide 3 g of Leu, because this dose is safe and well tolerated when consumed orally (Verhoeven et al. 2009), and similar doses (e.g., 3.4–5 g) have been reported to increase the MPS rate in young subjects (Stark et al. 2012; Wilkinson et al. 2013; Churchward-Venne et al. 2014).

Muscle cross-sectional area

A B-mode ultrasound Doppler (model MEDISON® X8; Gioânia, Goiás, Brazil) equipped with a 7.5-MHz linear-array probe was used to assess CSA of the VL and RF, according to a previously validated procedure (Lixandrão et al. 2014) (Fig. 2). Briefly, axial images of the VL and RF were obtained with the probe placed perpendicular to the tissue interface, without depressing the skin, under a thick layer of water-soluble transmission gel. Measures were taken on the dominant leg with the participants placed in the supine position on a bed. The upper border of the lower third of the distance between trochanter major and epicondylus lateralis of the femur was considered as the reference point. To avoid any erroneous influence of muscle swelling, images were obtained 48 h before starting the training program and 48 h after the last training session. Images were reconstructed using a PowerPoint program (Microsoft, Seattle, USA) and transferred to an image analysis software (ImageJ®, model 1.48v). Muscle CSA was outlined manually three times by the same blinded investigator in respect to treatments and time point, and the CSA was determined as the average of the three measures. To establish measurement reliability, the same experienced rater performed all measurements. Ultrasound has been validated in the previous studies (Schoenfeld et al. 2015a, b) as a reliable measure to hypertrophic changes. The previous analysis

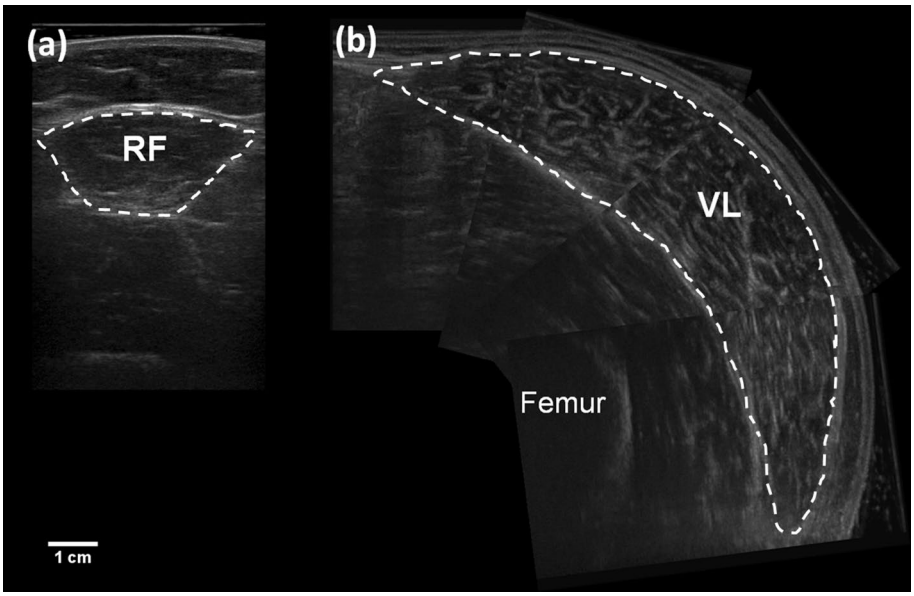

Fig. 2 Ultrasonography images of rectus femoris (**a**) and vastus lateralis (**b**) taken from a representative placebo subject showing cross-sectional area measurement

revealed a strong and significant intra-rater reliability (test–retest) for the RF (ICC: 0.98) and VL (ICC: 0.99) CSA measurements.

Statistical analyses

Data are expressed as mean ± SD. Data were tested for normality and homogeneity using Shapiro–Wilk's and Levene's tests, respectively. Baseline characteristics between groups were analyzed using an unpaired student's *t* test. A 2 (group: PLA vs. LEU) × 3 (time: basal, 4, and 8 weeks) ANOVA with repeated measures was used to evaluate the data across time and between groups for the training load. A 2 (group: PLA vs. LEU) × 2 (time: pre- and post-test) ANOVA with repeated measures was used to evaluate the data across time and between groups for the muscle CSA and nutritional intake. When significant differences were identified, post hoc analysis using a Bonferroni correction factor was employed to identify where these differences were located. The significance level was set at $P \leq 0.05$. Statistical analyses were performed using SPSS statistical analysis software (SPSS version 20.0; Chicago, IL, USA).

