



LEEDS  
BECKETT  
UNIVERSITY

---

Citation:

Ispoglou, T and King, RF and Polman, RC and Zanker, C (2011) Daily L-leucine supplementation in novice trainees during a 12-week weight training program. International journal of sports physiology and performance, 6 (1). 38 - 50. ISSN 1555-0265

Link to Leeds Beckett Repository record:

<https://eprints.leedsbeckett.ac.uk/id/eprint/1131/>

Document Version:

Article (Updated Version)

---

The aim of the Leeds Beckett Repository is to provide open access to our research, as required by funder policies and permitted by publishers and copyright law.

The Leeds Beckett repository holds a wide range of publications, each of which has been checked for copyright and the relevant embargo period has been applied by the Research Services team.

We operate on a standard take-down policy. If you are the author or publisher of an output and you would like it removed from the repository, please [contact us](#) and we will investigate on a case-by-case basis.

Each thesis in the repository has been cleared where necessary by the author for third party copyright. If you would like a thesis to be removed from the repository or believe there is an issue with copyright, please contact us on [openaccess@leedsbeckett.ac.uk](mailto:openaccess@leedsbeckett.ac.uk) and we will investigate on a case-by-case basis.

## Daily L-Leucine Supplementation in novice trainees during a 12-Week Weight Training Program

Original investigation

Theocharis Ispoglou, Leeds Metropolitan University

Roderick F. G. J. King, Leeds Metropolitan University

Remco C.J. Polman, Victoria University, Melbourne

Cathy Zanker, Leeds Metropolitan University

**Corresponding Author:** Theocharis Ispoglou, Carnegie Faculty of Sport and Education, Leeds Metropolitan University, Headingley Campus, Leeds, Fairfax Hall, UK, LS6 3QS. Telephone: +44 1138124604. Fax: +44 1138127575. Email: [t.ispoglou@leedsmet.ac.uk](mailto:t.ispoglou@leedsmet.ac.uk).

**Short running head:** Leucine supplementation

Abstract word count: 254

Text-only word count: 3970

Figures numbered: 0

Tables numbered: 3

**Purpose:** To investigate the effects of daily oral L-leucine ingestion on strength, bone mineral-free lean tissue mass (LTM) and fat mass (FM) of free living humans during a 12-week resistance-training program. **Methods:** Twenty six initially untrained men ( $n = 13$  per group) ingested either  $4 \text{ g}\cdot\text{d}^{-1}$  of L-Leucine (leucine group: age  $28.5 \pm 8.2$  y, body mass index  $24.9 \pm 4.2 \text{ kg}\cdot\text{m}^{-2}$ ) or a corresponding amount of lactose (placebo group: age  $28.2 \pm 7.3$  y, body mass index  $24.9 \pm 4.2 \text{ kg}\cdot\text{m}^{-2}$ ). All participants trained under supervision twice per week following a prescribed resistance training program using eight standard exercise machines. Testing took place at baseline and at the end of the supplementation period. Strength on each exercise was assessed by five repetition maximum (5-RM), and body composition was assessed by dual energy X-ray absorptiometry (DXA). **Results:** The leucine group demonstrated significantly higher gains in total 5-RM strength (sum of 5-RM in eight exercises) and 5-RM strength in five out of the eight exercises ( $P < 0.05$ ). The percentage total 5-RM strength gains were  $40.8\% (\pm 7.8)$  and  $31.0\% (\pm 4.6)$  for the leucine and placebo groups respectively. Significant differences did not exist between groups in either total percentage LTM gains or total percentage FM losses (LTM:  $2.9\% \pm 2.5$  versus  $2.0\% \pm 2.1$ , FM:  $1.6\% \pm 15.6$  versus  $1.1\% \pm 7.6$ ). **Conclusion:** These results suggest that  $4 \text{ g}\cdot\text{d}^{-1}$  of L-leucine supplementation may be used as a

nutritional supplement to enhance strength performance during a 12-week resistance training program of initially untrained male participants.

**Key words:** Ergogenic aids, resistance training, muscle growth, oral ingestion.

## **Introduction**

Leucine is one of the essential amino acids that belongs to the group of branched-chain amino acids (BCAA) and may play a specific and pertinent role in the regulation of protein turnover. It has been demonstrated clearly that leucine may act as nutrient signal to stimulate protein synthesis in animal studies,<sup>1, 2</sup> thus having the potential to enhance anabolism and act as an ergogenic aid. In humans, intravenous infusion of leucine may decrease protein degradation at rest,<sup>3</sup> whilst oral ingestion of leucine and essential amino acids may increase muscle protein synthesis at rest in both young and elderly individuals.<sup>4</sup> Addition of leucine to a carbohydrate/protein supplement post-exercise has been shown to elicit greater responses in the rate of muscle protein synthesis in healthy male subjects than a carbohydrate and a carbohydrate/protein supplement following a resistance exercise bout.<sup>5</sup> However, others have suggested that co-ingestion of leucine and whey protein before exercise does not result in a greater anabolic response than whey protein alone.<sup>6</sup> In terms of sports performance, leucine supplementation has been shown to increase time to exhaustion and the upper body power of rowers during six weeks of supplementation<sup>7</sup> to a greater extent than a placebo condition, whilst co-ingestion of protein and leucine during eight weeks of resistance training has shown to elicit further gains in one repetition maximum strength (1-RM) than a placebo (carbohydrate) and a control condition.<sup>8</sup>

