Leucine supplementation during caloric restriction in adults at risk of metabolic syndrome: An 8 week double blind randomized controlled trial

Kaveri Pathak
Curtin University

Yun Zhao
Emily Calton

Anthony James
Curtin University  https://orcid.org/0000-0002-0873-3714

Philip Newsholme
Curtin university  https://orcid.org/0000-0002-0500-6984

Jillian Sherriff
Curtin University

Mario Soares (✉ m.soares@sjri.res.in)
Curtin University  https://orcid.org/0000-0001-6071-0272

Article

Keywords: leucine, weight loss, fat free mass, metabolic syndrome, branched chain amino acid

Posted Date: January 17th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2319322/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background: Leucine (Leu) supplementation per se could benefit fat-free mass (FFM)/function and improve glucose metabolism.

Objectives: To determine whether leucine supplementation during caloric restriction blunted the loss of FFM, increased the loss of fat mass (FM) and impacted glucose tolerance.

Design: Thirty-seven adults, aged 20-65 y with increased waist circumference (>80 cm for females and >94 cm for males) and at least another component of metabolic syndrome (MetS) were studied in a parallel, double blind randomized control trial (RCT). Participants were allocated randomly to either an intervention (leucine – 3 g/d) or placebo (lactose - 2.67 g/d) group, while following an individualised calorie-restricted diet over an 8-week period. Body composition (DEXA), oral glucose tolerance test (OGTT), insulin and components of MetS were measured before and after the trial. Analysis of covariance assessed the effect of the leucine intervention on an intention-to-treat (ITT) principle. Bootstrapping method with 1000 bootstrap samples was used to derive parameter estimates, standard errors, p values, and 95% confidence intervals for all outcomes.

Results: Adjusted for baseline values and other covariates, FFM (p=0.045) and lean tissue mass (LTM) (p=0.050) were significantly higher following Leu. These outcomes were modified by a significant treatment x gender interaction that indicated Leu had the greater effect in men. Adjusted for body composition changes, there were no differences in insulin sensitivity, oral glucose tolerance, or MetS components.

Conclusion: Short-term leucine supplementation resulted in a greater preservation of FFM and LTM particularly in men.

Introduction

Non-communicable chronic disease (NCD) continues to be biggest challenge to public health, despite progress in primary prevention and in medical therapy. In Australia, deaths due to cancer and cardiovascular diseases have more than doubled from 23% in 1915 to 58% in 2015 (1), and NCDs accounted for 71% of global deaths (2). The incidence and prevalence of these diseases is closely linked to obesity, and weight loss is one of the best approaches to reduce their burden (3). Loss of FFM during weight loss is inevitable but an undesirable outcome of both surgical and non-surgical (diet, exercise, medications) weight-loss interventions. The proportion of FFM lost depends on the degree of caloric restriction, protein content, exercise, pharmaceutical and surgical intervention (4). This may be critical since FFM is strong predictor of REE and may increase risk of weight gain (5) and reduced FFM is a strong independent predictor of increased insulin resistance, which also is a risk factor for several chronic diseases (6).
Leucine is one the three amino acids involved in stimulating protein synthesis and activation of mRNA via activation of nutrient signalling (7). Leucine has been shown to independently upregulate the muscle protein synthetic machinery by activating the mechanistic target of rapamycin complex 1 (mTORC1), which is involved in the translation initiation process of muscle protein synthesis (8, 9).

One- third of our minimum daily requirement for essential amino acids is met by the three branched chain amino acids (BCAA): leucine, isoleucine and valine (10). A group of experts in the area have projected that the leucine requirements may be up to four times greater than that suggested in the landmark 1985 WHO/FAO/UNU report (11). High protein diets preserve muscle mass during weight loss (12), and consumption of whey protein is particularly effective for increasing muscle protein synthesis (13).

Leucine is found in relatively high quantities in whey protein (approx. 10%) and accelerates protein anabolism, cell growth and metabolism (8, 14) where leucine acts as a substrate but also a “trigger” in the process of protein synthesis (15). It is postulated that anabolic competence of ingested amino acids in muscle protein synthesis is characterised by essential amino acids profiles, bioavailability and mechanisms by which they are delivered to muscle tissues (16). Leucine’s capacity to influence insulin secretion (17) and improve insulin sensitivity and reduce cardiovascular risks (18) has been documented. Several studies on leucine supplementation indicate an increase in muscle mass (19) especially appendicular muscle mass (20), increased resting energy expenditure and lower RQ indicating greater fat oxidation (21, 22). Leucine may also increase satiety with an additional impact on glucose metabolism (23, 24) and stimulate energy and lipid metabolism by regulating uncoupling protein-3 (UCP-3) expression in metabolically active tissues such as skeletal muscle, brown adipose tissue (BAT) and white adipose tissue (WAT) (21, 25). The primary objective of this trial was to explore if short term leucine supplementation during a calorie-restricted diet would enhance fat loss and preserve FFM/LTM in obese adults.