Results

Participant characteristics

All participants (PLA, $N = 10$; LEU, $N = 10$) who began the 8-week RT program completed the study, and no subject reported adverse effects. The baseline characteristics of the subjects are presented in Table 1. All groups had similar ($P > 0.05$) baseline physical characteristics.

Macronutrients intake

The macronutrient intake for each group is presented in Table 2. No significant ($P > 0.05$) differences in the daily dietary intake were observed between the LEU and PLA groups before and after the 8-week intervention period (Table 2). In addition, both groups had an adequate intake of CHO and protein at pre- and post-training, according to the recommendations proposed by the American College of Sports Medicine (2016).

Muscle strength

The training load for each group is presented in Fig. 3. A significant ($P < 0.05$) time effect with no group-by-time interaction ($P > 0.05$) indicated a similar improvement in

Table 2 Dietary analysis

	LEU	PLA	ANOVA	*P* value
CHO (g/kg/day)				
Pre	3.3 ± 1.7	3.7 ± 1.3	Time	0.28
Post	3.1 ± 1.4	3.5 ± 1.2	Group × time	0.69
Protein (g/kg/day)				
Pre	1.6 ± 0.6	1.5 ± 0.6	Time	0.39
Post	1.7 ± 0.6	1.6 ± 0.5	Group × time	0.92
Fat (g/kg/day)				
Pre	1.2 ± 0.6	1.0 ± 0.4	Time	0.16
Post	1.4 ± 1.1	1.2 ± 0.9	Group × time	0.96

Values are mean ± SD. *PLA* Placebo group, *LEU* Leucine group. There was no significant main effect of time, or group-by-time interactions

muscle strength for the leg press (LEU: +33% vs. PLA: +37%; $P > 0.05$) and knee extension (LEU: +31% vs. PLA: 34%; $P > 0.05$) in the both groups from pre- to post-training.

Muscle hypertrophy

A representative RF and VL muscles axial image is shown in Fig. 2, and the corresponding data are presented in Fig. 4. A significant ($P < 0.05$) time effect demonstrated a similar increase in the VL (LEU: 8.9% vs. PLA: 9.6%; $P > 0.05$) and RF (LEU: +21.6% vs. PLA: + 16.4%; $P > 0.05$) CSA in the both groups from pre- to post-training.

Discussion

To our knowledge, this is the first study to examine the effects of free Leu supplementation on gains in muscle mass and strength during a supervised RT program in previously untrained young adults. Given that Leu supplementation enhances feeding and exercise-induced increases in rates of MPS (Churchward-Venne et al. 2012, 2014; Luiking et al. 2014), we hypothesized that free Leu supplementation would potentiate gains in muscle mass and strength in response to RT. The major finding of this study was that free Leu supplementation did not improve muscle mass and strength during chronic RT in previously untrained young individuals.

To ensure that any potential differences in muscle mass and strength between groups were not due to inherent differences in habitual diet, we evaluated macronutrient intake before and after the 8-week RT program. However, a limitation of the present study is that no dietary analyses were conducted to determine protein intake throughout the 8-week RT program, so we cannot exclude the possibility that the experimental groups had different protein intakes. However, the subjects were encouraged to maintain their habitual daily diet, thus allowing to investigation of the effects of adding Leu to an existing diet when combined with RT. This is similar to the manner that the Leu supplementation would likely be used by consumers. Both PLA and LEU groups had a sufficient protein (>1.2 g/kg/day) and CHO (>3 g/kg/day) intake, indicating that the anabolic response to RT may have already been maximized with little to no capacity

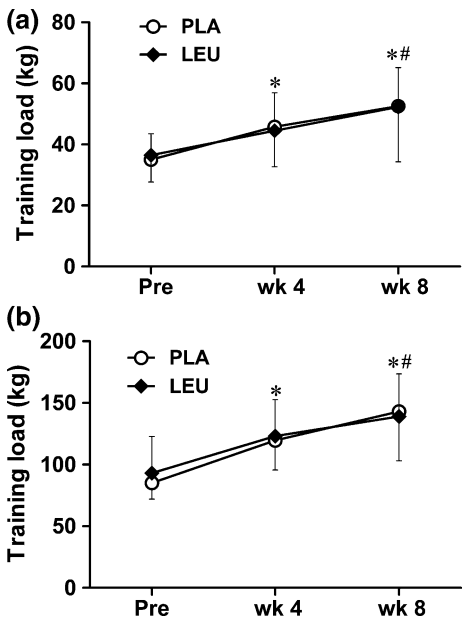

Fig. 3 Training load for knee extension (**a**) and leg press (**b**) exercises in the leucine (LEU, $N = 10$) and placebo (PLA, $N = 10$) groups throughout 8-week training program. $^{*}P < 0.05$ compared to pre-training values for both groups. $^{\#}P < 0.05$ compared to 4-week values for both groups