Bearing in mind the results of the aforementioned studies, it is probable that supplementation of leucine during resistance exercise training may be beneficial in terms of strength gains and muscle growth. Surprisingly, the capacity of orally ingested leucine alone to enhance strength and muscle mass in humans, whilst following an identical prescribed resistance-training program has not been tested. It has also been suggested that the recommended dietary intake of leucine of  $14 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{d}^{-1}$  (where BW = body weight) is inadequate in individuals who are physically active.<sup>9, 10</sup> At a protein intake of  $1.26 \text{ g}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{d}^{-1}$ , the serum concentrations of amino acids are lowered considerably during training of athletes.<sup>9</sup> Leucine supplementation of  $50 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{BW}\cdot\text{d}^{-1}$  appears to prevent the decrease in the serum leucine concentration during training.<sup>9</sup> Therefore, a dose of similar magnitude was utilised in the present study.

The potential of leucine to stimulate primarily protein synthesis provides the basis and justification to ingest leucine during resistance exercise training in order to enhance muscle growth and ultimately improve strength and personal performance. An increase in muscle size is generally accompanied by an increase in strength.<sup>11</sup> Strength gains may lead to improved performance in athletic disciplines and everyday life.<sup>12</sup> Gains in lean mass and losses in fat mass may

result in physiological adaptations that decrease the risk of diseases such as cardiovascular disease.<sup>13</sup> Therefore, the main aim of the present study is to investigate the capacity of 4 g·d<sup>-1</sup> oral ingestion of leucine alone to enhance strength, lean mass and decrease fat mass during a 12-week resistance-training program of initially untrained male participants. Our expectation was that supplementation with leucine would lead to further gains in muscle growth and consequently strength when compared to a placebo condition.

## **Methods**

### ***Participants***

Forty healthy males were recruited in stages. Participants who failed to follow the resistance training program and did not comply with our participation requirements described below were removed from the study. Thus, the required number of 13 participants per group (determined by power calculation using Machin's software version 2) was met. The volunteers were assigned to a placebo group (P) ( $28.2 \pm 7.3$  y;  $1.77 \pm 0.10$  m;  $78.4 \pm 16.8$  kg; BMI  $24.9 \pm 4.2$  kg·m<sup>-2</sup>) and a leucine supplemented group (L) ( $28.5 \pm 8.2$  y;  $1.76 \pm 0.05$  m;  $77.6 \pm 7.9$  kg; BMI  $24.6 \pm 3.2$  kg·m<sup>-2</sup>). Attempts were made to match pairs of participants as closely as possible on age and BMI. Medical history and lifestyle evaluation questionnaires were collected before the assignment of the participants to one of the two conditions. The participants had never taken part in any structured resistance training program or any other type of exercise training program in the past. Volunteers who had participated in any type of training the 6 months preceding the baseline measurements were also excluded. None of the participants had diabetes, high blood pressure, or symptomatic disease, including cardiovascular disease. All participants were free from medication and were not taking any other nutritional supplements during or before the intervention. The study was approved by the Leeds West Research Ethics Committee. All participants received information sheets and had the opportunity to ask questions about the potential benefits, risks and design of the study before informed consent was obtained.

### ***Experimental Design and Strength Training Protocol***

The design of the study was placebo-controlled and double blind. The participants agreed to take part exclusively in the assigned resistance training program. Both groups received identical prescribed resistance training over a period of 12 weeks. The participants were advised to maintain the same lifestyle and physical activity levels prior to the commencement of the experimental period. The standard machines used during the strength assessment and strength training sessions were as follows: Leg Press, Bench Press, Chest Cross, Pullover, Overhead Press, Preacher Curls, Triceps Press (All Nautilus, USA) and Prone Leg Curl (1-800-Nautilus Nitro, USA). All sessions took place in a University gym. The participants trained twice per week (with 2-3 rest days between sessions). According to the position stand of the American College of Sports Medicine (ACSM) the initial resistance for novice subjects should be in the range of 8-12 repetition maximum (RM) strength, and the training frequency 2-3 days per

week.<sup>14</sup> The duration of the training program was set at 12 weeks as muscle growth is more likely to become evident after 6-7 weeks of resistance training.<sup>15</sup>

