

## Systematic Review with Meta-Analysis

# The effectiveness of leucine on muscle protein synthesis, lean body mass and leg lean mass accretion in older people: a systematic review and meta-analysis

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(Submitted 11 February 2014 – Final revision received 18 July 2014 – Accepted 22 July 2014 – First published online 19 September 2014)

### Abstract

In the present study, we performed a meta-analysis to assess the ability of leucine supplementation to increase the muscle protein fraction synthetic rate and to augment lean body mass or leg lean mass in elderly patients. A literature search was conducted on Medline, Cochrane, EMBASE and Google Scholar databases up to 31 December 2013 for clinical trials that investigated the administration of leucine as a nutrient that affects muscle protein metabolism and muscle mass in elderly subjects. The included studies were randomised controlled trials. The primary outcome for the meta-analysis was the protein fractional synthetic rate. Secondary outcomes included lean body mass and leg lean mass. A total of nine studies were included in the meta-analysis. The results showed that the muscle protein fractional synthetic rate after intervention significantly increased in the leucine group compared with the control group (pooled standardised difference in mean changes 1.08, 95% CI 0.50, 1.67;  $P < 0.001$ ). No difference was found between the groups in relation to lean body mass (pooled standardised difference in mean changes 0.18, 95% CI  $-0.18$ , 0.54;  $P = 0.318$ ) or leg lean mass (pooled standardised difference in mean changes 0.006, 95% CI  $-0.32$ , 0.44;  $P = 0.756$ ). These findings suggest that leucine supplementation is useful to address the age-related decline in muscle mass in elderly individuals, as it increases the muscle protein fractional synthetic rate.

**Key words:** Essential amino acids: Elderly: Dietary supplements: Sarcopenia

Ageing is accompanied by a progressive decline in muscle mass and strength (sarcopenia) and is associated with a lower quality of life due to the reduced ability of an individual to perform daily living activities<sup>(1)</sup>. It also predisposes people to the development of chronic metabolic disorders such as diabetes and obesity<sup>(2)</sup>. The prevalence of sarcopenia differs by sex and living settings<sup>(3)</sup>. For example, age-related muscle loss has been reported to be prevalent in about 68% of elderly men and 21% of elderly women living in nursing homes<sup>(4,5)</sup>, but in about 10% of men and 33% of women living in the community<sup>(4,6)</sup>. Sarcopenia results in increased health care costs of approximately \$18.5 billion per year in the USA<sup>(6)</sup>.

Age-related muscle loss can result from a variety of modifiable factors including inadequate nutrition, oxidative

stress, low physical activity levels, inflammation and reduced hormone concentrations<sup>(7)</sup>. Studies have suggested that muscles of the elderly may have a blunted protein synthesis response to food ingestion<sup>(8–10)</sup>. A number of strategies to increase muscle mass in the elderly have been studied including different nutritional intervention strategies and physical exercise strategies<sup>(11–16)</sup>; however, the findings have been conflicting<sup>(11–24)</sup>.

The administration of dietary leucine increases muscle protein synthesis *in vivo* and in rodents<sup>(25)</sup>. It has been suggested that increasing leucine intake in the elderly may compensate for the blunted muscle protein synthesis response to food ingestion<sup>(9,26)</sup>. Several studies have found that increasing the amount of leucine in meals or in supplemental amino acid mixes increased the muscle protein synthesis response in the

**Abbreviation:** EAA, essential amino acids.

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elderly<sup>(9,26)</sup>. In addition, essential amino acids (EAA) and leucine supplementation (sometimes given as whey protein) have increased protein synthesis in muscles, and are considered as better strategies for offsetting muscle loss than intact protein<sup>(12,17,25,27,28)</sup>. In contrast, other studies did not find any association of increased ingestion of leucine with elevated muscle protein fraction synthetic rate, muscle mass or strength in the elderly<sup>(13,15,23)</sup>. A limited number of studies have evaluated the effect of acute and chronic leucine supplementation on lean body mass and/or leg lean mass in elderly populations, and, overall, there have been inconsistent findings of whether leucine supplementation increases these outcomes<sup>(29)</sup>.

Many of the studies evaluating the impact of leucine as a pharmacconutrient on age-related muscle loss have been small. To maximise the biostatistical power of controlled clinical trials, we performed a meta-analysis to assess the ability of leucine supplementation to increase muscle protein fraction synthetic rate, augment lean body mass or leg lean mass in elderly subjects.

## Methods

Medline, Cochrane, EMBASE and Google Scholar databases were searched up to 31 December 2013 for clinical trials that investigated the administration of leucine as a nutrient that affects muscle protein metabolism and lean body mass and leg lean mass in elderly subjects. Searches were conducted using the following terms: elderly; elder; older; aging; aged; geriatric; leucine; muscle; muscular; randomized. Randomised controlled trials in which the majority of subjects were elderly (age  $\geq 65$  years) and that investigated the efficacy of a clearly defined level of leucine were included in the meta-analysis. Included studies were published in English. Excluded studies were non-randomised controlled trials, letters, comments, editorials and case reports. Potential relevant studies were screened by two independent reviewers, and both had to agree on study inclusion. Any disagreement between the reviewers was resolved by a third reviewer.

