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ORIGINAL ARTICLE

Protein intake and lean body mass preservation during energy intake restriction in overweight older adults

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BACKGROUND: Dietary-induced weight loss is generally accompanied by a decline in skeletal muscle mass. The loss of muscle mass leads to a decline in muscle strength and impairs physical performance. A high dietary protein intake has been suggested to allow muscle mass preservation during energy intake restriction.

OBJECTIVE: To investigate the impact of increasing dietary protein intake on lean body mass, strength and physical performance during 12 weeks of energy intake restriction in overweight older adults.

DESIGN: Sixty-one overweight and obese men and women (63 ± 5 years) were randomly assigned to either a high protein diet (HP; 1.7 g kg^{-1} per day; $n = 31$) or normal protein diet (NP; 0.9 g kg^{-1} per day; $n = 30$) during a 12-week 25% energy intake restriction. During this controlled dietary intervention, 90% of the diet was provided by the university. At baseline and after the intervention, body weight, lean body mass (dual-energy X-ray absorptiometry), leg strength (1-repetition maximum), physical performance (Short Physical Performance Battery, 400 m) and habitual physical activity (actigraph) were assessed.

RESULTS: Body weight declined in both groups with no differences between the HP and NP groups (−8.9 ± 2.9 versus −9.1 ± 3.4 kg, respectively; $P = 0.584$). Lean body mass declined by 1.8 ± 2.2 and 2.1 ± 1.4 kg, respectively, with no significant differences between groups ($P = 0.213$). Leg strength had decreased during the intervention by 8.8 ± 14.0 and 8.9 ± 12.8 kg, with no differences between groups ($P = 0.689$). Physical performance as measured by 400 m walking speed improved in both groups, with no differences between groups ($P = 0.219$).

CONCLUSIONS: Increasing protein intake above habitual intake levels (0.9 g kg^{-1} per day) does not preserve lean body mass, strength or physical performance during prolonged energy intake restriction in overweight older adults.

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INTRODUCTION

Muscle mass and strength are main predictors of physical function and health status in older adults. During a period of energy intake restriction, the decline in body weight is accompanied by a significant loss of muscle mass and strength.[1,2] This decline in muscle mass and strength may even offset the metabolic benefits of the reduction of excess body fat. Novel strategies to preserve muscle mass during energy intake restriction are warranted.

It has been well established that nutrition is an important factor in regulating muscle mass maintenance.[3,4] Dietary protein intake stimulates muscle protein synthesis and allows postprandial muscle protein accretion.[5] It has been suggested that increasing the protein content of the diet might be an effective interventional strategy to preserve muscle mass during prolonged energy intake restriction, thereby maintaining strength and physical performance. Whereas several intervention studies show that increasing protein intake during a period of energy intake restriction can alleviate the loss of lean body mass (LBM) or increase the loss of fat mass (FM),[6–8] other studies have failed to confirm these findings.[9,10] The discrepancy in the literature may be attributed to differences in the selected study population, the applied nutritional intervention and/or compliance and adherence of the subjects to the diet.

Most of the previous work investigating the impact of different protein intakes on LBM preservation during energy intake restriction have been conducted in young to middle-aged adults.[7,8,11–16] However, the results of these studies may not be translated to the older population because of differences in muscle mass and the prevalence of anabolic resistance.[17–19] Another explanation for the discrepancy in the literature is the protein content of the applied intervention diets. Previous studies have compared the impact of a wide variety of protein intakes on LBM preservation during prolonged energy intake restriction, ranging from 0.6 to 0.8 versus 1.1 to 2.4 g protein kg^{-1} body weight per day.[8,9,20,21] Furthermore, the compliance and adherence to a dietary intervention requires careful attention, with nutrition being provided and consumed under strict supervision.[22,23]

In the present study, we investigate the impact of an increase in dietary protein intake on the preservation of muscle mass during prolonged energy intake restriction in overweight, older adults. Maintenance of habitual protein intake (0.9–1.0 g kg^{-1} per day) has previously been shown to be required for muscle mass preservation.[24,25] Therefore, we assess the impact of energy intake restriction on LBM preservation with protein intakes being set at 1.7 versus 0.9 g kg^{-1} per day. We hypothesize that a high protein (HP) intake can preserve LBM during energy intake restriction in overweight, older adults. To ensure proper compliance and adherence to the intervention diet, a strictly controlled dietary intervention is performed.