Five repetition maximum (5-RM) testing took place at baseline, at weeks 4, 8 and 12 of the experimental period. The participants warmed-up using a cycle ergometer (Startrac Pro, USA) for a period of 5-min followed by 5-min of dynamic arm and leg movements combined with gentle stretching of arms and legs. A 5-min rest was given between each trial to allow for adequate recovery. 5-RM was employed for assessment of strength in order to minimise the risk of injury because all subjects were untrained at baseline, and to determine rather than estimate the training loads during the strength workouts.<sup>16</sup> Multiple RM testing between 3-RM to 6-RM has also been shown to be a valid index of assessment of strength.<sup>16</sup> In addition to measuring 5-RM strength in all 8 exercises, total 5-RM was determined by the sum of 5-RM strength on each individual exercise. One week before the first 5-RM testing all participants were familiarised with the training equipment using light resistance.

The training protocol combined two different types of workouts shown to increase strength and muscle size.<sup>17</sup> The hypertrophy workout involved 3 sets of 10 repetitions with 1-min rest between sets; the training loads were adjusted in order to allow the participants to perform just 10 repetitions during the last set. During the strength workouts the resistance used was the 5-RM achieved during the previous assessment; participants in this case performed 4 x 5-RM with 3-min recovery between sets. If for any reason participants were unable to complete 5 repetitions during their final set, resistance was adjusted in a subsequent session to allow them to perform 5 repetitions during the final set.

#### ***Assessment of Body composition***

Total and regional (arms, legs and trunk) body composition was assessed at baseline and at the end of the supplementation period by dual energy X-ray absorptiometry (DXA). A DXA scanner (Prodigy Lunar, GE Medical Systems, serial No: 10395, USA) was used for the assessment of bone mineral-free lean tissue mass (LTM) and fat mass (FM). The testing took place in the Centre for Bone and Body Composition Research, Leeds University. DXA has been validated against other methods and has shown little bias based on age, fat distribution, activity levels, gender or race, whilst at the same time is a more sensitive method for assessing small and regional changes in body composition.<sup>18, 19</sup>

#### ***Diet and Supplements***

The participants were asked to maintain their habitual diet throughout the experimental period and were asked to keep diet diaries for a period of 4 days at the midpoint of the study to include two training, one weekend and one week non-training days. The parameters assessed for dietary analysis were: carbohydrate, protein, fat and alcohol intake. The CompEat program 5.7 (CompEat version 5, Nutrition systems, Grantham, UK) was used for the analysis of diary records. The

resting energy expenditure (REE) was calculated using the Harris-Benedict equation<sup>20</sup> and the total energy requirements were estimated by multiplying REE with a factor of 1.35.<sup>21</sup>

Supplementation was continuous for the duration of the study. On non-training days participants were asked to take the supplements in 3 equal doses during the day (morning, midday, evening). On training days, the supplements were ingested immediately following exercise. The doses were 4 g·d<sup>-1</sup> (approximately 50 mg·kg<sup>-1</sup>·BW·d<sup>-1</sup>) for leucine (L-Leucine white crystalline powder, Ajinomoto Co. Inc., Tokyo, Japan) and a corresponding amount of lactose (99% lactose monohydrate, Dairy Crest Ingredients, Surrey, UK). The composition of the placebo was not revealed to the participants. The leucine dose (4 g·d<sup>-1</sup>) resulted from the average weight for British men of 77.2 kg (Allied Dunbar National Fitness Survey);<sup>22</sup> 50 mg·kg<sup>-1</sup>·BW·d<sup>-1</sup> seems to be an adequate quantity to prevent a decrease in serum leucine concentration during intensive training of athletes.<sup>9</sup> There was good reason to believe that post-exercise supplementation in addition to regular supplementation through the day would be more likely to enhance any anabolic effect of leucine than daily supplementation alone. This is because previous research has shown that ingestion of protein and amino acid mixtures immediately after (ideally within the first hour) exercise can create a more anabolic environment conducive to muscle hypertrophy.<sup>23</sup> Continuity of supplementation was also of significant importance as protein turnover may remain elevated for up to 48-h following the end of exercise.<sup>24</sup> We expected that ingestion of leucine at different time points during non-exercising days (in combination with ingestion of nutrients through their normal diet) would create a sustained positive or a more positive protein balance conducive to muscle hypertrophy throughout the day.