## Data extraction

The following information was extracted from the studies that met the inclusion criteria: the name of the first author; year of publication; study design; demographics; leucine dosing regimen; exercise programme; muscle protein fractional synthetic rate; lean body mass; leg lean mass. Data were extracted by two independent reviewers, and a third reviewer was consulted if there were any uncertainties.

## Quality assessment and publication bias

The quality of the studies was evaluated using the Cochrane Risk of Bias Tool to assess the included studies<sup>(30)</sup>. Quality assessment was also performed by two independent reviewers, and a third reviewer was consulted for any ambiguities. Due to the small number of selected studies, it was inappropriate to use the funnel plot for the assessment of

publication bias. Therefore, five or fewer studies are not sufficient to detect funnel plot asymmetry<sup>(31)</sup>.

## Statistical analyses

The primary outcome for the meta-analysis was the protein fractional synthetic rate. Secondary outcomes included lean body mass and leg lean mass. Means and standard deviations or standard errors of means were used to summarise the outcomes before and after the intervention, and the change from baseline was used to evaluate the intervention effect. The standardised difference in mean changes with 95% CI for subjects treated with leucine supplements (leucine group) compared with those treated with placebo or other nutritional supplements (control group) was calculated for each study. Heterogeneity among the studies was assessed by calculating Cochran's  $Q$  and the  $I^2$  statistic<sup>(30,32)</sup>. For the  $Q$  statistic,  $P < 0.10$  was considered to indicate statistically significant heterogeneity. The  $I^2$  statistic indicates the percentage of the observed between-study variability caused by heterogeneity. Heterogeneity determined using the  $I^2$  statistic was defined as follows: 0–24%, no heterogeneity; 25–49%, moderate heterogeneity; 50–74%, large heterogeneity; 75–100%, extreme heterogeneity. If heterogeneity existed between studies (a  $Q$  statistic with  $P < 0.1$  or an  $I^2$  statistic  $> 50\%$ ), we performed the random-effects model (DerSimonian–Laird method)<sup>(33)</sup>. Otherwise, the fixed-effects model was used (Mantel–Haenszel method). The pooled standardised difference in mean changes was calculated, and a two-sided  $P$  value  $< 0.05$  was considered to indicate statistical significance. Sensitivity analysis was performed for all three outcomes based on the leave-one-out approach. All statistical analyses were performed using the statistical software Comprehensive Meta-Analysis, version 2.0 (Biostat).

## Results

Of the 525 studies identified, 492 were excluded and thirty-three underwent a full-text review. Of these, twenty-four

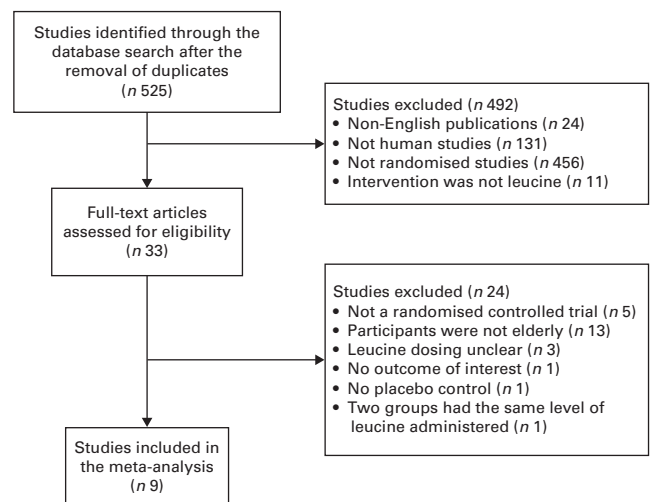


Fig. 1. Flow chart for study selection.

were eliminated because they were not randomised controlled trials ( $n = 5$ ), the participants were not elderly ( $n = 13$ ), the amount of leucine administered was not clear ( $n = 3$ ), the outcomes were not the ones being investigated in the present analysis ( $n = 1$ ), there was no placebo control ( $n = 1$ ) or treatment groups were given the same amount of leucine ( $n = 1$ ) (Fig. 1; for the details of the excluded studies, see the online supplementary material). Finally, nine studies met the inclusion criteria<sup>(9,13,14,15,17,23,24,34,35)</sup>.

**Quality assessment**

There was a low risk of data bias for the combination of the studies (Fig. 2) and for each individual study (Fig. 3), indicating that the data were of high quality.