Methods

Participant selection

Thirty seven obese men and women of European origin (20–65 years) with abdominal obesity as assessed by waist circumference (> 94 cm for males and > 80 cm for females) and at least one additional criterion for MetS were recruited for this study (26). We excluded anyone with a history of myocardial infarction (MI), stroke, type 1 diabetes, polycystic ovarian syndrome and thyroid disease. Those who had any intentional weight loss in last 6 months, were pregnant or lactating, on any medication that was likely to affect body composition, energy expenditure or food intake or on hormonal contraceptives or replacement therapy were also excluded. Type 2 diabetics with good glucose control (HbA1C < 6.5%) were included for the study. Those on calcium or vitamin D supplements ceased intake two weeks prior to first measurement day. Other medications for lowering lipids, glucose and blood pressure were noted, and monitored throughout for any change.

Study design and randomisation
This was an eight-week two-arm parallel, double-blind RCT of leucine/placebo supplementation with energy restricted diets (75% of estimated energy requirements). Random allocation software (27) was used to allocate participants to either experimental [leucine-3 g/d] and control groups [lactose 2.67 g/d]. Treatment codes were broken only on study completion. The investigator (KP) who carried out the trial, other researchers involved and all participants were unaware of the treatment allocation.

**Capsule manufacture and coding**

Both capsules were identical in shape, size and colour to make them look identical. Capsules were manufactured by an external compounding pharmacy (Pharmacy 777, Applecross, Perth, WA). All capsules were then coded and allocated by an investigator who was not involved in either data collection or in analysis (JS). Codes were unscrambled at the end of trial prior to data analysis.

**Study diets**

Resting metabolic rates (RMR) were measured in each participant via indirect calorimetry (Deltatrac II Metabolic Monitor; Datex-Ohmeda, Instrumentarium Corp, Helsinki, Finland). Energy requirements were calculated for both leucine/placebo groups as 75% of measured RMR multiplied by an activity factor of 1.5 for men and 1.3 for women as participants avoided vigorous exercise during the trial. Meals plans were provided to each person and ranged from 1000 to 2800 kcal/day and followed NHMRC guidelines for the target age group (Supplementary Table S1). We however restricted dairy to 1 serve per day, meat no more than 3 serves per week for men and 2 serves per week for women This was necessary to maintain total dietary leucine intake to 3 g/d. Calorie-calculated recipes with standard measurements of ingredients were provided to each participant for consistency and controlling portion sizes. Each participants were required to consume 6 capsules each day, two each with breakfast, lunch and dinner. The participants recorded their capsule consumption daily and noted any missed doses. Compliance to the trial was decided on either > 85% capsule ingestion and/or between 1–5% weight loss at the end of each month of the 8-weeks trial week. If the weights were stable or increased for two consecutive fortnightly visits, diet counselling was implemented.

**Anthropometric measurements and blood chemistry**

Measurements were recorded for weight, waist circumference and body composition using DEXA (Prodigy, Lunar Corporation) for reporting regional adiposity measurements and BIA to track compliance to caloric restriction during the trial (In Body 3.0, Biospace, Seoul, South Korea, multi frequency Bioimpedence Analysis). All the above-mentioned measurements were performed at the start, each fortnightly visit and at the end of 8 weeks for each participant except for DEXA which was done only twice: at the start and end of 8 weeks. Fasting and 2-hour (OGTT- 75 g glucose) postprandial venous blood was collected by trained phlebotomists at the start of the experiment and at the end of 8 weeks, centrifuged and stored at – 80 °C before sending to a hospital laboratory for analysis using validated ELISA techniques.