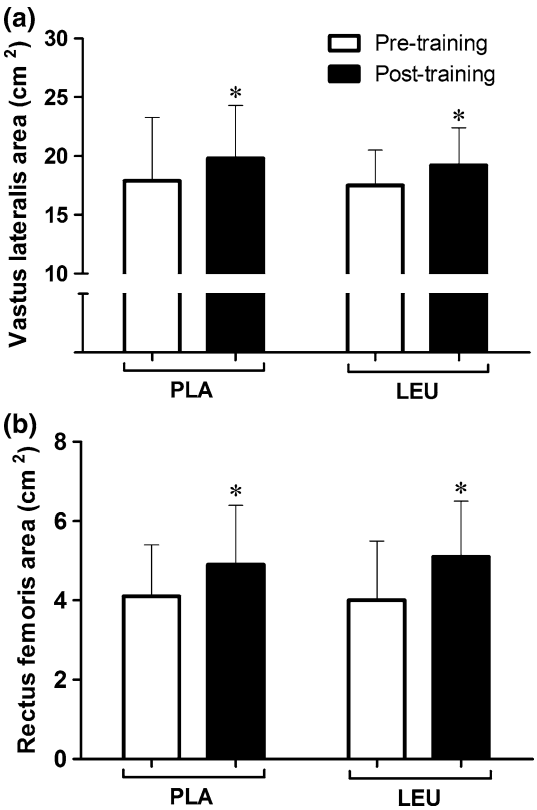

Fig. 4 Cross-sectional area of the vastus lateralis (**a**) and rectus femoris (**b**) in the leucine (LEU, $N = 10$) and placebo (PLA, $N = 10$) groups before and after the 8-week training program. $^{*}P < 0.05$ compared to respective pre-training values

for Leu treatment to confer a positive impact. The lack of effect of Leu supplementation on muscle strength and mass is, perhaps, not surprising considering previously published data on Leu supplementation plus other nutrients (e.g., amino acids and CHO) during RT in humans (Antonio et al. 2000; Williams et al. 2001; Ratamess et al. 2003; Chromiak et al. 2004). Antonio et al. (2000) showed no additional effect on muscle strength in EAA-supplemented group (average daily dose of 18.3 g of EAAs in pill form with 1.83 g of Leu per 10 g of EAA) compared to placebo group (cellulose) after 6 weeks of resistance and aerobic training (3×/week) in previously untrained young women. Likewise, Ratamess et al. (2003)found a similar increase in 1RM squat and bench press strength between an amino-acid-supplemented group (0.4 g kg/body mass, with 27 g of Leu per 100 g of amino acids) and placebo after a 4-week RT program in resistance-trained men. Williams et al. (2001) also found no difference in isometric, isokinetic, or 1RM strength gains between amino acid/glucose-supplemented group (containing 11% Leu) and a placebo after a 10-week RT program. The results of these studies and others (Williams et al. 2001; Chromiak et al. 2004) indicate that Leu supplementation combined with other amino acids and CHO does not result in greater muscle strength gains than RT alone. However, it is important to note in the above-mentioned studies that the Leu was consumed in combination with other amino acids and/or CHO compared to placebo (without the same mix of nutrients). Although these studies have reported no beneficial effects of Leu on muscle strength, the designs preclude the ability to examine the influence of Leu supplementation alone on muscular adaptations. Here, we demonstrate that Leu supplementation (3.0 g/day) alone does not promote any additional effect on muscle strength during RT in previously untrained young subjects consuming adequate protein intake.

In agreement with our observations, previous studies using a higher dose of free Leu (7.5 g/day) have also shown no additional effect on muscle strength in healthy elderly men (Verhoeven et al. 2009) and elderly men with type 2 diabetes (Leenders et al. 2011) after 12 and 24 weeks of nutritional intervention, respectively. Thus, the lack of additional effects of free Leu supplementation on muscle strength observed in our study and others (Verhoeven et al. 2009; Leenders et al. 2011) may not be attributed to a dose-dependent effect and/or different training regimes (e.g., volume, intensity, and exercise type). In addition, there was no additional effect of Leu supplementation (LEU group) on muscle mass gains compared with the RT alone (PLA group). This result is contradictory with the findings of previous animal (Anthony et al. 2000) and human (Koopman et al. 2006; Dreyer et al. 2008; Luiking et al. 2014) studies that investigated the effects of Leu supplementation on MPS rates. Considering that Leu intake has been shown to increase rates of MPS, it is unclear why Leu supplementation did not promote additional gains in muscle mass in the current study. There are two possibilities that might explain this paradox.