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SUBJECTS AND METHODS

Subjects

In total, 61 overweight and obese men ($n = 36$) and women ($n = 25$) were included in the study (see Figure 1 of Supplementary Information). All subjects were aged between 55 and 70 years, with a body mass index (BMI) between 27 and 40 kg m^{-2} and a waist circumference of ⩾102 cm for men and ⩾88 cm for women.[26] Subjects were excluded if they suffered from renal insufficiency (MDRD estimated glomerular filtration rate >60 ml min^{-1} per 1.73 m^2),[27] type 1 or type 2 diabetes (fasting glucose levels ⩾7 mmol l^{-1}),[28] cancer, chronic obstructive pulmonary disease, allergy to milk products or underwent a gastric bypass. Subjects were also excluded if they had severe loss of appetite, participated in a weight loss or heavy resistance-type exercise program 3 months before the intervention or if they used supplements or drugs known to interfere with energy balance. Women could only participate if they were postmenopausal (last period 1 year previous to study start). Written informed consent was obtained from all the subjects.

Design

The study is a randomized, fully controlled dietary intervention trial of 12 weeks. In total, 76 subjects were screened for current medical status, medical history, BMI, waist circumference, fasting glucose and renal insufficiency. In addition, the 1-repetition maximum (1-RM) was estimated during the screening as a familiarization session. Subjects were randomly allocated to either the HP group or the normal protein (NP) group. Randomization was carried out with permuted blocks, stratified by gender and BMI. After randomization, the following measurements were performed: body composition, the Short Physical Performance Battery (SPPB), handgrip strength, 400 m walk test, leg strength (1-RM leg press and leg extension), blood pressure, body weight and habitual physical activity. Subsequently, the subjects enrolled in the dietary intervention period that lasted for 13 weeks, consisting of one run-in week and 12 weeks of intervention. The subjects were not aware of the exact aim of the study to limit any possible effect on the outcome measures. The study is in accordance with the ethical standards with the Declaration of Helsinki of 1975 as revised in 1983 and approved by the Medical Ethical Committee of Wageningen University. The design and aim of the study was registered in the NIH clinical trial database (ClinicalTrials.gov).

Dietary intervention

One week after baseline measurements, subjects started with the run-in week. During the run-in week, subjects consumed 100% of their habitual energy intake (protein 14 Energy% (En%), fat 31 En%, carbohydrate 51 En %). Habitual energy intake was calculated from a validated 177-item food frequency questionnaire[29] and estimated by the Schofield formula in combination with a standard PAL (physical activity level) value of 1.6.[30] Menus were designed for 10 levels of energy intake, ranging from 7 to 16 MJ per day. Procedures during the run-in week were the same as during the intervention to familiarize the subjects with the intervention procedures and to standardize dietary conditions among groups. Moreover, if subjects lost or gained body weight during the run-in week, energy intake was adjusted to attain an adequate estimation of the habitual energy intake for the rest of the intervention. Directly after the run-in week, subjects enrolled in the dietary intervention.

The intervention diets were isocaloric compared with each other and energy intake restricted by 25% from baseline energy intake. Ninety percent of the diet was provided by the university and 10% was chosen by the subjects from a restricted list. Food products were weighed for each subject individually. The HP diet contained 1.7 g protein kg^{-1} per day and the NP diet contained 0.9 g kg^{-1} per day. Protein was replaced by carbohydrates in the NP group, the fat content was equal in both diets. Subjects consumed a supplement two times per day, containing either 20 g protein for the HP group (50% whey, 40% casein, 10% soy) or 25 g carbohydrate for the NP group. The intervention diets were designed and calculated by experienced research dietitians using the Dutch food composition table.[31] On weekdays, subjects came to the university to consume their hot meal (during afternoon or evening). The rest of the diet (breakfast, bread meal, snacks and beverages) and all meals for Saturdays and Sundays were provided in take-home packages, together with the meal planning. Subjects were carefully instructed how to prepare the hot meals for the weekend. Protein intake was evenly divided over the day with an average 34 ± 4 g (ranging from 25 to 41 g) protein per meal in the HP group and 16 ± 1 g (ranging from 15 to 18 g) protein per meal in the NP group. A sample daily meal plan and an overview of the macronutrient distribution are provided in the Supplementary Information. To cover the remaining 10% of daily energy intake, subjects were obliged to choose foods that were low in fat and protein content (max 0.6 g protein per portion) from a list with allowed food products. They recorded these foods in a diary in which they also noted any deviations from the study protocol, illnesses and use of drugs.

