Long-term leucine supplementation does not increase muscle mass or strength in healthy elderly men[1–3]

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ABSTRACT

Background: It has been reported that the blunted muscle protein synthetic response to food intake in the elderly can be normalized by increasing the leucine content of a meal.

Objective: The objective was to assess the effect of 3 mo of leucine supplementation on muscle mass and strength in healthy elderly men.

Design: Thirty healthy elderly men with a mean (±SEM) age of 71 ± 4 y and body mass index (BMI; in kg/m^2) of 26.1 ± 0.5 were randomly assigned to either a placebo-supplemented ($n = 15$) or leucine-supplemented ($n = 15$) group. Leucine or placebo (2.5 g) was administered with each main meal during a 3-mo intervention period. Whole-body insulin sensitivity, muscle strength (one-repetition maximum), muscle mass (measured by computed tomography and dual-energy X-ray absorptiometry), myosin heavy chain isoform distribution, and plasma amino acid and lipid profiles were assessed before, during, and/or after the intervention period.

Results: No changes in skeletal muscle mass or strength were observed over time in either the leucine- or placebo-supplemented group. No improvements in indexes of whole-body insulin sensitivity (oral glucose insulin sensitivity index and the homeostasis model assessment of insulin resistance), blood glycated hemoglobin content, or the plasma lipid profile were observed.

Conclusion: Long-term leucine supplementation (7.5 g/d) does not augment skeletal muscle mass or strength and does not improve glycemic control or the blood lipid profile in healthy elderly men. This trial was registered at clinicaltrials.gov as NCT00807508. *Am J Clin Nutr* 2009;89:1468–75.

INTRODUCTION

The process of age-related loss of muscle mass and function, or sarcopenia, is accompanied by a reduction in physical performance, the loss of functional capacity, and an increased likelihood of developing chronic metabolic diseases such as obesity and type 2 diabetes (1, 2). It has been suggested that a disruption in the regulation of muscle protein turnover is one of the major causes of sarcopenia (3, 4). Aging has been associated with a decline in basal mixed muscle and/or myosin heavy chain (MHC) protein synthesis rates in some (3, 5–7) but not in all (4, 8–10) studies. Furthermore, it has been speculated that the elderly are less sensitive to the major muscle protein anabolic stimuli, such as food intake and/or physical activity. In accordance, more recent work suggests that the elderly show a blunted muscle protein synthetic response to dietary protein intake (4, 11, 12).

Recent studies have shown that the attenuated muscle protein synthetic response to food intake in the elderly can be compensated for by increasing the leucine content of a meal (12, 13). In a recent study, Katsanos et al (12) report a greater muscle protein synthetic response after the ingestion of 6.7 g essential amino acids in young than in elderly subjects. However, when the leucine content of this essential amino acid mixture was increased from 26% to 41%, the elderly had a muscle protein synthetic response similar to that of the young. The authors concluded that increasing the leucine content of a meal reverses the blunted muscle protein synthetic response to amino acid or protein intake in the elderly. The latter was confirmed by Rieu et al (13), who reported substantially higher muscle protein synthesis rates after the ingestion of leucine-enriched food by elderly men. The proposals that leucine inhibits muscle protein breakdown (14, 15) and/or stimulates postprandial muscle protein synthesis has been attributed to its capacity to stimulate mRNA translation initiation via insulin-dependent and independent pathways (16).

The recent in vivo human data (12, 13) suggest that leucine supplementation with each main meal represents an effective nutritional strategy to augment skeletal muscle mass and/or function in the elderly. However, human intervention studies on the clinical benefits of prolonged leucine supplementation in the elderly are lacking. The present study investigated the effect of 3 mo of leucine supplementation with each main meal (7.5 g/d) on skeletal muscle mass and strength and on glycemic control in healthy elderly men.

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SUBJECTS AND METHODS

Subjects

A total of 30 healthy elderly men (71 ± 4 y) were selected to participate in a 12-wk nutritional intervention program. The medical history of all subjects was evaluated, and an oral-glucose-tolerance test and resting electrocardiogram were performed. Exclusion criteria included (silent) cardiac or peripheral vascular disease, orthopedic limitations, and/or type 2 diabetes (17). All subjects were living independently and had not participated in any intervention program for ≥5 y. Medication use was limited to mild antihypertensive drugs. After being selected, the subjects were randomly assigned to either a leucine- or a placebo-supplemented group. One subject in the placebo group dropped out of the study because of hospitalization after an injury. The characteristics of the subjects are provided in **Table 1**. All subjects were informed about the nature and possible risks of the experimental procedures before their written informed consent was obtained. The study conformed to the principles of the Declaration of Helsinki and was approved by the local Medical Ethical.