The volume of each drink was approximately 150 mL and consisted of 4 parts water, one part squash (Kia-Ora, Coca Cola Enterprises Ltd) and the daily supplement doses; the energy value excluding the supplements was approximately 16 kcal (11 kcal·100 mL<sup>-1</sup>). The squash was added in the drinks in an attempt to disguise the taste of the supplements and in particular the bitterness of leucine. The energy value of 4 g·d<sup>-1</sup> of lactose was 16 kcal (400 kcal·100 g<sup>-1</sup>), whilst the energy value of 4 g·d<sup>-1</sup> of leucine was 24.8 kcal (6.2 kcal·g<sup>-1</sup>). Powders were dispensed in plastic food bags that were sealed, placed in opaque envelopes, which were labelled as A, or B and then distributed to the participants. The participants were instructed to shake well the mix in the supplied beakers (black colour). As a result there were no residues of leucine or lactose left in the beakers. Furthermore, as the experimental groups were discreet groups, neither the participants in the leucine group nor the participants in the placebo group knew what the drink tasted like in the opposite group.

#### ***Ratings of Perceived Exertion and Seven Day Physical Activity Recall***

Ratings of perceived exertion (RPE) were recorded at the end of each exercise within each training bout to compute session RPE (Average of RPE in 8 exercises). The 6-20 Borg scale has been used in resistance training studies to

monitor exercise intensity<sup>25</sup> and has been found to be a very useful tool to distinguish between different training intensities. In addition to RPE, a seven-day physical activity recall (7-day PAR) interview took place at the end of the supplementation period. The purpose of the 7-day PAR, a reliable index in assessing activity levels<sup>26</sup> was to explore the activity levels of the two groups towards the end of the experimental period.

### ***Blood Samples***

Blood samples were drawn by venepuncture after a minimum of 10-h overnight fast at the beginning and at the end of the supplementation period to assess liver function (liver enzymes, bilirubin and albumin) and blood profile (standard full blood count). Subjects remained seated in a reclined position for 5-min before the sample was drawn from one of the brachial, medial cubital or radial veins. The samples were drawn in succession into a Gold 3.5-mL (13 x 75 mm) BD SST II Vacutainer (BD Vacutainer Systems Preanalytical Solutions, UK) and a purple EDTA 4-mL Vacuette (Vacuette, Greiner Bio-One, Austria). The samples were transferred to the department of Clinical Biochemistry and Immunology, Leeds General Infirmary) for subsequent analysis. No other tests were conducted on blood sampling days.

### ***Statistical analysis***

Data are presented as means ( $\pm$  standard deviations). Independent t-test were employed to test for significant differences between groups in percentage strength and percentage LTM and FM differences (the difference between the absolute values expressed as percentage of the baseline value). In addition, effect sizes were computed by using the method of Cohen. Independent t-test were also employed for analysing 7-Day PAR data and mean differences of blood data (post-exercise absolute values minus baseline absolute values). Comparison of RPE data (average for each training session) were made using a 2-Way ANOVA (condition\*training sessions) with repeated measures across the training period for both the hypertrophy and strength training workouts. The alpha level of statistical significance was set at  $P < 0.05$ . Data were analysed using the statistical package for the social sciences, version 16.0 (SPSS, Inc., Chicago, USA).

## **Results**

### ***Strength variables***

Table 1 provides the descriptive statistics at baseline and end of the supplementation period. The percentage gains from baseline until the end of the experimental period in 5-RM strength for both groups ranged between 19% and 60% with a gain of approximately 30-40% in the majority of exercises (Table 1). On average the percentage 5-RM strength gains for the L group were approximately 10% higher than the P group. Statistical analysis on the mean percentage gains across the 12-week experimental period showed that the L group had significantly greater percentage mean gains than the P group in 5 out of 8

exercises and total strength ( $P < 0.05$ ) (Table 1). The statistical analysis showed significant differences between groups for leg press ( $t_{24} = -2.79$ ;  $P = 0.010$ ), bench press ( $t_{24} = -2.45$ ;  $P = 0.02$ ), pullover ( $t_{24} = -2.33$ ;  $P = 0.03$ ), preacher curls ( $t_{24} = -3.17$ ;  $P = 0.004$ ), triceps press ( $t_{24} = -3.47$ ;  $P = 0.002$ ) and total strength ( $t_{24} = -4.06$ ;  $P < 0.001$ ). No significant differences between conditions were observed for leg curls ( $P = 0.19$ ), chest cross ( $P = 0.08$ ) and overhead press ( $P = 0.35$ ).

#### *Body Composition*

Table 2 reports the body composition variables at baseline and week 12. The mean gains in total bone mineral-free lean tissue mass (LTM) and losses in total fat mass (FM) were 1.08 kg ( $\pm 1.1$ ), 1.53 kg ( $\pm 1.3$ ) and 0.41 kg ( $\pm 1.4$ ), 0.93 kg ( $\pm 3.0$ ) on average for P and L groups respectively. Independent t-test showed that these percentage changes were not significantly different between groups (LTM:  $t_{24} = -0.94$ ;  $P = 0.36$  and FM:  $t_{24} = 0.10$ ;  $P = 0.92$ ). Similarly, significant differences did not exist between groups in LTM or FM of arm, leg and trunk regions (Table 2).

