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Original article

Leucine co-ingestion improves post-prandial muscle protein accretion in elderly men

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SUMMARY

Background & aims: It has been speculated that the amount of leucine in a meal largely determines the post-prandial muscle protein synthetic response to food intake. The present study investigates the impact of leucine co-ingestion on subsequent post-prandial muscle protein accretion following the ingestion of a single bolus of dietary protein in elderly males.

Methods: Twenty-four elderly men (74.3 ± 1.0 y) were randomly assigned to ingest 20 g intrinsically L-[1-^{13}C]phenylalanine-labeled casein protein with (PRO + LEU) or without (PRO) 2.5 g crystalline leucine. Ingestion of specifically produced intrinsically labeled protein allowed us to create a plasma phenylalanine enrichment pattern similar to the absorption pattern of phenylalanine from the ingested protein and assess the subsequent post-prandial incorporation of L-[1-^{13}C] phenylalanine into muscle protein.

Results: Plasma amino acid concentrations increased rapidly following protein ingestion in both groups, with higher leucine concentrations observed in the PRO + LEU compared with the PRO group ($P < 0.01$). Plasma L-[1-^{13}C]phenylalanine enrichments increased rapidly and to a similar extent in both groups following protein ingestion. Muscle protein-bound L-[1-^{13}C]phenylalanine enrichments were significantly greater after PRO + LEU when compared with PRO at 2 h (72%; 0.0078 ± 0.0010 vs. 0.0046 ± 0.00100 MPE, respectively; $P < 0.05$) and 6 h (25%; 0.0232 ± 0.0015 vs. 0.0185 ± 0.0010 MPE, respectively; $P < 0.05$) following protein ingestion. The latter translated into a greater muscle protein synthetic rate following PRO + LEU compared with PRO over the entire 6 h post-prandial period (22%; 0.049 ± 0.003 vs. 0.040 ± 0.003% h^{-1}, respectively; $P < 0.05$).

Conclusion: Leucine co-ingestion with a bolus of pure dietary protein further stimulates post-prandial muscle protein synthesis rates in elderly men.

1. Introduction

Aging is associated with a progressive loss of skeletal muscle mass.[1] The loss of muscle mass reduces strength, impairs functional capacity, and increases the risk of developing chronic metabolic diseases.[1] Age related changes in skeletal muscle mass are attributed to a disruption in the regulation of skeletal muscle protein synthesis and/or breakdown.[2] Since basal muscle protein synthesis[3–7] and breakdown[8] rates do not seem to differ between young and elderly individuals, investigators have begun to look at the muscle protein synthetic response to the main anabolic stimuli, food intake and physical activity.[7,9]

Protein turnover in skeletal muscle tissue is highly responsive to nutrient intake in healthy young individuals.[10] Protein ingestion strongly increases muscle protein synthesis rates,[10,11] an effect that is mainly attributed to the stimulatory effect of the essential amino acids,[12,13] and leucine in particular.[14–19] Several studies indicate that senescent muscle is less sensitive to the anabolic properties of amino acids,[4,7,20–22] a concept that is now commonly referred to as anabolic resistance of muscle protein synthesis in the elderly.[23] Leucine has been reported to stimulate muscle protein synthesis in an insulin dependent and independent manner.[24,25] Consequently, it has been suggested that increasing the leucine content of a meal may effectively compensate for the blunted muscle protein synthetic response to food intake in the elderly.[16,26] In agreement, Katsanos et al. reported that increasing the leucine content of an

Abbreviations: FSR, fractional protein synthetic rate.

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amino acid mixture (from 26 to 41%; or from 1.7 to 2.8 g leucine) normalizes the post-prandial muscle protein synthetic response in the elderly when compared with the young.[26] These findings were supported by Rieu et al. who observed considerably higher myofibrillar muscle protein synthetic rates following continuous ingestion of mixed meals supplemented with leucine in elderly men.[16] As a consequence, it has been suggested that increasing the leucine content of a meal represents an effective strategy to augment the muscle protein synthetic response to food intake in the elderly and, as such, may be used to attenuate the loss of muscle mass with aging.

So far, there are no data available to confirm the proposed efficacy of leucine co-ingestion to increase post-prandial muscle protein accretion following the ingestion of a single meal like bolus of dietary protein in elderly individuals. Therefore, in the present study, we assess post-prandial muscle protein accretion following ingestion of a single 20 g bolus of dairy protein with or without additional crystalline leucine (2.5 g) in healthy older males. We applied specifically produced intrinsically labeled protein to create a plasma phenylalanine enrichment pattern similar to the absorption pattern of phenylalanine from the ingested protein and assess the subsequent post-prandial incorporation of L-[1-^{13}C] phenylalanine into muscle protein. Intrinsically labeled casein protein was obtained by infusing cows with large quantities of L-[1-^{13}C]phenylalanine, collecting milk and purifying the casein fraction. This process resulted in dairy protein with the high L-[1-^{13}C]phenylalanine enrichment level (37.4 MPE) required to allow measurable increases in L-[1-^{13}C]phenylalanine enrichment in the mixed muscle protein pool following ingestion of only 20 g dietary protein.[27] The present study provides proof-of-principle that addition of free leucine with a single bolus of dairy protein can further augment post-prandial muscle protein accretion in older adults.