**Intervention protocol**
The study was conducted by one investigator (KP), and to allow easier project management, the selected participants were split into two groups by studying them in two phases. Phase 1 \([n = 18: 9\text{ in placebo and } 9\text{ in the intervention group}]\) was completed at the end of one year well before the Christmas break, and Phase 2 \([n = 19]\) was started well after the following New Year holidays. Participants meeting inclusion criteria and willing to participate were invited for the orientation to the centre where they were informed about the study, including the tests/measurements needed to be performed, the equipment to be used, capsule consumptions and meal plans to be followed. Prior to their first measurement day all participants were asked to consume the dinner which confirmed to the meal plan provided by us during the trial for consistency. A date for baseline measurements and commencement of capsules was decided.

Participants attended the research centre following at least 10 hours of overnight fasting, 8 hours of sleep and abstinence from alcohol and vigorous exercise for at least 36 hours prior to the measurement. They were instructed to not shower in the morning. On arrival, their weight and waist circumference were measured after emptying their bladder and changing into a standard dressing gown. They were then asked to rest in bed for 30 min in the supine position, in a 25°C maintained insulated chamber. RMR was measured twice for 25 min each with a 10-minute rest over the next hour. We used the second measurement for our analysis. Fasting bloods were then drawn by trained phlebotomists for blood chemistry followed by ingestion of standard glucose drink (75 g). At the conclusion of two hours bloods were drawn again for postprandial measurements. Body composition using DEXA and BIA machines was measured at the end. All participants were offered beverages and light refreshments before they left the premises. All the participants also completed diet records and physical activity questionnaires on both measurement days (at start and after 8 weeks). This was to assure their compliance to diet and physical activity guidelines. Capsules sufficient for four weeks were provided on the first measurement day and refilled during their fortnightly visits. Dietary counselling was provided if poor compliance was identified during fortnightly visits. Additional phone calls were made to check diet and capsule compliance. All participants returned their capsule containers at the end of 8 weeks.

**Statistical Methods**

**Sample size calculations**

Sample size was calculated utilising GPower version 3.1.9.2 (28). Based on 2x2 repeated measures, to detect a small effect of 0.25 with a power of 80% at the 5% significance level and 0.5 correlation between measures, the total number of participant was estimated as 34 (17 for each group).

**Analysis**

All analyses were performed by using IBM SPSS Statistics for Windows (version 26 Armonk, NY: IBM Corp). Normality was assessed and a natural logarithm transformation was applied for skewed variables. To investigate the effect of leucine supplementation (Treatment) at 8 weeks on body composition and fasting metabolic parameters, we performed an analysis of covariance (ANCOVA) (via General Linear
Model (GLM) univariate procedure in SPSS), using the outcome variables at 8 weeks as the dependent variable, with an adjustment of the baseline values. In our analyses, age, gender, phase and caloric deficit, kJ/d were adjusted as confounders and two potential interaction effects between treatment and gender (treatment*gender), and between treatment and phase (treatment*phase), were assessed in the ANCOVA. As normality could not be achieved even after transformation for several outcome variables, bootstrapping method with 1000 bootstrap samples was used for deriving robust estimates of standard errors, regression coefficients and corresponding 95% confidence intervals. A p-value of less than 0.05 was accepted as being statistically significant at 5% level. In addition, a “Change” variable of each main outcome variable between the values at baseline and at 8 weeks was calculated and used in multivariable linear regression analysis for verifying the findings achieved via the ANCOVA (data not shown). Both analyses revealed similar direction and magnitude of the intervention effect, and hence only those based on the ANCOVA were reported in the paper.

Results

Out of 77 interested candidates 38 passed eligibility criteria for this study and were recruited and randomised for allocation to the experimental groups. However, one participant withdrew before start of the study due to work commitments. Fifteen participants from each group completed the study. Three participants in the intervention group and 4 participants from the placebo group were lost to follow-up due to either work commitments or inability to adhere to the diet regime. In this study, ITT methodology was used to include any participants missing final measurements but attended the trial for 4 weeks or more (Figure 1). The placebo group included 12 females (63.2 %) and 7 males (36.8%) and the intervention group consisted of 13 females (72.2%) and 5 males (27.8%). Baseline characteristics for the 37 total participants are presented in Table 1 and both groups were similar in all characteristics and metabolic measures.