Study design

Subjects were supplemented with either 2.5 g leucine or a placebo with each main meal (breakfast, lunch, and dinner) during a 12-wk intervention period. Before, during, and after the nutritional intervention, anthropometric measurements (height, body weight, leg volume (18)), strength assessment (one repetition maximum; 1RM), computed tomography (CT), and dual-energy X-ray absorptiometry (DXA) were performed and muscle biopsies, blood samples, and records of dietary intakes were collected.

Diet and physical activity before testing

Standardized meals were provided to all subjects the evening before each test day (44 kJ/kg; 60% of energy from carbohydrate, 28% of energy from fat, and 12% of energy from protein). The subjects were instructed to refrain from strenuous physical activity for ≥3 d before testing began. Dietary intake was recorded for 2 d before muscle biopsy and blood sample collection to standardize food intake before muscle biopsy and blood sampling during the postintervention measurements. To assess potential changes in daily food intake during the intervention period, the subjects recorded 3-d weighted dietary intake records (Thursday through Saturday) before the onset of the intervention program and in week 11 of the intervention. Dietary records were analyzed with Eetmeter Software (version 1.4.0; Voedingscentrum, The Hague, Netherlands). On all test days, the subjects arrived at the laboratory by car or public transportation after fasting overnight.

TABLE 1
Characteristics of the subjects[1]

	Placebo (n = 14)	Leucine (n = 15)
Body weight (kg)	78.1 ± 2.4	77.6 ± 2.3
Height (m)	1.72 ± 0.01	1.73 ± 0.01
BMI (kg/m^2)	26.3 ± 0.6	25.9 ± 0.6
Plasma glucose (mmol/L)	5.54 ± 0.11	5.68 ± 0.18
Hb A_{1c} (%)	5.9 ± 0.1	5.8 ± 0.1
HOMA-IR	1.5 ± 0.2	1.7 ± 0.2

[1] All values are means ± SEMs. Hb A_{1c}, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance. Data were analyzed by using an independent-sample t tests. No significant differences were observed between groups.

Supplementation

The subjects were studied over a 12-wk period during which they were supplemented with either 7.5 g/d leucine (Ajinomoto, Tokyo Japan) or a placebo (wheat flour; Verstegen, Rotterdam, Netherlands). The subjects were provided the supplements in a double-blinded manner and ingested 5 capsules (500 mg) with each main meal (breakfast, lunch, and dinner). The different capsules could not be distinguished by scent, color, or taste.

Strength

Maximal strength was assessed by 1RM strength tests on leg-press and leg-extension machines (Technogym, Rotterdam, Netherlands). During a familiarization trial, the proper lifting technique was demonstrated and practiced, after which maximum strength was estimated by using the multiple repetitions testing procedure (19). In an additional session, ≥1 wk before muscle biopsy collection, each subject's 1RM was determined as described previously (20). The 1RM test was repeated after cessation of the intervention program.

CT scan

The anatomical cross-sectional area of the quadriceps muscle was measured with a 16-slice CT scanner (IDT 8000; Philips Medical Systems, Best, Netherlands) before and after cessation of the intervention period. While the subjects lay supine with their legs extended and their feet secured, a 3-mm thick axial image (scanning characteristics: 120 kV, 300 mA, 0.75-s rotation time, and 500-mm field of view) was taken midway between the anterior superior iliac spine and the bottom of the patella. The CV for repeated scans was 0.6%. Images were loaded onto a personal computer by using AGFA IMPAX imaging software (version 5.2; AGFA Health Care, Brussels, Belgium). The muscle area of the right leg was selected between −29 and 150 Hounsfield units (21), after which the quadriceps muscle was selected by manual tracing. The quadriceps area was calculated by using Lucia 4.81 software (Nikon Instruments Europe, Badhoevedorp, Netherlands). All analyses were performed by 2 investigators blinded to subject coding; intraclass correlation coefficients for inter- and intrainvestigator reliability were 0.997 and 0.998, respectively.

DXA scan

Directly after CT was performed, body composition and bone mineral content were measured with DXA (Lunar Prodigy Advance; GE Health Care, Madison, WI). Whole-body and regional lean mass, fat mass, and bone mineral content were determined by using the system's software package enCORE 2005 (version 9.15.00).