**Study characteristics**

Among the nine included studies, six were randomised controlled trials with parallel treatment arms<sup>(9,13,15,17,35)</sup> and three were randomised cross-over trials<sup>(23,24,34)</sup> (Table 1). The total number of patients in the studies ranged from eight to fifty-seven, and the duration of intervention ranged from hours to 6 months (Table 1). Of these studies, four<sup>(9,23,24,35)</sup> investigated the acute effect of leucine and administered leucine only once. The other five studies<sup>(13,15,17,34)</sup> administered leucine as a long-term supplement with the length of intervention ranging from 10 d<sup>(14)</sup> to 6 months<sup>(15)</sup> (Table 1). Across the studies, the amount of leucine given for long-term supplementation ranged from 2.8 to 16.1 g/d, and for acute administration, it ranged from 2.6 to 17.6 g/d (Table 1). Among these studies, two<sup>(23,24)</sup> included exercise as part of the intervention (Table 1). In five of the studies<sup>(13,15,23,24,35)</sup>, all the subjects were male and five studies<sup>(9,13,17,23,24)</sup> included only healthy (or healthy and lean) subjects (Table 1).

All of the included studies utilised a stable isotope infusion test to assess the muscle protein fractional synthetic rate from the mixed skeletal muscle protein (Table 2) and evaluated body composition by using dual-energy X-ray absorptiometry. The studies used the same measure of muscle protein fractional synthetic rate (%/h) and supplements were administered orally. In two studies that compared EAA with placebo,

the muscle protein fractional synthetic rate was approximately 0.07 before long-term supplementation both for placebo and EAA, but decreased for placebo after 10 d to 3 months of treatments (Fig. 2)<sup>(14,17)</sup>. There was little effect of long-term supplementation on lean body mass or leg lean mass (Table 2)<sup>(13,14,17,25)</sup>. In the four studies that used acute leucine administration, three studies showed a greater increase in muscle protein fractional synthetic rate from baseline with leucine supplementation than the control (Table 2)<sup>(24,35,36)</sup>.

**Muscle protein fractional synthetic rate**

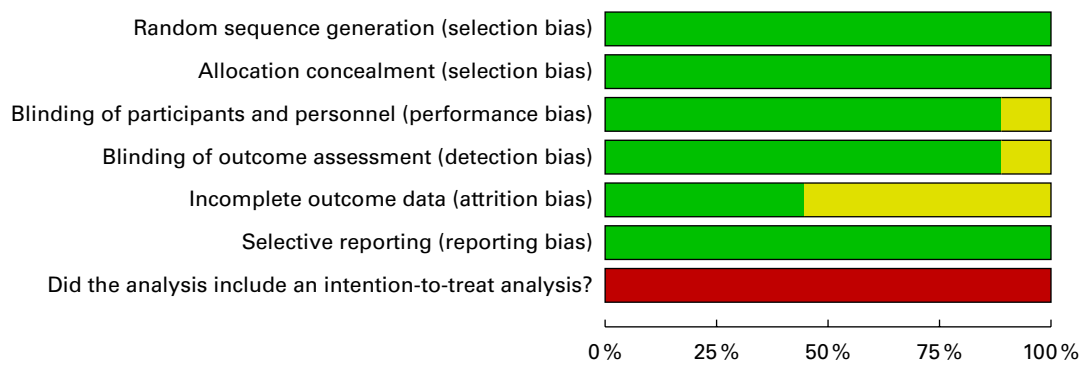
Of the studies included in the meta-analysis, four assessed the effect of leucine on the fractional synthetic rate of muscle protein: three reported values before and after intervention<sup>(9,14,17)</sup> and one reported the fractional synthetic rate as the change from baseline<sup>(35)</sup> (Table 2). After pooling of data, there was no significant heterogeneity found across the studies (heterogeneity test:  $Q = 4.36$ ,  $df = 3$ ,  $P = 0.225$ ;  $I^2 = 31.16\%$ ); therefore, a fixed-effects model of analysis was used. The results indicated that the muscle protein fractional synthetic rate after intervention significantly increased in the leucine group compared with the control group (pooled standardised difference in mean changes 1.08, 95% CI 0.50, 1.67;  $P < 0.001$ ; Fig. 4).

**Lean body mass**

For the analysis of lean body mass, we included the four studies that reported lean body mass values both before and after leucine administration<sup>(13-15,17)</sup>. There was no significant heterogeneity found among the studies (heterogeneity test:  $Q = 2.37$ ,  $df = 3$ ,  $P = 0.499$ ;  $I^2 = 0.0\%$ ); therefore, a fixed-effects model of analysis was used. The results showed that the change in lean body mass after intervention did not significantly differ between the leucine group and the control group (pooled standardised difference in mean changes 0.18, 95% CI -0.18, 0.54;  $P = 0.318$ ; Fig. 5).