#### *Dietary intake*

The daily percentage contribution of different nutrients and alcohol to total energy as well as the total energy intakes were similar for both groups (Table 3). One participant from the P and 2 participants from the L group failed to submit their diet diaries. The P group met 98.2% ( $\pm 24.6$ ) and the L group 89.8% ( $\pm 18.2$ ) of the estimated energy requirements. The dietary grams of protein per kilogram of body weight were 0.88 g ( $\pm 0.28$ ) and 0.90 g ( $\pm 0.15$ ) for P and L groups respectively. There were no significant differences between the 2 groups in either absolute or percentage terms.



Table 1: Mean strength expressed as five repetition maximum strength (5-RM) at baseline and at the end of the supplementation period in 8 resistance exercises and total strength.

Exercises	Placebo (n = 13)			Leucine (n = 13)			Effect Size
	Baseline	Week 12	% Mean Gain	Baseline	Week 12	% Mean Gain	
Leg Press (kg)	171.1 ± 28.4	216.9 ± 35.1	27.0 ± 7.8	169.7 ± 26.3	232.3 ± 33.3	37.4 ± 11.0**	0.5
Bench Press (kg)	71.3 ± 12.0	93.6 ± 12.9	31.9 ± 8.7	71.5 ± 19.7	99.7 ± 22.4	41.5 ± 10.9*	0.4
Leg Curls (kg)	58.1 ± 12.4	68.3 ± 12.0	18.6 ± 7.5	58.2 ± 12.7	71.0 ± 12.5	23.0 ± 9.1	0.3
Chest Cross (kg)	56.5 ± 12.8	82.6 ± 14.4	48.2 ± 10.7	56.0 ± 17.4	87.2 ± 20.4	59.8 ± 19.9	0.3
Pullover (kg)	71.1 ± 14.3	89.1 ± 15.4	26.3 ± 7.7	68.6 ± 15.7	92.3 ± 19.3	35.4 ± 11.9*	0.4
Overhead Press (kg)	36.1 ± 6.9	53.0 ± 8.8	48.0 ± 14.7	39.3 ± 12.9	58.9 ± 14.8	53.8 ± 16.0	0.2
Preacher Curls (kg)	47.3 ± 9.0	60.4 ± 9.1	29.0 ± 9.0	46.6 ± 8.8	64.4 ± 9.9	39 ± 6.9**	0.5
Triceps press (kg)	75.3 ± 11.1	102.6 ± 12.4	36.9 ± 7.2	76.0 ± 17.1	113.6 ± 18.1	51.7 ± 13.6**	0.6
Total Strength (kg)	587.1 ± 89.6	766.6 ± 103.7	31.0 ± 4.6	586.0 ± 115.3	819.5 ± 129.3	40.8 ± 7.4**	0.6

\* Denotes significant difference in percentage gain between the placebo and Leucine condition (\* P < 0.05; \*\*P < 0.01).

Table 2: Bone mineral-free lean tissue mass (LTM), fat mass (FM) and soft tissue mass (STM = LTM + FM) by DXA at baseline and at the end of the experimental period.

Body composition Variables	Placebo (n=13)			Leucine (n=13)			Effect Size
	Baseline	Week 12	% Change	Baseline	Week 12	% Change	
Arm FM (kg)	1.6 ± 1.0	1.5 ± 1.0	-3.1 ± 12.7	1.8 ± 0.8	1.7 ± 0.6	-3.8 ± 12.2	0.02
Leg FM (kg)	6.8 ± 3.0	6.5 ± 2.8	-4.4 ± 7.1	7.1 ± 1.7	6.9 ± 1.3	-1.1 ± 13.8	0.1
Trunk FM (kg)	11.3 ± 6.2	11.2 ± 5.7	-1.3 ± 8.6	12.1 ± 4.9	11.5 ± 4.0	-1.5 ± 18.5	0.006
Arm LTM (kg)	6.6 ± 0.9	7.0 ± 0.9	6.1 ± 5.6	6.5 ± 0.9	7.0 ± 1.1	6.5 ± 3.4	0.04
Leg LTM (kg)	20.0 ± 2.7	20.4 ± 2.8	1.8 ± 2.5	19.6 ± 2.6	20.0 ± 2.4	1.9 ± 2.7	0.02
Trunk LTM (kg)	27.6 ± 5.0	27.9 ± 4.9	1.3 ± 3.1	25.9 ± 2.6	26.7 ± 2.9	2.9 ± 4.3	0.2
Total FM (Arm+Leg+Trunk) (kg)	19.7 ± 9.9	19.3 ± 9.4	-1.1 ± 7.7	21.0 ± 7.3	20.1 ± 5.6	-1.6 ± 15.6	0.02
Total LTM (Arm+Leg+Trunk) (kg)	54.2 ± 7.9	55.2 ± 8.0	2.0 ± 2.1	52.0 ± 5.6	53.6 ± 5.9	2.9 ± 2.5	0.2
Total STM	76.7 ± 16.7	77.4 ± 16.1	1.1 ± 2.2	75.8 ± 7.7	76.4 ± 6.6	1.0 ± 4.0	0.02

Table 3: Average daily dietary intakes over a period of 4 days (2 training days, 1 weekend day and 1 non-training weekday) in the middle of the training period. Total energy Intake (TEI).