Blood samples

Before and after 2, 4, 8, and 12 wk of intervention, fasting blood samples were collected to determine basal plasma glucose and insulin concentrations, plasma amino acid and lipid profiles, serum creatinine, and blood glycated hemoglobin (Hb A_{1c})

content. Blood (10 mL) was collected into EDTA-containing and serum tubes and immediately centrifuged at 1000 × *g* for 10 min at 4°C (plasma) or 15 min at 18°C (serum). Aliquots of plasma or serum were immediately frozen in liquid nitrogen and stored at −80°C until further analysis. Plasma glucose concentrations were analyzed with a COBAS FARA semiautomatic analyzer (Uni Kit III; Roche, Basel, Switzerland). Plasma insulin concentrations were determined by using an Insulin RIA Kit (LINCO Research Inc, St Charles, MO). For the amino acid analyses, plasma was deproteinized on ice with 100 μL 24% (wt:vol) 5-sulfosalicylic acid and mixed, and the clear supernatant fluid was collected after centrifugation. Plasma amino acid concentrations were analyzed with a dedicated amino acid analyzer (LCA10; Shimadzu Benelux, Den Bosch, Netherlands) by using an automated precolumn derivatization procedure and a ternary solvent system. The reagents used to determine plasma triglycerides, total cholesterol, and HDL cholesterol were from ABX Diagnostics (Montpellier, France). Plasma free fatty acid concentrations were analyzed with the NEFA C test kit from Wako Chemicals (Neuss, Germany). Because plasma triacylglycerol concentrations were <4.5 mmol/L, plasma LDL cholesterol could be calculated as LDL cholesterol = total cholesterol − HDL cholesterol – triacylglycerol/2.2 (in mmol/L). Serum creatinine concentrations were measured by using the Jaffe rate method on a Synchron LX Systems analyzer (Beckmann Coulter Inc, Fullerton, CA). To determine the blood Hb A_{1c} content, 3 mL blood was collected into EDTA-containing tubes and analyzed by HPLC (Variant II 4; Bio-Rad, Munich, Germany).

Whole-body insulin sensitivity

Whole-body insulin sensitivity and/or the oral glucose tolerance were assessed on the basis of fasting blood glucose and insulin concentrations by using the homeostasis model assessment of insulin resistance (HOMA-IR) (22). Furthermore, the oral glucose insulin sensitivity index (OGIS) was calculated from the data derived in the oral-glucose-tolerance test (17).

Muscle biopsies

Three days before the onset of nutritional intervention and 4 d after the postintervention strength assessment, percutaneous needle biopsy samples (50–80 mg) were collected from the right vastus lateralis muscle of each subject in the morning after an overnight fast. Any visible nonmuscle tissue was immediately removed from the biopsy samples, after which the muscle biopsy samples were frozen in liquid nitrogen and stored at −80°C until further analyses.

Muscle tissue analyses

Muscle tissue samples were analyzed for the expression of the different MHC isoforms (I, IIa, and IIx). A 5% muscle homogenate was prepared by dispersion (Polytron, Lucerne, Switzerland) in 250 mmol/L sucrose, 2 mmol/L EDTA, and 10 mmol/L tris (hydroxymethyl) aminomethane (pH 7.4) for 1 min. The homogenates were centrifuged for 10 min at 10,000 × g at 4°C. The pellet was resuspended in 3 volumes of ice-cold extraction buffer (100 mmol/L $Na_4O_7P_2 \cdot 10\ H_2O$ (pH 8.5), 5 mmol/L EDTA, and 1 mmol/L dithiothreitol), incubated on ice for 30 min and centrifuged for 10 min at 10,000 × *g* at 4°C. The supernatant fluid was used for MHC isoform analysis. Electrophoresis gels were run for 22 h by using a Protean II xi Cell system (Bio-Rad, Veenendaal, Netherlands) at 10 mA, with voltage increasing to a maximum of 350 V. Approximately 0.3 μg protein was loaded per lane. The gels were silver-stained, scanned, and photographed with a scanning densitometer (Fluor-S Multimager; Bio-Rad), after which the protein bands were quantified by using Quantity software (Bio-Rad). The contents of type I, IIa, and IIx MHC isoforms were expressed relative to the total content (23).