**Leg lean mass**

Of the studies included in the meta-analysis, three<sup>(13-15)</sup> reported leg lean mass findings from both before and after



**Fig. 2.** Risk of bias for the included studies. ■, Low risk of bias; ■, unclear risk of bias; ■, high risk of bias. A colour version of this figure can be found online at <http://www.journals.cambridge.org/bjn>

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Did the analysis include an intention-to-treat analysis?
Björkman (2011) <sup>(34)</sup>	+	+	+	+	+	?	-
Deutz (2011) <sup>(35)</sup>	+	+	+	+	+	+	-
Dillon (2009) <sup>(17)</sup>	+	+	+	+	+	?	-
Ferrando (2010) <sup>(14)</sup>	+	+	+	+	+	+	-
Katsanos (2006) <sup>(9)</sup>	+	+	+	?	?	?	-
Koopman (2006) <sup>(24)</sup>	+	+	+	+	+	?	-
Koopman (2008) <sup>(23)</sup>	+	+	+	+	+	+	-
Leenders (2011) <sup>(15)</sup>	+	+	+	+	+	?	-
Verhoeven (2009) <sup>(13)</sup>	+	+	+	+	+	+	-

**Fig. 3.** Summary of risk of bias for the included studies. Green, low risk of bias; yellow, unclear risk of bias; red, high risk of bias. A colour version of this figure can be found online at <http://www.journals.cambridge.org/bjn>

intervention. There was no significant heterogeneity found across the studies (heterogeneity test:  $Q = 0.59$ ,  $df = 2$ ,  $P = 0.752$ ;  $I^2 = 0.0\%$ ); consequently, a fixed-effects model was used. There was no significant difference in change in leg lean mass after intervention between the subjects treated with leucine or placebo (pooled standardised difference in mean changes  $0.006$ ,  $95\%$  CI  $-0.32, 0.44$ ;  $P = 0.756$ ; Fig. 6).

**Sensitivity analysis**

Sensitivity analysis was performed, in which the results were analysed when one study was removed in turn. The direction and magnitude of the pooled estimates for muscle protein fractional synthesis rate (Fig. 7(a)) and lean body mass and leg lean mass (Fig. 7(b) and (c)) did not vary substantially with the removal of any study from the analysis, indicating that one study did not influence the findings.

**Discussion**

The age-associated loss of skeletal muscle mass is an important factor in the loss of functional performance and the ability to maintain a healthy lifestyle in the elderly<sup>(25)</sup>. Sarcopenia is influenced by a combination of factors including poor diet and sedentary lifestyle<sup>(37)</sup>. In one study<sup>(9)</sup>, it was found that increasing the leucine content in an amino acid mixture (from 26 to 41%) could compensate for the blunted response to amino acid ingestion in the elderly, raising the idea that addition of leucine may be an effective strategy to normalise the postprandial response of muscle protein synthesis in the elderly. The present meta-analysis investigated whether leucine is an effective pharmaconutrient that could influence muscle protein fractional synthesis rates, lean body mass and leg lean mass in elderly subjects. We found that the addition of leucine increased the protein fractional synthesis rate compared with the control. Higher levels of leucine did not significantly affect lean body mass or leg lean mass even after long-term supplementation.

Leucine can have an impact on muscle mass in several ways including being a building block for protein synthesis, and also as a nutritional signal that acts via mTOR (mammalian target of rapamycin) in an insulin-dependent and -independent signalling cascade. The mTOR signalling pathway stimulates translation and protein synthesis by the phosphorylation of the translation initiation factor 4E-BP1 (eukaryotic translation initiation factor 4E-binding protein 1)<sup>(25)</sup>. Rodent studies indicated that the EAA leucine may represent an effective pharmaconutrient with the largest anabolic properties<sup>(25)</sup>, and leucine concentrations are thought to be the primary stimulus driving the postprandial response to muscle protein synthesis<sup>(38)</sup>.

The effect of leucine on muscle protein metabolism is complex, as it influences both muscle protein synthesis and degradation, and this complexity may confound study findings. A prior study showed that intravenous infusion of leucine ( $0.14\text{ g/kg}$  body weight over a 7 h period) in healthy subjects resulted in decreased protein degradation by about  $35\%$ <sup>(39)</sup>. Several studies have shown a significant increase ( $35\text{--}50\%$ ) in the rates of muscle protein synthesis in healthy males with intravenous administration of amino acids, with leucine being an important component<sup>(40–42)</sup>. However, other studies have not detected an effect of increased leucine administration on muscle protein synthesis<sup>(23,43)</sup>. The discrepancy among these studies may be due to the differences in the amount of leucine administered, the time of administration and the population studied. Of the studies included in the present meta-analysis, both the levels of leucine and the duration of dosing differed. For example, five studies had long-term dosing ( $>10\text{ d}$ ) and four had short-term dosing ( $\leq 8\text{ h}$ ). However, one of the studies with short dosing time found an improvement in the muscle protein fractional synthesis rate<sup>(9)</sup>, suggesting that the length of dosing is not the only factor influencing the results. Instead, the study showed that increasing the proportion of leucine in a mixture of EAA may increase the muscle protein fractional synthesis rate<sup>(36)</sup>.