Dietary analysis

To analyze the amount of energy and macronutrients in the diet as provided by the university, one complete diet for each group was collected throughout the intervention. The collected duplicates were stored at −20 °C until analysis. After the study, all duplicate meals were pooled, homogenized and analyzed. Total energy, fat, protein, dry matter, ash and fiber were analyzed and available carbohydrates were calculated. The nutrient composition of the remaining 10% of energy intake was calculated from the food dairies.

Compliance

Compliance was assured via daily contact (weekdays) with the investigators and dietitians. Dietitians supervised during meal time to ensure that the complete meal was consumed. Food diaries were discussed once per 2 weeks to ensure that the remaining 10% of energy intake was properly consumed. Body weight was measured two times per week on a digital balance with indoor clothing, without shoes and with empty pockets. If body weight changed >2 kg within the first weeks of the intervention, energy intake was adjusted.

Body composition

Fasted body weight was measured at baseline and at the end of the study to the nearest 0.1 kg using a digital balance (Seca, Bascule, MT, USA) with indoor clothing, without shoes and with empty pockets. Waist circumference was measured between the lower rib margin and the iliac crest, accurate to 0.5 cm, using a non-stretchable tape. Height was measured to the nearest 0.1 cm using a microtoise. Total LBM (primary outcome), appendicular LBM and FM was determined using dual-energy X-ray absorptiometry (model DPX-L; Lunar Radiation Corp, Madison, WI, USA; software version 1.31).

Muscle strength

Maximum leg strength was assessed by 1-RM strength tests on leg press and leg extension machines (Technogym, Rotterdam, The Netherlands). During a first familiarization session during the screening, the proper lifting technique was demonstrated and practiced, after which maximum strength was estimated using the multiple repetitions testing procedure for leg press and leg extension. Handgrip strength of the dominant hand was measured using a hydraulic hand dynamometer (Jamar, Jackson, MI, USA). Three consecutive measures of handgrip strength were recorded to the nearest 0.5 kg with subjects sitting in an upward position and the arm in a 90° angle. The maximum strength effort was reported.

Physical performance

Physical performance was assessed by two different tests, the Short Physical Performance Battery (SPPB) and a 400 m walk test. The SPPB consists of three components: balance, gait speed and chair rise ability. Scores of 0 to 4 were based on categories of performance in the balance test, on the time necessary to complete the walk, and on the time needed to perform the chair rise test. A summary performance score of 0 to 12 was calculated by summing the scores of the tests. The 400 m walk test assessed the time it takes to walk 400 m. Directly after finishing the test, subjects had to score the rate of perceived exertion from 6 to 20 points.

Physical activity

Subjects were asked to maintain their habitual physical activity level during the intervention. Physical activity was measured in counts per minute (c.p.m.) using a small-sized triaxial accelerometer (Actigraph GT3X; Actigraph LLC, Pensacola, FL, USA), worn on the waist. Subjects wore the actigraph during wakefulness for 7 consecutive days and were instructed not to wear them while bathing or swimming. The actigraph method enables a reliable and objective assessment of daily physical activity.[32–34] Activity counts were assessed at 1-min intervals. Data were included if subjects wore the actigraph for a minimum of 5 days and at least 10 h per day.

Blood pressure

Blood pressure was measured in the morning after 10 min of rest with an Omron HEM-907 (Omron Healthcare, Lake Forest, IL, USA) device. At baseline and end, four measurements were carried out, with an interval of 2 min, of which the first measurement was discarded. The mean value of the three subsequent measurements for systolic and diastolic blood pressure was taken.