<i>Dietary Variables</i>	<i>Placebo (n = 12)</i>	<i>Leucine (n = 11)</i>
Carbohydrate intake (kcal)	1180.7 ± 344.9	1012.2 ± 210.6
Protein intake (kcal)	393.7 ± 108.6	355.0 ± 67.2
Fat intake (kcal)	853.5 ± 215.3	718.2 ± 165.7
Alcohol intake (kcal)	96.6 ± 163.8	182.7 ± 290.2
Carbohydrate (% of TEI)	48.2 ± 7.4	46.3 ± 7.5
Protein (% of TEI)	16.0 ± 2.5	16.5 ± 3.5
Fat (% of TEI)	34.8 ± 4.9	33.2 ± 8.0
Alcohol (% of TEI)	4.0 ± 6.6	6.9 ± 10.1
TEI (kcal)	2450.6 ± 554.0	2204.8 ± 444.8

### *RPE*

Ratings of perceived exertion (RPE) during each hypertrophy workout (session RPE) ranged from 16.2 ( $\pm 1.9$ ) to 17.6 ( $\pm 1.2$ ) and 16.1 ( $\pm 1.9$ ) to 17.8 ( $\pm 1.3$ ) for P and L groups respectively. During the strength workouts session RPE ranged from 16.2 ( $\pm 1.4$ ) to 18.3 ( $\pm 0.8$ ) and 16.3 ( $\pm 1.6$ ) to 18.1 ( $\pm 1.1$ ) for P and L groups respectively. Significant differences did not exist between groups. There was neither a condition main effect ( $P = 0.499$  for hypertrophy and  $P = 0.865$  for strength workouts) nor an interaction main effect ( $P = 0.175$  for hypertrophy and  $P = 0.540$  for strength workouts).

### *Seven day physical activity recall*

The physical activity recall data showed no differences in the number or intensity in work related and/or other activity levels between both groups.

### *Blood*

All blood variables were within the normal range (as provided by the Department of Clinical Biochemistry and Immunology, Leeds General Infirmary). No significant differences between groups were found.

## **Discussion**

The resistance training program for the novice weight trainers employed in the current study was associated with positive adaptations in strength and LTM for both the P and L groups. The magnitude of gains for both groups was in the expected range of approximately 20-40% for moderately trained and untrained individuals.<sup>14</sup> Daily ingestion of leucine during 12 weeks of resistance training was accompanied by significantly greater gains in 5-RM strength in 5 out of 8 resistance exercises and 5-RM total strength compared to similar training with a placebo.

The gains observed in strength in both groups in the present study are most likely the result of the prescribed supervised resistance training program. Participants were clearly instructed to refrain from any lifestyle or activity modifications (other than the weight training sessions) during the experimental period. Hence, differences between the two conditions are likely to be attributed to the ingestion of leucine supplementation. The groups engaged in exactly the same type and volume of supervised resistance exercises and received the same training stimulus during these sessions as indexed by similar RPE ratings for both the hypertrophy and strength workouts. Also, participants did not differ in activity or exercise levels towards the end of the intervention program nor did they differ in their dietary intake. Of course, differences in genetics or muscle fibre distribution might result in inter-individual differences in progress. Because genotype has a bearing on the magnitude of gains in strength and/or FFM on its own right, recruitment of identical twins might be the best way to test the effectiveness of leucine as an ergogenic aid. However, the control mechanisms in place in the present study provide support for the notion that leucine supplementation might act as an ergogenic aid in novice weight trainers.

Our hypothesis that leucine supplementation would lead to greater gains in strength due to additional gains in muscle growth was supported by the strength data but only weakly supported by the LTM data (Table 2). The leucine group had slightly higher percentage gains and losses in total LTM and FM respectively than the P group but none of these differences were significant. However, the small effect size (Table 2) in trunk and total LTM suggests that a study with a longer duration and a higher training frequency may have resulted in significant differences between groups in LTM. The current study design was implemented to improve adherence to the study protocol. Despite the fact that participants were only required to engage in training twice per week only 26 out of 40 participants completed the required number of training sessions. This resulted in a drop-out of 35%. An aim of future studies would be to improve adherence to training programs.