First, the human studies (Koopman et al. 2006; Dreyer et al. 2008; Luiking et al. 2014) that found an increase in rates of MPS following Leu supplementation used a combination of nutrients (e.g., EAAs, whey protein, and/or CHO), and the placebo group was not equivalent to the amount and type of nutrients. For example, Dreyer et al. (2008) investigated the effects of a beverage containing Leu-enriched EAAs compared to control group after a single bout of RT. Koopman et al. (2006) conducted a study with young and elderly men that consumed a beverage containing CHO plus protein and free Leu (CHO + Pro + Leu) compared with the ingestion of CHO only. In addition, Luiking et al. (2014) examined the effects of a high whey protein, Leu-enriched supplement (20-g whey protein and 3-g total Leu) compared to an isocaloric milk protein control (6 g milk protein), immediately after a unilateral resistance exercise. The experimental design of these studies does not enable the authors to discern the isolated effects of Leu supplementation, thereby suggesting that other nutrients (e.g., amino acids, proteins, and/or CHO) might have contributed to the increase in MPS. This hypothesis is consistent with the previous studies that showed a further increase in MPS or muscle mass after consumption of a nutrients mixture (e.g., amino acids and/or proteins) containing Leu (Koopman et al. 2006; Dreyer et al. 2008; Churchward-Venne et al. 2012; Luiking et al. 2014) but not Leu alone (Verhoeven et al. 2009; Leenders et al. 2011), suggesting that the efficacy of Leu may depend on the presence of other amino acids. This could explain, at least partially, the lack of additional effects of free Leu supplementation on muscle mass observed in our study.

Second, the lack of effects of Leu supplementation on muscle mass may be due to the fact that our participants were already consuming a daily protein intake known to saturate rates of MPS and had a maximal capacity of MPS. Previous animal and human studies that showed an additional effect of Leu supplementation on MPS have investigated subjects with dietary restriction (e.g., food-deprived rats) (Anthony et al. 2000) or reduced capacity of MPS (e.g., aging or cancer cachexia) (Katsanos et al. 2006; Dardevet et al. 2002; Peters et al. 2011). For example, Anthony et al. (2000) showed that free Leu administration (1.35 g/kg body weight) promoted greater increase in MPS rate and stimulation of mTOR signaling pathway in skeletal muscle of food-deprived rats. In addition, it has been shown that acute Leu supplementation attenuates muscle wasting in cancer cachexic mice (Peters et al. 2011) and restores

the postprandial stimulation of MPS in old rats (Dardevet et al. 2002). Taken together, the results of these studies suggest that Leu intake may be a favorable strategy to increase MPS in conditions in which there is severe protein deficit (e.g., food restriction) or reduced capacity of MPS (e.g., aging or cancer). However, Leu supplementation failed to increase the hypertrophic response in healthy young men after 8 weeks of RT (Coburn et al. 2006). Moreover, Katsanos et al. (2006) reported a maximization in rates of MPS in elderly subjects, but not young, after ingestion of Leu-enriched EAAs (41% Leu) (Katsanos et al. 2006), suggesting that anabolic effects of Leu supplementation may be dependent of capacity of prior protein synthesis—given that elderly present a blunted MPS response (i.e., anabolic resistance) to food intake (Rennie 2005; Cuthbertson et al. 2005; De Bandt 2016). Therefore, the lack of a significant effect of free Leu supplementation on muscle mass in our health subjects may be due to adequate dietary protein intake and maximal capacity of MPS, indicating that Leu supplementation may be necessary only for individuals with insufficient protein intake and limited capacity of MPS.

In conclusion, our data indicate that Leu supplementation alone does not increase skeletal muscle mass and strength during RT in untrained healthy young adult subjects who consume adequate habitual dietary protein intake. Further studies are required to determine whether Leu supplementation alone is capable of stimulating the anabolic signaling pathways in human skeletal muscle during long-term RT programs.

Compliance with ethical standards

Conflict of interest No conflicts of interest, financial or otherwise, are declared by the author(s).

Human rights statement This research involved human participants, who were carefully informed of the purpose, procedures, benefits, risks, and discomfort of the investigation, and signed an informed consent document approved by the Institutional Review Board of the University (Protocol No: 44487715.6.0000.0108–CAAE).

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