In this paper we report the results based on the ANCOVA, in which the effect of treatment on the outcome variables post supplementation (at the end of 8 weeks) was assessed by adjusting for respective baseline values (and other confounders). Weight loss among Leu (94.0 ±21.43 vs 90.6 ± 22.32 kg) and placebo (92.5 ± 19.18 vs 88.21 ± 90.61 kg) groups were similar at the end of intervention period. For body composition parameters, there was a significant between-group difference in FFM following 8 weeks of weight loss (placebo: 51.99 ± 2.19 kg vs Leu: 52.95 ± 2.13 kg, p=0.045) and LTM trended towards significance (placebo: 49.24 ± 2.10 kg vs Leu: 50.17 ± 2.05 kg, p=0.050) (Table 2). There was a treatment x gender interaction for both variables (FFM: p=0.040; LTM: p=0.045), and the estimated marginal means showed that compared to their placebo counterparts, males of the LEU group had a higher FFM and LTM while females had similar FFM and LTM (Figure 2 and 3).

There was no marked improvement in insulin sensitivity (fasting and postprandial glucose and insulin, McAuleys ISI, Stumvoll fasting and postprandial), and MetS component post supplementation (Table 3). However, we observed a significant difference in postprandial insulin (p=0.025) and Stumvoll index(p=0.041) between the phases, respectively. There was also a significance interaction found
between treatment and phase for MetS component (p=0.037), and a further comparison found that the MetS component in the Leu group was lower by 0.628 in the first phase, while was higher by 0.459 in the second phase, on average compared to that in the placebo group, respectively.

Discussion

There is a close relationship of excessive adiposity to insulin resistance and hence MetS. It follows that weight loss per se would elicit significant improvements in insulin sensitivity (29). However loss of lean tissue mass is common during weight loss, and that would impinge on insulin sensitivity. Preserving lean mass during intentional weight loss is hence important (30). Supplementation with BCAA during caloric restriction, particularly leucine, may serve the purpose of LTM retention (20, 30). We are unaware of any published study that has addressed these issues in MetS, while exploring the changes in body composition and insulin sensitivity markers following leucine supplementation during caloric restriction.

A systematic review examined the loss in FFM with various weight loss strategies, such as caloric restriction, exercise or surgery (31). Linear regression analysis revealed very low calorie diets resulted in significant loss in % FFM ($r^2 = 0.31; P = 0.006$) compared to exercise and surgical interventions. The loss was greater among men (27 ± 7%) than women (20 ± 8%, $P = 0.08$) (4). A more recent study provided the range of expected loss of FFM to total decrease in weight as 35–40% for men and 30–35% for women (31).

The present study demonstrates that although both groups lost weight at the end of the intervention period, leucine supplementation dampened the drop in FFM and LTM associated with a calorie-restricted diet (Table 2). This is consistent with a recently published RCT that employed a much higher dose (10 g/d) of leucine (32). We however observed a gender bias in our outcomes where men retained more FFM and LTM following weight loss, than women (Tabl2 2, Fig. 2a & b). This is a novel finding, since to the best of our knowledge no trials have reported such findings. It appears that this gender effect on LTM was reflected in the non-appendicular tissue mass compartment, rather than the appendicular tissue mass (Table 2). The latter compartment is mainly skeletal muscle, while the former is comprised of all the organ tissue masses (and some residual skeletal muscle). Leucine acts on a variety of tissues (33) and while it is expected to stimulate protein synthesis in skeletal muscle, in organ tissues like the liver, it serves instead to dampen protein degradation (34). Under basal, weight stable conditions gender differences in protein turnover have been reported in individual studies, but a review of evidence negates the presence of a gender bias (35). In effect better control over subject selection, methodology employed (phenylalanine versus leucine trace), and accounting for relative body fatness in the analysis seemed necessary before a gender bias in protein turnover was accepted (35). Given the present weight loss scenario together with leucine supplementation, is difficult to explain the observations of preserved organ mass in men, seen here.

Other studies suggest that the extent of loss of FFM and FM with leucine supplementation may be dependent on many factors such as age, gender, health status of individuals, dosage and length of
supplementation and presence of energy restriction with and without exercise (4, 32, 36). One study on mice and another on elderly men and women with rheumatoid arthritis both suggest that gain in fat mass can be blunted with leucine supplementation (37). However, due to either small sample size, dose, variation in age of subjects or duration we did not notice any difference in FM between the groups (Table 3). Increasing age has a negative relationship with the ability to utilise protein consumed via regular meals (38) and further increases protein requirements among elderly (39). In an acute supplementation study, the authors claimed that leucine supplementation improved muscle protein syntheses (FSR rate) in elderly males (40) which is similar to another study that included oral supplementation of leucine with meals for 2 weeks (41). Surprisingly, both studies observed no alterations in either body composition or insulin responses. This is in alignment to another animal study (42) and those with elderly men ((71 ± 4 y)) with prolonged supplementation (7.5 g /d) for 24 weeks (43). This did not change whether on habitual diets or energy restriction. From this we may interpret that increase in muscle protein synthesis may not always get translated into increased fat free mass since the rates of protein synthesis and degradation are finely coupled.