Statistics

The sample size was based on a power calculation with an expected difference in LBM of 1.4 ± 1.6 kg between the HP and NP group.[35] With a power of 80% and a significance level (α) of 0.05, at least 23 participants are required per group. Sixty-one subjects were included to allow for drop-outs.

Data are presented as means ± s.d. To check if there were baseline differences between the groups, an independent sampled *t*-test was used. A paired *t*-test was used to locate if parameters changed over time. After checking the assumptions, analysis of covariance (ANCOVA) was applied to assess the differences between groups over time with diet group as the independent variable, end parameters as the dependent variable and BMI, gender and baseline parameters as covariates. Data were analyzed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). Results were considered statistically significant at the 0.05 level.

RESULTS

Subjects

An overview of the subjects' baseline characteristics is presented in Table 1. A total of 61 subjects participated in the intervention with a mean age of 63 ± 4.8 years and BMI of 31.2 ± 3.0 kg m^{-2}. One person in the HP group withdrew during the run-in week, because of the time constraints of the intervention (Supplementary Figure 1). No significant differences were observed in baseline values between groups.

Dietary intake

The actual composition of the intervention diets based on chemical analysis of the duplicate meals, food diaries and supplements is presented in Table 2. The HP diet group consumed on average 7.93 MJ per day with 27 En% from fat, 35 En% from carbohydrates and 34 En% from protein (1.69 g protein kg^{-1} per day). The NP diet group consumed on average 7.80 MJ per day with 24 En% from fat, 51 En% from carbohydrate and 19 En% from protein (0.92 g protein kg^{-1} per day). Habitual dietary protein intake measured by the food frequency questionnaire was 1.1 ± 0.4 g kg^{-1} per day.

Body composition

Body weight declined over time for both groups (HP: 92.8 ± 11.0–83.9 ± 10.1 kg; $P < 0.01$; NP: 90.5 ± 10.0–81.5 ± 9.7 kg; $P < 0.01$). However, no significant differences in weight loss were detected between the diet groups ($P = 0.584$). Waist circumference declined from 108 ± 8 to 97 ± 7 cm ($P < 0.01$) in the HP group and from 107 ± 7 to 97 ± 7 cm in the NP group ($P < 0.01$), with no significant differences observed between groups ($P = 0.921$). Similarly, BMI decreased in both groups: from 31.3 ± 3.0 to 27.5 ± 5.8 kg m^{-2} ($P < 0.01$) in the HP group and from 31.0 ± 2.9 to 27.9 ± 3.1 kg m^{-2} ($P < 0.01$) in the NP group. No significant difference between the diet groups was found ($P = 0.395$). LBM also changed, as it declined over time in both the HP group (from 54.8 ± 12.2 to 53.1 ± 11.4 kg; $P < 0.01$) and in the NP group (from 54.5 ± 9.3 to 52.4 ± 9.1 kg; $P < 0.01$), without a significant difference between the two groups ($P = 0.213$). In both groups, appendicular LBM decreased: from 23.8 ± 5.5 to 23.1 ± 5.4 kg and from 23.8 ± 4.8 to 22.8 ± 4.6 kg in the HP and NP group, respectively ($P < 0.01$), without a significant difference between the two diet groups ($P = 0.122$). Changes in body composition are presented in Figures 1 and 2.

Table 1. Baseline subjects' characteristics

	High protein group, n = 31	*Normal protein group,* n = 30
Male/female (*n*)	18/13	18/12
Age (years)	63 ± 4.8	62 ± 4.8
Body weight (kg)	92.8 ± 11.0	90.5 ± 10.0
BMI (kg/m^2)	31.3 ± 3.0	31.0 ± 2.9
Lean body mass (kg)	54.8 ± 12.2	54.5 ± 9.3
Fat mass (% BW)	37.7 ± 8.4	36.1 ± 8.0
Fat mass (kg)	34.4 ± 7.2	32.5 ± 8.2
Waist circumference (cm)	108 ± 8.4	107 ± 6.6
Fasting glucose (mmol l^{-1})	5.7 ± 0.5	5.8 ± 0.5

Abbreviations: BMI, body mass index; BW, body weight. Data represented as means ± s.d. No baseline differences between groups are observed with an independent sampled *t*-test.