There are several reasons for the observed increase in muscle protein synthesis but not in lean muscle mass or leg

**Table 1.** Characteristics of the studies included in the meta-analysis

Author (year)	Study type	Condition or diagnosis of elderly people	Supplement comparison	Leucine content	Length of the intervention	Exercise programme	Cases (n)	Mean age (years)	Male (%)
<b>Long-term leucine supplementation</b>									
Björkman (2011) <sup>(34)</sup>	RCT cross-over trial	Polymyalgia rheumatica	Test supplement v. control supplement	Difference between two groups: 2.3 g/d	2 months	Low-intensity home-based exercise	23 v. 24	69.9 v. 69.1	13 v. 8.3
Leenders (2011) <sup>(15)</sup>	RCT	Type 2 diabetes	Leucine v. placebo	7.5 v. 0 g/d	6 months	No	29 v. 28	71 v. 71	100 v. 100
Ferrando (2010) <sup>(14)</sup>	RCT	Bed rest	EAA v. placebo	16.146 v. 0 g/d	10 d	No	10 v. 11	71 v. 68	10 v. 54.5
Dillon (2009) <sup>(17)</sup>	RCT	Healthy	EAA v. placebo	2.78 v. 0 g/d	3 months	No	7 v. 7	67 v. 69	0 v. 0
Verhoeven (2009) <sup>(13)</sup>	RCT	Healthy	Leucine v. placebo	7.5 v. 0 g/d	3 months	No	15 v. 14	All 71	100 v. 100
<b>Acute leucine administration</b>									
Deutz (2011) <sup>(35)</sup>	RCT	Cancer	Experimental medical food v. conventional medical food	7.8 v. 2 g	Once	No	12 v. 12	68.8 v. 66.6	100 v. 100
Koopman (2008) <sup>(23)</sup>	Randomised cross-over trial	Healthy and lean	CHO + PRO + leucine v. CHO + PRO	17.6 v. 4.7 g	Once	30 min, moderate intensity	8	73	100 v. 100
Katsanos (2006) <sup>(9)</sup>	RCT	Healthy	41% leucine v. 26% leucine	2.8 v. 1.7 g	Once	No	10 v. 10	66.5 v. 66.7	50 v. 70
Koopman (2006) <sup>(24)</sup>	Randomised cross-over trial	Healthy and lean	CHO + PRO + leucine v. CHO	17.6 v. 0 g	Once	30 min, moderate intensity	8	75	100 v. 100

RCT, randomised controlled trial; EAA, essential amino acids; CHO, carbohydrate; PRO, protein hydrolysate.

Effect of leucine on muscle protein in elderly

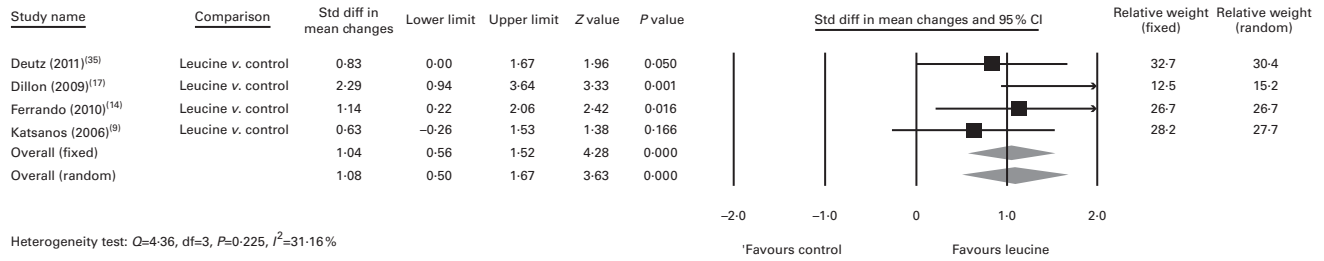
**Table 2.** Primary and secondary outcomes from the studies included in the meta-analysis (Mean values and standard deviations or standard errors)