The dietary intakes of the participants were under the estimated energy requirements but all within the 15% expected underestimation<sup>27</sup> when food diaries are used. Ingestion of extra amino acids through the diet, which may have favoured one of the groups in terms of muscle protein synthesis, was not the case since the protein intake per kilogram of body weight was identical in both groups. Thus, the most likely explanation of the marginally higher gains in LTM of the L group, supported by a small effect size in LTM and accompanied by significantly greater gains in 5-RM strength, was leucine supplementation. We acknowledge that four days of diet records may not be adequate to estimate actual intakes across the twelve weeks. However, it has been shown that as dietary record duration increases, the accuracy and recording declines.<sup>28</sup>

We can only speculate about the mechanisms of action since the data collected is not appropriate for this purpose. Co-ingestion of leucine and protein has shown to elicit a greater anabolic response in untrained individuals than carbohydrate alone or carbohydrate/protein,<sup>5</sup> whilst removal of leucine in the presence of all other amino acids can decrease protein synthesis by 40%.<sup>29</sup> Thus, it is probable that leucine supplementation further enhanced the rates of muscle protein synthesis in our L group when compared to diet alone and to some extent this is supported by the small effect size in LTM. Others have suggested that addition of leucine to a whey protein supplement before exercise does not result in greater anabolic responses than whey protein alone.<sup>6</sup> However, this needs to be further investigated since neither a whey alone nor a leucine alone group were included in their study. The role of amino acids, obtained from either endogenous or exogenous sources, in enhancing the rate of protein synthesis cannot be ignored; as in some conditions leucine enhances protein synthesis only in the presence of these other amino acids.<sup>30</sup> However, it is unlikely our participants were depleted of any amino acids since protein intake was adequate and similar in both groups and supplements were co-ingested with food during the non-exercising days. It is also unlikely the significant gains in 5-RM strength of L group was the result of amelioration of central fatigue since leucine ingestion elevates plasma leucine and BCAA

concentrations without affecting plasma free-tryptophan and the ratio of free-tryptophan to BCAA ratio.<sup>7</sup>

Although the dose used in the current study ( $4 \text{ g}\cdot\text{d}^{-1}$ ) seems to be an adequate amount to effect positive adaptations during 12 weeks of combined resistance training and supplementation in novice weight trainers, different dosages of leucine (higher or lower) should also be tested in future studies. Older individuals seem to require higher doses of leucine for stimulation of protein synthesis.<sup>4</sup> However, more evidence-based research to test the efficacy and safety of different dosages is needed, before prescription of higher dosages. In our study, there was no evidence to suggest that  $4 \text{ g}\cdot\text{d}^{-1}$  leucine ingestion for a period of up to 12 weeks during resistance training negatively affected the health of the male participants as indicated by tests of liver function and full blood counts.

### **Practical applications**

The additional strength gains in the L group support the use of leucine as a diet supplement to improve strength and ultimately performance in everyday life and athletic disciplines. In cases where supplementation is deemed necessary (e.g. high intensity, high volume exercise), supplementation could be considered and complement an appropriate diet. An investigation of special significance would be to test the hypothesis that leucine supplementation may be of benefit in diseases in which lean body mass is compromised by atrophy such as type II diabetes based on supporting evidence that leucine can attenuate body wasting.<sup>2</sup>

### **Conclusion**

In conclusion, the resistance training program employed in the current study increased strength of initially untrained male participants regardless of whether they ingested leucine or a placebo. However, those participants ingesting leucine had the greatest increase in strength. The data therefore suggest that regular ingestion of leucine while undertaking a resistance training program may accentuate strength gains. However, given the small effect size in LTM, more research is needed before any concrete conclusions can be made regarding the efficacy of leucine as an ergogenic aid.

### **Acknowledgements**

The research was funded by the Greek National Foundation Scholarships (IKY). Special thanks to the men who participated in this research study and the laboratory technician Brian Oldroyd (Centre for Bone and Body Composition, University of Leeds) for his assistance with the research.