Muscle strength

Leg strength declined over time in both groups, without a significant difference between the groups (for leg press $P = 0.689$; for leg extension $P = 0.296$). In accordance, handgrip strength declined over time in the HP group ($P = 0.013$), but not in the NP group ($P = 0.215$), with no significant differences observed between groups ($P = 0.210$). Strength and physical performance data are presented in Table 3.

Table 2. Diet composition

	High protein group, n = 31		*Normal protein group,* n = 30	
	g per day	*En%*	*g per day*	*En%*
Energy (MJ per day)	7.93	100	7.80	100
Energy (kcal per day)	1894	100	1863	100
Total fat	57	27	51	24
Total carbohydrate	170	35	236	51
Total protein	157	34	84	19
Protein (in g kg^{-1} per day)	1.69	34	0.92	19
Fiber	21.3	2	33.9	4
Alcohol	7.7A	3	7.1	3

Abbreviation: En%, Energy%.

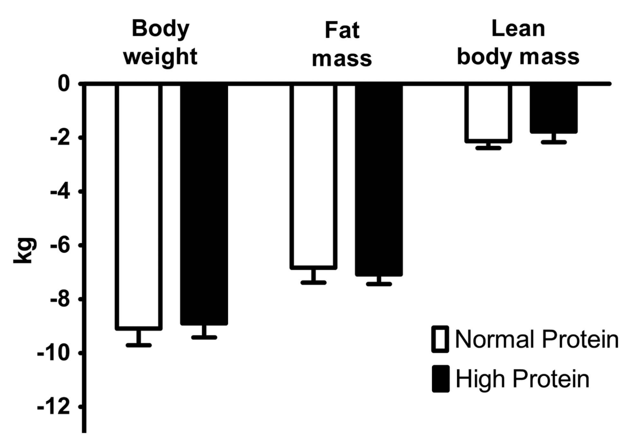

Figure 1. Body composition changes after 12 weeks in the NP and HP group. Paired *t*-tests showed that body weight, FM and LBM all changed over time. No differences are found between groups, as indicated by an analysis of covariance (ANCOVA). No differences in loss of LBM are found between men and women ($P = 0.50$) and between overweight and obese subjects ($P = 0.52$). Data are represented as mean ± s.d.

Physical performance

The total scores of the SPPB did not change over time (11.5 ± 0.7 to 11.6 ± 0.5 points; $P = 0.132$) and no significant differences were observed between groups ($P = 0.483$). The 400 m walking velocity increased from 1.45 ± 0.19 to 1.49 ± 0.21 ms^{-1} ($P < 0.01$) without a difference between the two groups ($P = 0.219$). Strength and physical performance data are presented in Table 3.

Physical activity

Habitual physical activity did not change over time in the HP group ($P = 0.748$; baseline value 916 ± 203 c.p.m.) or the NP ($P = 0.176$; baseline value 825 ± 258 c.p.m.). No significant differences in habitual physical activity were observed between groups ($P = 0.685$).

Blood pressure

Systolic blood pressure decreased over time from 139 ± 18 to 132 ± 15 mmHg ($P < 0.01$) in both groups. Diastolic blood pressure decreased from 82 ± 9 to 77 ± 8 mmHg ($P < 0.01$) in both groups. No significant differences between groups were observed for systolic ($P = 0.279$) and diastolic ($P = 0.170$) blood pressure.

DISCUSSION

In the present study, we observed a nearly 10 kg reduction in body weight following 12 weeks of 25% energy intake restriction in overweight, older adults. The reduction in body mass was accompanied by a 2 kg decline in LBM and as much as a 10% decline in leg strength. Increasing dietary protein intake from 0.9 to 1.7 g kg^{-1} per day during the intervention did not preserve LBM nor did it prevent the decline in muscle strength associated with prolonged energy intake restriction.