Statistics

Data are expressed as means ± SEMs. Baseline characteristics between groups were compared by means of an independent-samples *t* test. Data from before and after the intervention were analyzed by using repeated-measures analysis of variance with time as a within-subjects factor and treatment as a between-subjects factor. Statistical significance was set at $P < 0.05$. All calculations were performed by using the Statistical Package for the Social Sciences 15.0 (SPSS Inc, Chicago, IL).

RESULTS

Subjects

Characteristics of the subjects before the intervention are provided in Table 1. A total of 29 subjects completed the intervention program ($n = 15$ in the leucine group; $n = 14$ in the placebo group). No differences were observed between groups before the intervention. The subjects had normal glucose tolerance and blood Hb A_{1c} concentrations.

Diet

Energy intake and macronutrient composition, calculated from the dietary intake records, did not differ between groups at baseline and did not change over time during the experimental period. The average daily energy intake was 8.2 MJ/d, consisting of 51 ± 1% of energy from carbohydrate, 32 ± 1% of energy from fat, and 17 ± 1% of energy from protein. The average daily protein intakes before the supplementation period were 0.99 ± 0.07 and 0.99 ± 0.04 g/kg body wt in the leucine and placebo groups, respectively. No differences were observed between groups after the supplementation period (0.97 ± 0.07 and 1.01 ± 0.05, respectively; $P = 0.7$). Leucine supplementation did not result in changes in daily energy intake or in the macronutrient composition of the diet.

Muscle strength

At baseline, 1RM for leg press and leg extension did not differ between groups (170 ± 8 compared with 172 ± 6 kg and 85 ± 3 compared with 85 ± 3 kg in the leucine and placebo groups, respectively). No changes in 1RM leg press or leg extension were observed over time during the 12-wk intervention period. No significant differences were observed between groups.

Body composition

Whole-body and leg fat mass and fat-free mass (by DXA) and leg volume and upper leg muscle cross-sectional area (by CT) did

not differ between groups before the intervention (**Table 2**). No changes in body composition or muscle mass were observed over time, and no significant differences were observed between groups.

Glycemic control

Measures of glycemic control are presented in **Table 3**. At baseline, no differences in whole-body insulin sensitivity and/or in oral glucose tolerance were observed between groups. Basal glucose and insulin concentrations, the HOMA-IR index, blood Hb A_{1c} concentrations, and OGIS did not change over time. No significant differences were observed between groups.

Blood lipid profile

Blood lipid profiles are provided in **Table 4**. At baseline, no differences were observed between groups. Plasma triglyceride and total, LDL-, and HDL-cholesterol concentrations did not change significantly over time in either group.

Creatinine clearance

Serum creatinine concentrations were within the normal range before the intervention (1.08 ± 0.05 and 1.08 ± 0.04 mg/dL in the placebo and leucine groups, respectively) and did not change after the intervention (1.09 ± 0.05 and 1.05 ± 0.04 mg/dL, respectively). No differences were observed between groups. Creatinine clearance did not change over time in either group: 79.4 ± 3.7 and 89.6 ± 7.3 mL · min^{-1} · m^{-2} before the intervention and 68.4 ± 6.6 and 75.3 ± 6.2 mL · min^{-1} · m^{-2} after the intervention in the placebo and leucine groups, respectively ($P > 0.05$).

Amino acid profiles

Amino acid profiles are provided in **Table 5**. No differences were observed between groups in plasma amino acid concentrations before the intervention. Basal plasma valine concentrations declined by 25 ± 2% within 2 wk of supplementation in the leucine group, after which concentrations remained stable: from 197 ± 7 (150–249) to 157 ± 7 (106–196) and to 161 ± 10 (99–236) μmol/L after 2 and 12 wk of intervention, respectively ($P < 0.05$). Basal plasma leucine and isoleucine concentrations did not change over time and did not differ between groups.

TABLE 2
Body composition[1]

	Placebo ($n = 14$)		Leucine ($n = 15$)	
	Before	After	Before	After
Lean mass (kg)	55.8 ± 0.9	56.2 ± 1.1	54.6 ± 1.0	55.0 ± 1.5
Fat mass (kg)	19.8 ± 1.7	19.2 ± 2.0	20.0 ± 1.4	20.0 ± 1.3
Body fat (%)	24.5 ± 1.7	23.9 ± 1.9	25.3 ± 1.2	25.4 ± 1.2
Leg lean mass (kg)	17.6 ± 0.4	18.0 ± 0.4	17.1 ± 0.5	17.6 ± 0.4
Leg fat (%)	18.9 ± 1.5	19.4 ± 1.6	19.6 ± 1.2	19.8 ± 1.2
CSA (cm^2)	71 ± 3	71 ± 3	71 ± 2	71 ± 2
Leg volume (L)	7.5 ± 1.9	7.5 ± 1.7	8.1 ± 3.0	7.8 ± 4.1

[1] All values are means ± SEMs. CSA, cross-sectional area. Data were analyzed by using repeated-measures ANOVA. No significant differences were observed between groups or over time.