2. Materials and methods

2.1. Participants

Twenty-four healthy elderly men (74.2 ± 1.0 y) participated in the present study. The participants were randomly assigned to an experiment in which a single meal like bolus of protein with (PRO + LEU) or without (PRO) additional crystalline leucine was ingested. Participants' characteristics are presented in Table 1. Exclusion criteria were BMI >30 kg m^{-2}, diabetes, all co-morbidities interacting with mobility and muscle metabolism of the lower limbs (e.g. arthrosis, arthritis, spasticity/rigidity, all neurological disorders and paralysis), use of anticoagulants, blood diseases, phenylketonuria, allergy for lidocain and participation in any regular exercise program. All participants were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained. This study was approved by the Medical Ethics Committee of the Maastricht Medical Centre.

Table 1
Participants' characteristics.

	PRO	PRO + LEU
n	12	12
Age (y)	74.3 ± 1.2	74.2 ± 0.7
Weight (kg)	78.4 ± 1.9	79.4 ± 3.4
BMI (kg/m^2)	25.7 ± 0.4	25.6 ± 0.8
Systolic blood pressure (mm Hg)	140 ± 5	144 ± 4
Diastolic blood pressure (mm Hg)	73 ± 3	73 ± 3
Fat (% of body weight)	22.3 ± 1.3	22.6 ± 1.5
Lean body mass (kg)	58.5 ± 1.1	58.4 ± 1.8
Basal plasma glucose (mmol L^{-1})	5.4 ± 0.1	5.5 ± 0.1
Plasma glucose OGTT t = 120 min (mmol L^{-1})	6.3 ± 0.5	6.3 ± 0.6
Basal plasma insulin (mU L^{-1})	21.0 ± 2	23.0 ± 4.0
Plasma insulin OGTT t = 120 min (mU L^{-1})	93.0 ± 19	120.0 ± 16
HbA1c (%)	5.7 ± 0.1	5.6 ± 0.1
HOMA	5.1 ± 0.5	5.7 ± 0.9
OGIS (mL min^{-1} m^{-1})	347 ± 17	337 ± 22

Values represent means ± SEM. HbA1c: glycated hemoglobin; OGTT: oral glucose tolerance test; HOMA: homeostasis model assessment; OGIS: oral glucose insulin sensitivity. No significant differences between groups.

2.2. Pretesting

Before selection for the study, all participants participated in a routine medical screening. During this session, participants completed a health and activity questionnaire and underwent an oral glucose tolerance test (OGTT) to screen for type 2 diabetes according to the criteria set out by the World Health Organization.[28] Venous plasma glucose and insulin concentrations determined during the OGTT were used to determine the oral glucose insulin sensitivity (OGIS) index[29] and the homeostasis model assessment (HOMA) index.[30] Prior to the OGTT, body weight and height were measured, and body composition was determined by dual-energy X-ray absorptiometry (DEXA, Discovery A; Hologic, Bedford, USA).

2.3. Diet and activity before testing

All participants consumed a standardized meal (33 ± 2 kJ kg^{-1} body weight, providing 44 energy% (En%) carbohydrate, 22 En% protein, and 34 En% fat the evening prior to the experiment. All volunteers were instructed to refrain from strenuous physical activity and to keep their diet as constant as possible for 2 d prior to the experiment.

2.4. Protocol

At 0800, following an overnight fast, participants arrived at the laboratory by car or public transport. A polytetrafluoroethylene catheter was inserted into a heated dorsal hand vein after which the hand was placed in a hot box (60 °C) for arterialized blood sampling.[31] After a basal arterialized blood sample was collected, a baseline muscle biopsy sample was obtained from the *vastus lateralis* muscle. Participants then ingested a single bolus of test drink containing 20 g intrinsically L-[1-^{13}C]phenylalanine-labeled casein with (PRO + LEU) or without (PRO) 2.5 g crystalline leucine (Frutarom, Belgium). The consumption of the test drink with or without the additional leucine signified the beginning (t = 0 min) of a 6 h post-prandial period. Arterialized blood samples were subsequently collected at t = 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min. A second muscle biopsy sample was taken from the same limb, through a new incision, >2 cm distal from the first incision, at t = 120 min. A third muscle biopsy was collected from the contralateral leg at t = 360 min. Arterialized venous blood samples were collected into EDTA-containing tubes and centrifuged at 1000*g* for 10 min at 4 °C. Aliquots of plasma were frozen in liquid nitrogen and stored at −80 °C until further analysis. Muscle biopsy samples were obtained from the middle region of the *vastus lateralis*, 15 cm above the patella and ~3 cm below entry through the fascia using the percutaneous needle biopsy technique.[32] Muscle biopsy samples were carefully dissected and freed from any visible non-muscle material and then immediately frozen in liquid nitrogen and stored at −80 °C for further analysis.

2.5. Preparation of intrinsically labeled protein

Intrinsically L-[1-^{13}C]phenylalanine-labeled casein protein was obtained by infusing a Holstein cow with large quantities of L-[1-^{13}C]phenylalanine, collecting milk, and purifying the casein fraction as described previously.[27] The L-[1-^{13}C]phenylalanine enrichment in the casein fraction averaged 37.4 moles percent