**Leucine and insulin sensitivity**

FFM is metabolically active tissue and has been linked with insulin-stimulated glucose uptake in central and peripheral tissues (44). Circulating insulin has the capacity to enhance protein synthesis (45). Some studies show positive association between FFM and insulin sensitivity in older adults (46, 47) and following calorie-restricted weight loss (48). Overall, whilst there are animal studies to suggest leucine supplementation may contribute to improved insulin sensitivity (21), evidence in humans is lacking (36). In this study we did not observe any improvement in glucose tolerance or surrogate markers of insulin sensitivity after controlling for changes in body composition (Table 3). There is the possibility of multiple mechanisms being involved in leucine's improvement of glucose metabolism. One of them is the potential of a combined action of leucine and its metabolites - α-ketoisocaproate (α-KIC) and β-hydroxy-β-methylbutyrate (HMB)- that are formed in skeletal muscle, on increasing protein synthesis and regulating glucose homeostasis Interestingly, it has also been observed that improved BCAA intake augments levels of plasma BCAA which are inversely related to insulin sensitivity(49). On the other hand, weight loss induces insulin sensitivity that reduces proteolysis and thereby decreases plasma BCAA (50). Hence, the debate of improved insulin sensitivity and high protein/BCAA diets remains equivocal, and further investigation is required.

**Strengths & Limitations**

There are limitations to this RCT. The short duration of 8 weeks may not have allowed the full impact of leucine on body composition, particularly body fat, as well as the detection of ongoing changes in insulin sensitivity markers. We did not purposefully select equal number of men and women. The gender bias observed needs replication as the sample size of men was small. The strengths of the investigation are a very strong trial design where both participants and investigators were blinded to the intervention, testing
of compliance to all aspects of intervention every fortnight, an individualized weight loss strategy, provision of sample menus and telephone contact to assist adherence to study requirements.

Conclusions

Leucine supplementation during weight loss may prevent the loss of FFM and LTM in those at risk of MetS, but this effect was restricted to males. There was no improvement in glucose tolerance or insulin sensitivity on accounting for the changes in body composition.

Declarations

Acknowledgments: MJS acknowledges infrastructure support from the School of Population Health. The authors thank the participants for their time and involvement in the trial

Author Contribution Statement: MJS, JS and PN conceived the idea and obtained funding. MJS, PN, JS, KP, TJ & EC planned the intervention. KP conducted the study and collated the results, prepared first draft of the manuscript including tables. EC assisted KP in data collection. JS directed the blinding of trial. YZ conducted the statistical analysis and prepared figures. KP, MJS, YZ, PN, EC, TJ & JS finalised the manuscript for submission. All authors read and agreed the final submitted manuscript.

Data Availability Statement: The raw data used in this paper is freely available for non-commercial purposes only. The institution’s human ethics committee must endorse the formal request, and researchers may contact Mario Soares m.soares@curtin.edu.au in the first instance.

Funding: The study was partly funded by a seed grant from the School of Population Health, Curtin University, Bentley Campus.

Ethical Approval: The study was approved by Curtin University Human Research Ethics Committee (HR 108/2013). The trial was registered with Australian New Zealand Clinical Trials Registry (ACTRN 12616001528448). Informed consent was collected from all participants who agreed to participate in the study.

Competing Interests: MJS is the Editor- in Chief of the EJCN. All other authors declare that there are no competing financial or other conflicts of interests in relation to this study.

References


46. Myette-Côté É, Doucet É, Prud'homme D, Rabasa-Lhoret R, Lavoie J-M, Brochu M. Changes in glucose disposal after a caloric restriction-induced weight loss program in obese postmenopausal women:


Tables

Tables 1-3 is available in the Supplementary Files section.

Figures
Figure 1

CONSORT Flow Diagram - Recruitment, allocation and intervention
Figure 2

Estimated marginal mean (with 95% CI) of fat free mass (FFM) at the end of 8 weeks trial for leucine and placebo groups by gender

Estimated marginal mean (with 95% CI) of lean mass at the end of 8 weeks trial for leucine and placebo groups by gender
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- Table2.xlsx
- Table3.xlsx