TABLE 3
Glycemic control[1]

	Placebo ($n = 14$)					Leucine ($n = 15$)				
	0	2	4	8	12	0	2	4	8	12
Plasma glucose (mmol/L)	5.54 ± 0.11	5.63 ± 0.13	5.62 ± 0.12	5.75 ± 0.13	5.49 ± 0.10	5.69 ± 0.18	5.77 ± 0.18	5.86 ± 0.15	5.85 ± 0.17	5.66 ± 0.16
Plasma insulin (mU/L)	6.04 ± 0.76	6.08 ± 0.82	4.66 ± 0.54	6.37 ± 1.04	6.15 ± 1.25	6.73 ± 0.68	8.22 ± 0.91	9.03 ± 1.45	8.34 ± 1.44	7.37 ± 1.17
Hb A_{1c} (%)	5.8 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1
HOMA-IR	1.51 ± 0.21	1.55 ± 0.23	1.19 ± 0.16	1.66 ± 0.29	1.54 ± 0.33	1.74 ± 0.21	2.12 ± 0.26	2.38 ± 0.4	2.19 ± 0.39	1.89 ± 0.32
$Glucose_{120\ min}$ OGTT	7.16 ± 0.67	—	—	—	5.44 ± 0.49	6.76 ± 0.5	—	—	—	6.76 ± 0.65
OGIS (mL · min^{-1} · m^{-2})	876 ± 40	—	—	—	939 ± 40	924 ± 44	—	—	—	906 ± 42

[1] All values are means ± SEMs. HOMA-IR, homeostasis model assessment of insulin resistance; OGIS, oral glucose insulin sensitivity; OGTT, oral-glucose-tolerance test; Hb A_{1c}, glycated hemoglobin. Data were analyzed by using repeated-measures ANOVA. No significant differences were observed between groups or over time.

TABLE 4
Blood lipid profile over time[1]

	Placebo ($n = 14$)					Leucine ($n = 15$)				
	0 wk	2 wk	4 wk	8 wk	12 wk	0 wk	2 wk	4 wk	8 wk	12 wk
Free glycerol (μmol/L)	114 ± 11	82 ± 8	83 ± 9	90 ± 10	91 ± 7	104 ± 9	81 ± 8	76 ± 9	70 ± 5	87 ± 8
Total glycerol (μmol/L)	908 ± 125	909 ± 117	936 ± 113	978 ± 116	983 ± 138	946 ± 82	1055 ± 68	1125 ± 118	987 ± 89	943 ± 61
HDL cholesterol (mmol/L)	1.59 ± 0.11	1.71 ± 0.12	1.72 ± 0.11	1.67 ± 0.12	1.6 ± 0.11	1.47 ± 0.13	1.52 ± 0.12	1.48 ± 0.13	1.54 ± 0.12	1.44 ± 0.1
LDL cholesterol (mmol/L)	3.29 ± 0.24	3.29 ± 0.26	3.34 ± 0.24	3.25 ± 0.24	3.19 ± 0.18	3.35 ± 0.26	3.55 ± 0.31	3.49 ± 0.31	3.54 ± 0.28	3.43 ± 0.26

[1] All values are means ± SEMs. Data were analyzed by using repeated-measures ANOVA. No significant differences were observed between groups or over time.

Muscle tissue analyses

At baseline, no differences in the relative distribution of the MHC isoforms were observed between the placebo and leucine groups: 59 ± 3% compared with 64 ± 2% for MHC-I, 31 ± 2% compared with 30 ± 2% for MHC-IIA, and 10 ± 1% compared with 6 ± 1% for MHC-IIX, respectively. After 3 mo of supplementation, the relative distribution of the MHC isoforms had not changed in the placebo and leucine groups: 65 ± 4% compared with 65 ± 3% for MHC-I, 28 ± 3% compared with 30 ± 3% for MHC-IIA, and 7 ± 1% compared with 6 ± 1% for MHC-IIX, respectively.