Twelve weeks of energy intake restriction (25%) successfully reduced body weight by 9.0 ± 3.1 kg in this group overweight older adults ($P < 0.01$; Figure 1). These results are in line with previous work showing that 12 weeks of 20–33% energy intake reduction induced a 7–10 kg decline in body weight in overweight and obese adults.[6,12,20,36] The loss of body weight in the present study was attributed to a decline in both FM (7.0 ± 2.5 kg) and LBM (2.0 ± 1.8 kg; $P < 0.01$; Figure 1). The changes in LBM were correlated with the changes in appendicular LBM ($R = 0.697$; $P < 0.01$), the latter ranging from as much −3.8 to +1.9 kg (Figure 2). The overall decline in LBM was accompanied by a concomitant −8.9 ± 13.3 kg decline in leg strength (−42 to +16 kg; $P < 0.01$). Despite the reduction in leg strength, physical performance assessed with the 400 m walking speed test showed a significant improvement following dietary intervention (Table 3). This was at least partly attributed to the decline in FM ($R = −0.243$; $P = 0.06$). The SPPB score did not change over time, probably because the high baseline scores that limit the range for improvement (average score 11.5 ± 0.7 of 12 points maximum). Energy intake restriction improved blood pressure, BMI and waist circumference in both groups.

Despite the benefits of prolonged energy intake restriction to reduce body FM and improve metabolic function, the loss of skeletal muscle mass and strength is particularly undesirable in an older population prone to sarcopenia.[37] Therefore, effective dietary strategies should be developed to preserve muscle mass during a period of energy intake restriction. During a period of energy intake restriction, muscle protein synthesis rates usually decrease,[8] which would lead to the loss of LBM. However, this decline in muscle protein synthesis rates can be attenuated by the intake of 27 g whey protein.[38] Increasing the protein content of the diet might therefore be an effective strategy to preserve muscle mass during prolonged energy intake restriction, thereby preserving strength and physical performance. This is confirmed by previous work showing that an HP diet can preserve LBM during prolonged energy intake restriction.[6–8,11,12] In the present study, we tested our hypothesis that a higher protein intake preserves LBM during prolonged energy intake restriction in overweight, older adults. We compared the impact of a higher protein diet (1.7 g protein kg^{-1} per day; 34 En% protein) with an NP diet (0.9 g protein kg^{-1} per day; 19 En% protein) on LBM, muscle strength and physical performance (Table 2). Despite substantial declines in LBM in both the normal and HP groups (2.1 ± 1.4 and 1.8 ± 2.2 kg, respectively), we observed no differences between groups ($P = 0.213$; Figure 2). These findings are in contrast with some,[6–8] but certainly not all[9,10] studies. The apparent discrepancy may be attributed to differences in the level of dietary control,[7–9,11–16,18,20] the protein content in the diets,[15,16,18] the duration of the intervention period,[7,8,11,39] the level of physical activity[10,13,35] or the specific population that is studied.[7–9,11–16,20]

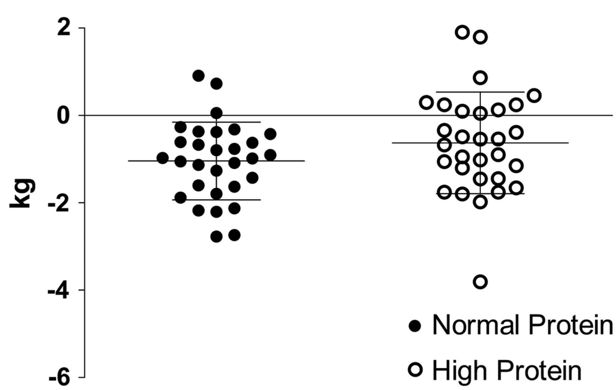

Figure 2. Individual changes in appendicular LBM following 12 weeks of energy intake restriction with or without an increase in protein intake. Appendicular LBM changed over time, indicated by a paired *t*-test. ANCOVA testing showed no significant differences between groups. Data are represented as mean ± s.d.