Author (year)	Supplement comparison	Time point for assessing muscle protein fractional synthetic rate	Precursors of the stable isotope infusion test	Muscle protein fractional synthetic rate (%/h)		Time point for assessing body composition	Lean body mass (fat-free mass) (kg)		Leg lean mass (kg)	
				Before	After		Before	After	Before	After
Long-term leucine supplementation										
Björkman (2011) <sup>(34)</sup>	Test supplement v. control supplement	NA	NA	NA	NA	5 months	NA	NA	NA	NA
Leenders (2011) <sup>(15)</sup>	Leucine v. placebo	NA	NA	NA	NA	6 months	61.9 (SEM 1.1) v. 62.2 (SEM 1.3)	62 (SEM 1) v. 62.2 (SEM 1.3)	19 (SEM 0.4) v. 19.3 (SEM 0.5)	19 (SEM 0.4) v. 19.4 (SEM 0.4)
Ferrando (2010) <sup>(14)</sup>	EAA v. placebo	10 d	L-[ring- <sup>13</sup> C <sub>6</sub> ]Phe	0.069 (SEM 0.005) v. 0.077 (SEM 0.008)	0.07 (SEM 0.008) v. 0.051 (SEM 0.007)	10 d	43 (SEM 0.2) v. 46.8 (SEM 0.3)	42.1 (SEM 0.2) v. 45.3 (SEM 0.3)	13.3 (SEM 0.07) v. 14.5 (SEM 0.1)	12.5 (SEM 0.07) v. 13.6 (SEM 0.1)
Dillon (2009) <sup>(17)</sup>	EAA v. placebo	3 months	L-[ring- <sup>2</sup> H <sub>5</sub> ]Phe	0.062 (SD 0.006) v. 0.06 (SD 0.004)	0.08 (SD 0.007) v. 0.066 (SD 0.002)	3 months	43.5 (SD 2.8) v. 40.7 (SD 2.4)	45.2 (SD 3) v. 41 (SD 2.8)	NA	NA
Verhoeven (2009) <sup>(13)</sup>	Leucine v. placebo	NA	NA	NA	NA	3 months	54.6 (SEM 1) v. 55.8 (SEM 0.9)	55 (SEM 1.5) v. 56.2 (SEM 1.1)	17.1 (SEM 0.5) v. 17.6 (SEM 0.4)	17.6 (SEM 0.4) v. 18 (SEM 0.4)
Acute leucine administration										
Deutz (2011) <sup>(35)</sup>	Experimental medical food v. conventional medical food	5 h	L-[ring- <sup>13</sup> C <sub>6</sub> ]Phe	Change from baseline: 0.02312 (SD 0.03069) v. -0.00839 (SD 0.04374)	NA	NA	NA	NA	NA	NA
Koopman (2008) <sup>(23)</sup>	CHO + PRO + leucine v. CHO + PRO	6 h	L-[ring- <sup>13</sup> C <sub>6</sub> ]Phe	NA	0.081 (SEM 0.003) v. 0.082 (SEM 0.006)	NA	NA	NA	NA	NA
Katsanos (2006) <sup>(9)</sup>	41 % leucine v. 26 % leucine	6.5 h	L-[ring- <sup>2</sup> H <sub>5</sub> ]Phe	0.038 (SEM 0.007) v. 0.044 (SEM 0.003)	0.056 (SEM 0.008) v. 0.049 (SEM 0.006)	NA	NA	NA	NA	NA
Koopman (2006) <sup>(24)</sup>	CHO + PRO + leucine v. CHO	6 h	L-[ring- <sup>13</sup> C <sub>6</sub> ]Phe	NA	0.072 (SEM 0.006) v. 0.043 (SEM 0.003)	NA	NA	NA	NA	NA

NA, not available; EAA, essential amino acids; CHO, carbohydrate; PRO, protein hydrolysate.

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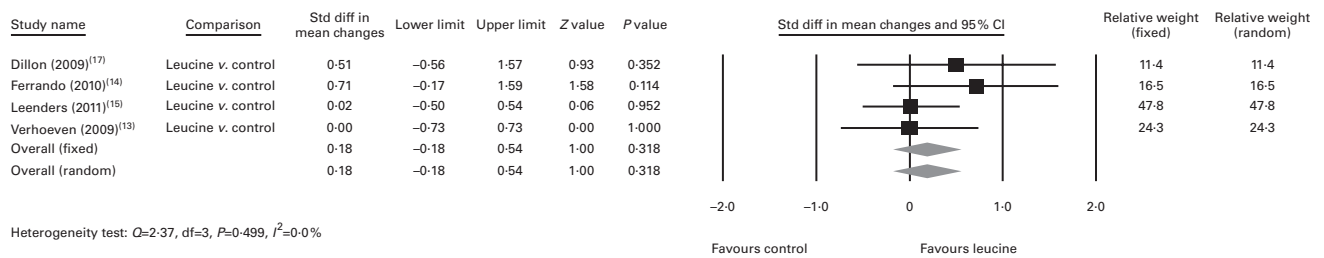
**Fig. 4.** Forest plot showing the results for the meta-analysis of standardised difference (Std diff) in mean changes of muscle protein fractional synthetic rates after leucine v. control intervention.

lean mass with leucine supplementation. One idea is consistent with the ‘anabolic threshold concept’, suggesting that in elderly people, changes in key signalling pathways and changes in catabolic factors and oxidative stress may have negative effects on amino acid or insulin signalling pathways that play a role in the stimulation of muscle anabolism after food intake<sup>(44)</sup>. These changes may lead to ‘anabolic resistance’ of muscle, such that there is a requirement for higher anabolic stimuli to promote maximal anabolism and protein retention. Another idea, which is not mutually exclusive, is that the muscle becomes refractory (or ‘full’) when exposed to the persistent levels of amino acid concentrations, independent of the mode of amino acid delivery<sup>(22)</sup>. Ingestion of leucine or a protein meal transiently increases myofibrillar protein synthesis after an approximate 45 min delay from intake, for about 45–90 min after which synthesis rapidly declines to pre-intake rates<sup>(45)</sup>. The increase in muscle protein synthesis probably results from the activation of processes that regulate mRNA translation. The decline in protein synthesis occurs even in the continued presence of amino acids, suggesting that muscles have a mechanism for regulating the synthesis of new proteins.