DISCUSSION

The present study was designed to assess the effect of 3 mo of leucine supplementation on skeletal muscle mass, strength, and/or glycemic control in healthy elderly men. Leucine supplementation did not increase whole-body fat-free mass, increase the upper leg cross-sectional area, or change muscle fiber type composition. Furthermore, leucine supplementation was not accompanied by changes in glycemic control and/or blood lipid profiles in healthy elderly men.

Recent work has reported a blunted muscle protein synthetic response to food intake in the elderly (11–13, 24). Recent in vivo human studies suggest that increasing the leucine content of a meal augments the postprandial muscle protein synthetic response in the elderly (12, 13). Consequently, it has been suggested that long-term leucine supplementation with each main meal represents an effective nutritional strategy for augmenting muscle mass and strength in the elderly (25). Previously, Katsanos et al (12) reported a substantial 0.008%/h increase in postprandial muscle protein synthetic rate for 2.5 h after the leucine content of an oral amino acid mixture was increased in 10 healthy elderly men. Extrapolation of these data toward the effect of leucine supplementation with each main meal for a 3-mo period would theoretically result in a 1.7-kg (range: 1.4–2.1 kg) gain in muscle mass. Consequently, an increase in whole-body lean tissue mass of ≈3% could be expected in the leucine supplemented group. With a population size of 15, an α level of 0.05, and a power of 0.8, the limit for a statistically detectable change in muscle mass would be ≈0.75 kg, which is easily detected by DXA scanning (with a CV for lean tissue <0.5%). Even when using the lower boundary of the proposed gain in muscle mass (1.4 kg), the power in the present study would still be 0.95. Therefore, in case prolonged leucine supplementation led to a clinically relevant gain in muscle mass, the present study design would easily detect such changes.

In the present study, 3 mo of leucine supplementation did not induce any changes in muscle mass on a whole-body, limb, and/or muscle tissue level in healthy elderly men (Table 2). No changes in body composition (by DXA) were observed in both the leucine and placebo groups (Table 2). In accordance, upper leg muscle cross-sectional area did not change during the intervention. The latter was further supported by the absence of changes in MHC expression in muscle tissue obtained before and after 3 mo of intervention. The close relation between muscle mass and upper leg cross-sectional area and muscle strength has been well established (2, 26). In line with the absence of any changes in muscle mass, we observed no changes in 1RM muscle strength in either condition. Consequently, our data consistently showed that long-

TABLE 5
Amino acid concentrations over time[1]