## References

1. 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):2413-2419.
2. Gomes-Marcondes MC, Ventrucchi G, Toledo MT, Cury L, Cooper JC. A leucine-supplemented diet improved protein content of skeletal muscle in young tumor-bearing rats. *Braz J Med Biol Res.* 2003;36(11):1589-1594.
3. Nair KS, Schwartz RG, Welle S. Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am J Physiol.* 1992;263(5 Pt 1):E928-934.
4. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab.* 2006;291(2):E381-387.
5. Koopman R, Wagenmakers AJ, Manders RJ, et al. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *Am J Physiol Endocrinol Metab.* 2005;288(4):E645-653.
6. Tipton KD, Elliott TA, Ferrando AA, Aarsland AA, Wolfe RR. Stimulation of muscle anabolism by resistance exercise and ingestion of leucine plus protein. *Appl Physiol Nutr Metab.* 2009;34(2):151-161.
7. Crowe MJ, Weatherson JN, Bowden BF. Effects of dietary leucine supplementation on exercise performance. *Eur J Appl Physiol.* 2006;97(6): 664-672.
8. Coburn JW, Housh DJ, Housh TJ, et al. Effects of leucine and whey protein supplementation during eight weeks of unilateral resistance training. *J Strength Cond Res.* 2006;20(2):284-291.
9. Mero A, Pitkanen H, Oja SS, Komi PV, Pontinen P, Takala T. Leucine supplementation and serum amino acids, testosterone, cortisol and growth hormone in male power athletes during training. *J Sports Med Phys Fitness.* 1997;37(2):137-145.
10. Hood DA, Terjung RL. Amino acid metabolism during exercise and following endurance training. *Sports Med.* 1990;9(1):23-35.
11. Bruce SA, Phillips SK, Woledge RC. Interpreting the relation between force and cross-sectional area in human muscle. *Med Sci Sports Exerc.* 1997;29(5):677-683.
12. Stone MH, Fleck SJ, Triplett NT, Kraemer WJ. Health and performance-related potential of resistance training. *Sports Medicine.* 1991;11(4):210-231.
13. Hurley BF, Hagberg JM, Goldberg AP, et al. Resistive training can reduce coronary risk factors without altering VO<sub>2</sub>max or percent body fat. *Med Sci Sports Exerc.* 1988;20(2):150-154.

14. Kraemer WJ, Adams K, Cafarelli E, et al. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc.* 2002;34(2):364-380.
15. Phillips SM. Short-term training: when do repeated bouts of resistance exercise become training? *Can J Appl Physiol.* 2000;25(3):185-193.
16. Baechle TR, Earle RW, Wathen D. *Resistance training.* 2 ed: Human Kinetics; 2000.
17. Kraemer WJ, Dziados JE, Marchitelli LJ, et al. Effects of different heavy-resistance exercise protocols on plasma beta-endorphin concentrations. *J Appl Physiol.* 1993;74(1):450-459.
18. Houtkooper LB, Going SB, Sproul J, Blew RM, Lohman TG. Comparison of methods for assessing body-composition changes over 1 y in postmenopausal women. *Am J Clin Nutr.* 2000;72(2):401-406.
19. Lohman TG, Zhao C. Dual-Energy X-Ray Absorptiometry. In: Heymsfield SB, Lohman TG, Wang J, Going SB, eds. *Human Body Composition.* 2 ed. USA: Human Kinetics; 2005:63-77.
20. Lynch CJ, Halle B, Fujii H, et al. Potential role of leucine metabolism in the leucine-signaling pathway involving mTOR. *Am J Physiol Endocrinol Metab.* 2003;285(4):E854-863.
21. Douglas CC, Lawrence JC, Bush NC, Oster RA, Gower BA, Darnell BE. Ability of the Harris Benedict formula to predict energy requirements differs with weight history and ethnicity. *Nutr Res.* 2007;27(4):194-199.
22. *Allied Dunbar national fitness survey. Main findings.* [S.l.]: Allied Dunbar[in association with]Health Education Authority[and]Sports Council; 1992.
23. Levenhagen DK, Gresham JD, Carlson MG, Maron DJ, Borel MJ, Flakoll PJ. Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. *Am J Physiol Endocrinol Metab.* 2001;280(6):E982-993.
24. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol.* 1997;273(1):E99-107.
25. Gearhart RF, Jr., Goss FL, Lagally KM, et al. Ratings of perceived exertion in active muscle during high-intensity and low-intensity resistance exercise. *J Strength Cond Res.* 2002;16(1):87-91.
26. Hayden-Wade HA, Coleman KJ, Sallis JF, Armstrong C. Validation of the telephone and in-person interview versions of the 7-day PAR. *Med Sci Sports Exerc.* 2003;35(5):801-809.
27. Livingstone MB, Black AE. Markers of the validity of reported energy intake. *J Nutr.* 2003;133 Suppl 3:895S-920S.
28. Gersovitz M, Madden JP, Smiciklas-Wright H. Validity of the 24-hr dietary recall and seven-day record for group comparisons. *J Am Diet Assoc.* 1978;73(1):48-55.
29. Vary TC, Jefferson LS, Kimball SR. Amino acid-induced stimulation of translation initiation in rat skeletal muscle. *Am J Physiol.* 1999;277(6):E1077-1086



30. Zhang XJ, Chinkes DL, Wolfe RR. Leucine supplementation has an anabolic effect on proteins in rabbit skin wound and muscle. *J Nutr.* 2004;134(12):3313-3318.