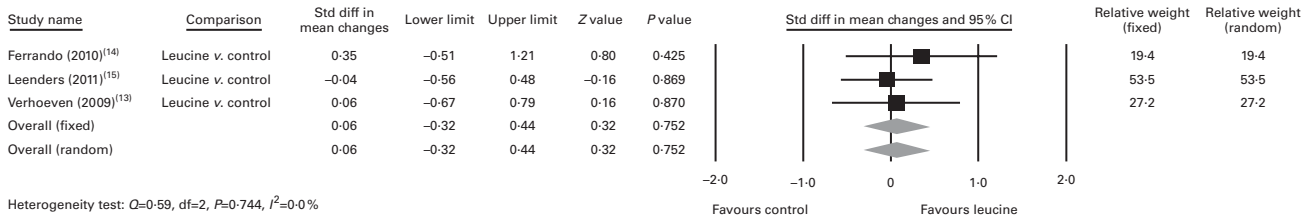
The long-term effect of leucine on muscle mass is not clear. Leucine supplementation is known to interact with other key pathways such as insulin signalling and glucose metabolism pathways<sup>(46)</sup>. Long-term treatment with leucine attenuates insulin secretory dysfunction of human diabetic islets via the up-regulation of certain key metabolic genes, and *in vivo* leucine administration improves glycaemic control in human subjects and rodents with type 2 diabetes<sup>(47)</sup>. These findings may have implications for the association between leucine supplementation and type 2 diabetes. In addition, leucine may also attenuate adiposity by increasing fatty oxidation

and mitochondrial biogenesis in adipocytes and muscle tissue. In one study, it has been suggested that leucine may be useful in the management of obesity and obesity-related co-morbidities by increasing fat oxidation and reducing oxidative stress and inflammation<sup>(48)</sup>. Muscle contraction also appears to influence muscle protein synthesis; it strongly stimulates muscle protein synthesis and also increases muscle protein degradation, but to a lesser extent, resulting in an improved net muscle protein balance<sup>(25)</sup>. The studies of Koopman *et al.*<sup>(23,24)</sup> investigated the effect of leucine administration following physical activity in elderly men who received ample amounts of dietary protein on whole-body protein turnover and muscle protein synthetic rate compared with the administration of controls. They found that muscle fraction synthetic rates were not different between the groups. These findings suggest that leucine supplementation and exercise did not further elevate the rates of muscle protein synthesis in elderly men who received ample amounts of protein. The lack of enhancement from additional leucine and exercise may reflect the fact that the subjects were already receiving ample amounts of protein in their diets<sup>(25)</sup>. The influence of protein intake in a subject’s diet in the course of a study on whether leucine supplementation does or does not affect muscle protein synthesis is a confounding factor that may, at least in part, explain the discrepancies among the studies. It is possible that long-term leucine supplementation would be more clinically relevant in malnourished elderly or in specific clinical subpopulations<sup>(25)</sup>.

The longest study included in the present meta-analysis was 3 months in duration, raising the issue of the effects of longer-term administration of leucine on muscle protein synthesis and other metabolic processes. In two studies that were not



**Fig. 5.** Forest plot showing the results for the meta-analysis of standardised difference (Std diff) in mean changes of lean body mass after leucine v. control intervention.



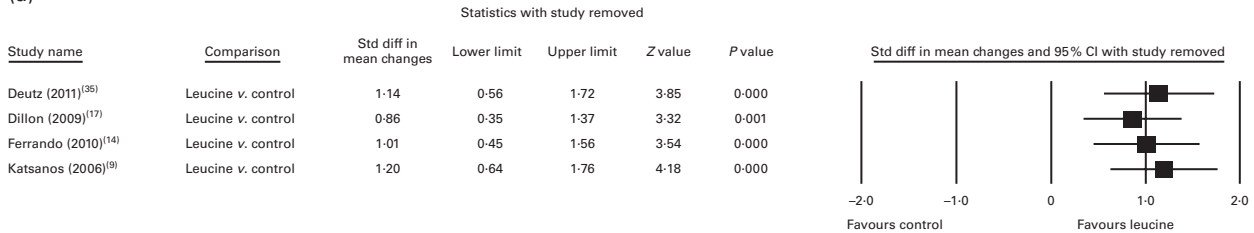
**Fig. 6.** Forest plot showing the results for the meta-analysis of standardised difference (Std diff) in mean changes of leg lean mass after intervention: leucine v. control.