Amino acid and group	0 wk	2 wk	4 wk	8 wk	12 wk
	μmol/L	*μmol/L*	*μmol/L*	*μmol/L*	*μmol/L*
Glutamic acid					
Placebo	88.3 ± 8.0	78.9 ± 7.3[2]	80.8 ± 5.2[2]	84.1 ± 9.4[2]	97.4 ± 7.2[2]
Leucine	103.2 ± 9	81.5 ± 5.4[2]	81.1 ± 7.2[2]	77.6 ± 5.1[2]	98.7 ± 9.4[2]
Asparagine					
Placebo	44.5 ± 3.9	52.6 ± 1.7	52.5 ± 1.9	55.4 ± 2.3	53.5 ± 4.7
Leucine	47.2 ± 2	48.9 ± 2.7	51.7 ± 5.4	48.3 ± 2.5	46.3 ± 2.0
Serine					
Placebo	94 ± 5	93 ± 5	96 ± 4	99 ± 5	100 ± 5
Leucine	102 ± 3	97 ± 3	92 ± 5	95 ± 4	97 ± 4
Glutamine					
Placebo	544 ± 26	603 ± 25	576 ± 28	594 ± 24	598 ± 26
Lleucine	561 ± 20	569 ± 22	534 ± 35	560 ± 18	533 ± 16
Histidine					
Placebo	71.9 ± 4.1	75.4 ± 3.5	74.1 ± 3.1	75.2 ± 3.4	77.4 ± 3.3
Leucine	76.2 ± 3.0	77.7 ± 3.6	77.1 ± 5.0	75.0 ± 4.9	72.9 ± 3.6
Glycine					
Placebo	205 ± 14	223 ± 16[2]	226 ± 16[2]	237 ± 17[2]	239 ± 18[2]
Leucine	209 ± 11	218 ± 12	212 ± 13	212 ± 13	205 ± 11
Threonine					
Placebo	104 ± 5	114 ± 5	114 ± 6	122 ± 5[2]	125 ± 5[2]
Leucine	107 ± 5	104 ± 5	109 ± 5.4	103 ± 5[3]	100 ± 6[3]
Citrulline					
Placebo	31.2 ± 3.1	31.9 ± 2.2	33.0 ± 3.3	34.6 ± 3.4	34.3 ± 2.8
Leucine	31.1 ± 2.7	35.9 ± 2.5	32.9 ± 2.8	32.1 ± 3.1	35.3 ± 2.9
Arginine					
Placebo	84.2 ± 5.0	92.3 ± 4.8	87.6 ± 4.4	95.9 ± 4.9	93.8 ± 4.2
Leucine	89.8 ± 3.9	89.5 ± 4.1	95.7 ± 4.6	87.7 ± 3.8	82.9 ± 3.1[2]
Alanine					
Placebo	287 ± 19	315 ± 17	306 ± 24	337 ± 17	317 ± 18
Leucine	308 ± 17	332 ± 21	350 ± 18	328 ± 16	292 ± 18
Tyrosine					
Placebo	51.2 ± 2.3	56.8 ± 2.9	59.5 ± 3.6	59.4 ± 3.1	60.3 ± 3.3
Leucine	55.9 ± 2.0	54.9 ± 3.9	59.1 ± 2.7	59.2 ± 2.9	58.1 ± 2.1
Valine					
Placebo	192 ± 7	201 ± 9	208 ± 10	207 ± 9	207 ± 8
Leucine	197 ± 7	157 ± 7[2,3]	157 ± 8[2,3]	161 ± 10[2,3]	161 ± 10[2,3]
Methionine					
Placebo	20.2 ± 1.2	21.9 ± 0.9[2]	21.8 ± 1.2	23.5 ± 1.3[2]	23.2 ± 1.0[2]
Leucine	21.9 ± 1.2	23.3 ± 1.4	24.5 ± 1.2[2]	23.3 ± 1.4	21.6 ± 0.9
Isoleucine					
Placebo	56.8 ± 1.9	59.4 ± 3.3	59.6 ± 2.9	61.3 ± 2.7	62.4 ± 2.7
Leucine	57.7 ± 2.2	52.2 ± 2.8	55.6 ± 2.8	51.9 ± 3.1	52.3 ± 3.4
Phenylalanine					
Placebo	49.2 ± 2.3	51.1 ± 2.1	52.4 ± 2.0	53.5 ± 2.4	54.0 ± 1.9
Leucine	52.5 ± 1.8	54.5 ± 2.7	54.9 ± 2.3	53.8 ± 2.5	52.8 ± 2.1
Tryptophan					
Placebo	40.1 ± 2.4	41.8 ± 2.1	41.8 ± 2.4	41.2 ± 2.2	51.7 ± 8.0
leucine	43.1 ± 1.6	45.7 ± 2.5	45.6 ± 2.3	43.9 ± 2.5	43.3 ± 1.9
Leucine					
Placebo	104 ± 3	107 ± 7	111 ± 5	114 ± 5	114 ± 4
Leucine	106 ± 3	118 ± 7	129 ± 12	118 ± 9	116 ± 7
Ornithine					
Placebo	52.1 ± 2.1	53.4 ± 2.8	52.5 ± 2.8	54.6 ± 3.0	57.0 ± 2.7
Leucine	55.3 ± 1.6	50.9 ± 2.4	52.7 ± 2.6	48.1 ± 2.3	51.0 ± 2.7
Lysine					
Placebo	175 ± 8	184 ± 11	184 ± 11	194 ± 11[2]	199 ± 10[2]
Leucine	194 ± 9	184 ± 8	194 ± 7	178 ± 9	177 ± 7

[1] All values are means ± SEMs. Data were analyzed by using repeated-measures ANOVA. A significant time × treatment interaction was observed for glycine, threonine, arginine, valine, methionine, and lysine ($P < 0.05$).
[2] Significantly different from baseline, $P < 0.05$.
[3] Significantly different from placebo at the same time point, $P < 0.05$.

term leucine supplementation with each main meal did not augment muscle mass and/or strength in healthy elderly men.

The anabolic property of leucine has been attributed to its capacity to stimulate mRNA translation initiation via insulin-independent and insulin-dependent pathways (16, 27). Leucine can also strongly augment glucose-induced insulin secretion by directly stimulating the pancreatic β cell (28). Previous studies in our laboratory have established the insulinotropic properties of leucine when co-ingested with carbohydrate and/or protein (29, 30). The latter has since been applied as an effective nutritional strategy to lower postprandial blood glucose excursions and improve glycemic control in vivo in patients with type 2 diabetes (30, 31). Therefore, we speculated that prolonged leucine supplementation with each main meal would also improve glycemic control by increasing whole-body muscle mass and/or by improving postprandial glycemic control. The latter is supported by Zhang et al (32), who reported that long-term leucine supplementation prevents hyperglycemia and improves insulin sensitivity in mice fed a high-fat diet. The present study was the first placebo-controlled study to assess the proposed effect of prolonged leucine supplementation on glycemic control in humans. Therefore, we also measured blood Hb A_{1c} concentrations and performed an oral-glucose-tolerance test before and directly after the supplementation period. However, no changes in any of the variables that were used to assess glycemic control and/or whole-body glucose tolerance were observed (basal glucose and insulin concentrations, blood Hb A_{1c} concentrations, HOMA-IR, and OGIS (Table 3).

Generally, improvements in postprandial blood glucose homeostasis are accompanied by improvements in the blood lipid profile. In accordance, Zhang et al (32) also observed a reduction in plasma total cholesterol and LDL-concentrations after leucine supplementation in mice fed a high-fat diet. In line with the lack of changes in glycemic control, we observed no changes in basal, fasting plasma LDL and HDL cholesterol, triglyceride, and/or free fatty acid concentrations in the present study (Table 4). The apparent discrepancy between the rodent data presented by Zhang et al (32) and our in vivo human data are likely explained by the fact that the improvements in glycemic control and lipidemia in the mice supplemented with leucine were only observed when mice were fed a high-fat diet. From the present study, we concluded that prolonged leucine supplementation does not improve glycemic control and/or the blood lipid profile in healthy elderly men. It might be of interest to study the proposed protective effects of leucine supplementation under conditions of excess fat and/or total energy intake. Furthermore, future studies assessing the proposed benefits of leucine co-ingestion with each main meal in type 2 diabetes patients are warranted.

It has been well-established (33) that leucine administration lowers plasma concentrations of the other branched-chain amino acids (valine and isoleucine). In accordance, we observed a 25 $\pm$ 2% decline in basal plasma valine concentrations within 2 wk of leucine supplementation, after which concentrations remained stable. Whether this decline in fasting plasma valine concentration is of clinical relevance remains debatable, because the basal concentrations did not further decline and remained within a normal physiologic range. Plasma isoleucine concentrations did not show any changes over time.

The absence of any changes in muscle mass and strength after long-term leucine supplementation with each main meal seems to be in contrast with recent studies that assessed the acute muscle protein synthetic response to the ingestion of a leucine-enriched amino acid mixture (12) or meal (13) in elderly subjects. The apparent discrepancy might be explained by the overall leucine and/or protein intake in these healthy elderly men. Habitual protein intake in our subjects averaged 78 $\pm$ 3 g/d, or 1.0 $\pm$ 0.1 g $\cdot$ kg body $wt^{-1} \cdot d^{-1}$. The latter value is well above dietary guidelines for daily protein intakes in the elderly (34–39). Moreover, recent data by Campbell et al (36) show that a daily protein intake of 0.85 g protein$\cdot$ kg body $wt^{-1} \cdot d^{-1}$ is adequate for both young and old subjects. Therefore, it could be speculated that the total habitual protein intake and the associated leucine intake ($\approx$6.3 $\pm$ 0.3 g/d) were already sufficient to maximize the meal-induced muscle protein synthetic response in our subjects.

It could be speculated that an even longer intervention period would have resulted in a different study outcome. The latter is unlikely because previous reports on the acute postprandial muscle protein synthetic response to the ingestion of leucine-enriched foods (12, 13) suggest that a substantial increase in muscle mass should be achieved within 3 mo of leucine supplementation. Furthermore, whether long-term leucine supplementation might be an effective nutritional strategy in the more frail elderly population, with a less than optimal dietary protein intake, remains to be determined. Nonetheless, the present study clearly shows that previous reports on the acute stimulating properties of an increased leucine content of a meal on the postprandial muscle protein synthetic response do not translate into an effective long-term interventional strategy to augment skeletal muscle mass in healthy elderly men.

In conclusion, leucine supplementation with each main meal (7.5 g/d) does not represent an effective nutritional strategy to increase muscle mass, strength, and/or glycemic control in healthy elderly men.

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