included in the present meta-analysis, the effect of long-term leucine supplementation was evaluated. Zeanandin *et al.*<sup>(49)</sup> evaluated the effects of 6 months of dietary leucine administration on insulin signalling and sensitivity in elderly rats (18 months of age). Rats were fed a 15% protein diet with or without 4.5% leucine. They found that the mTOR pathway was not significantly altered in muscle, and glucose tolerance was not changed. No change in skeletal muscle mass was observed, although perirenal adipose tissue mass accumulated in the leucine-supplemented mass. These findings suggest that the effect of leucine is somewhat tissue specific. Guo *et al.*<sup>(50)</sup> assessed the metabolic effects of leucine supplementation in an obese/diabetic mouse model, and found that leucine

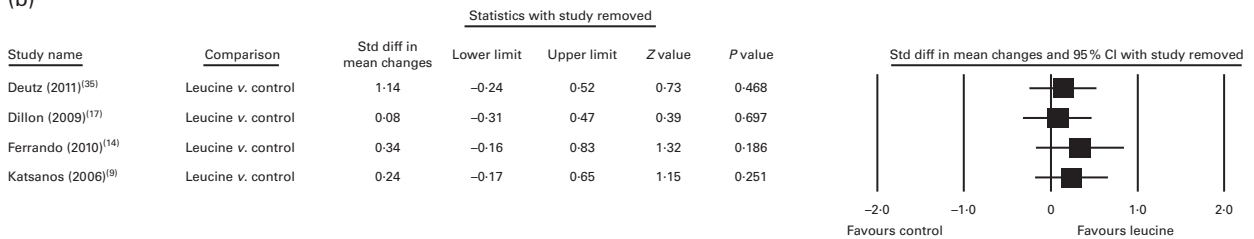
supplementation for 8 months significantly improved glycaemic control, and that the effects of leucine probably acts by multiple mechanisms in different tissues.

All the studies included in the meta-analysis were randomised controlled trials. However, the studies investigated different populations, leucine levels and dosing regimens. This heterogeneity in experimental designs and subject populations could have influenced the findings, and indicates the need for additional studies with more similar experimental designs to address whether leucine supplementation can be used for the normalisation of protein synthesis in the elderly. The pooled results for muscle protein fractional synthetic rates (pooled standardised difference in mean changes 1.08, 95% CI

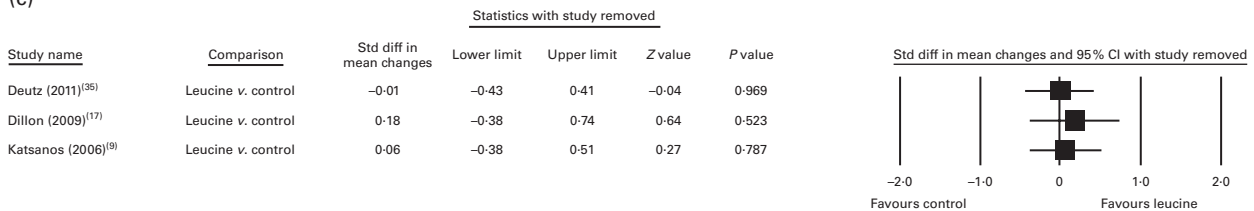
(a)



(b)



(c)



**Fig. 7.** Results of sensitivity analysis for the examination of the influence of individual studies on pooled estimates as determined using the leave-one-out approach: (a) muscle protein fractional synthetic rate; (b) lean body mass; (c) leg lean mass.



0.50, 1.67;  $P < 0.001$ ) were determined from a combination of acute and long-term supplementation outcomes. Among the four studies comparing the changes in muscle protein fractional synthetic rates between two groups, two used long-term supplementation<sup>(14,17)</sup> and two employed acute administration of leucine supplementation<sup>(35,36)</sup>. However, all the four studies reported significant differences in the fractional synthetic rate of muscle protein between the intervention and control groups. Thus, our findings suggest that either long-term or acute leucine supplementation could increase the muscle protein fractional synthetic rate.

In conclusion, we found that ingestion of leucine significantly increased the muscle protein fractional synthetic rate in elderly individuals, and thus may be of benefit to address sarcopenia in this population.

### Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S0007114514002475>

### Acknowledgements

The present study was supported by the Science and Technology Project of Zhejiang Province (grant no. 2013C33122).

The authors' contributions are as follows: Z.-r. X. contributed to the manuscript preparation and literature search, and was the guarantor of the integrity of the entire study and responsible for the definition of the intellectual content; Z.-j. T. contributed to the definition of the intellectual content, literature research and data acquisition; Q. Z. performed the data analysis and statistical analyses; Q.-f. G. contributed to the manuscript editing; Y.-m. Y. contributed to the study concept, study design and manuscript review.

There are no conflicts of interest.

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