Table 3. Muscle strength and physical performance before and after intervention

	High protein group				*Normal protein group*			
	n	*Baseline*	*End*	*Δ*	n	*Baseline*	*End*	*Δ*
1-RM leg press (kg)	28	152 ± 44	143 ± 39	− 9 ± 14[a]	25	157 ± 33	148 ± 30	− 9 ± 13[a]
1-RM leg extension (kg)	27	93 ± 31	91 ± 29	− 2 ± 8	26	98 ± 25	94 ± 25	− 4 ± 4[a]
Handgrip strength (kg)	30	40 ± 11	37 ± 9	− 3 ± 6[a]	30	41 ± 10	40 ± 11	− 1 ± 5
SPPB (score)	30	11.6 ± 0.7	11.7 ± 0.5	0.1 ± 0.7	30	11.4 ± 0.9	11.6 ± 0.6	0.1 ± 0.7
400 m walk ($m s^{-1}$)	30	1.46 ± 0.19	1.50 ± 0.20	0.05 ± 0.09[a]	29	1.45 ± 0.19	1.47 ± 0.22	0.02 ± 0.08

Abbreviations: ANCOVA, analysis of covariance; 1-RM, 1-repetition maximum; SPPB, Short Physical Performance Battery. Data are represented as means ± s.d. ANCOVA testing showed no significant differences between groups. [a]Data with represent significant differences over time ($P < 0.01$), indicated with a paired *t*-test.

Through our strict dietary control, whereby we provided all subjects with 90% of their prescribed energy intake, the influence of possible confounders is limited. Dietary compliance was assured by daily contact (on weekdays) with the investigators and dietitians and body weight measurements performed two times weekly. No subject failed to consume the supplement more than two times during the entire intervention period as reported in the food intake diaries. Moreover, the results confirm the high level of compliance of the subjects in both groups showing a 10% reduction in body weight with a concomitant decrease in waist circumference of 10.3 ± 4.3 cm. In addition, by collecting duplicate meals, we were also able to analyze the actual macronutrient composition of the diets, thereby knowing the exact protein content of diets consumed in both intervention groups. Furthermore, people generally reduce overall energy intake when the level of dietary protein is increased,[40] because of the satiating effect of protein.[41] By providing the subjects with nearly their entire diet in the current study, energy intake remained equal in both intervention groups (7.66 MJ per day in the HP group, 7.38 MJ per day in the NP group). Consequently, the NP and HP diet groups lost exactly the same amount of body weight (-9.1 ± 3.4 and 8.9 ± 2.9 kg, respectively). In addition, it is well known that underreporting of energy intake is common in dietary interventions.[23,42] Overweight subjects typically underestimate energy (and macronutrient) intake, which can make data interpretation difficult.[43] The results of the current study cannot be influenced by underreporting because intake of energy and macronutrients does not rely on food intake diaries, but is based on macronutrient analyses of the diet as provided by the university.

The current study aimed to investigate the additional effect of an HP diet over an NP diet containing $0.9\,g\,kg^{-1}$ per day, the latter representing habitual protein intake.[24,25] Several previous studies consider intakes of $1.1\,g\,kg^{-1}$ per day as an HP diet,[9,20,44] and intakes of $0.8\,g\,kg^{-1}$ per day or lower as normal to low protein diets.[7,8,14,16,20] The present study demonstrates 20% loss of LBM expressed as a proportion of total weight lost in the HP group—an effect also observed in the comparable literature.[6,12] Surprisingly, we demonstrate that the NP group experienced a lower percentage of LBM loss (23% of total weight lost) compared with the results of the aforementioned studies showing a 35% loss of LBM with similar protein intakes of 18%.[6,13] Thus, comparing our data with the literature suggests that maintaining habitual protein intake may be more important compared with increasing protein intake beyond habitual protein intake levels as a means to preserve LBM during energy intake restriction. It is hereby important that protein intake during energy intake restriction is expressed in En%, as well as in $g\,kg^{-1}$ per day. When energy intake is reduced and protein intake is expressed only in En%, protein intake might falsely be defined as normal while absolute protein intake is decreased.

The protein intake in the current study was on average 34 g per meal and evenly distributed throughout the day, which is in accordance with the current guidelines for elderly.[45,46] Specifically during energy restriction, an evenly distributed protein intake with lower protein amounts per meal appears to be more effective or as good as a skewed protein intake pattern in preserving muscle protein synthesis rates or LBM.[47,48] Details about the foods and macronutrient distribution are provided in Tables 1–3 of the Supplementary Information.

The current study had an intervention period of 12 weeks and is therefore in line with previous work that showed a positive effect of an HP diet on LBM.[6,12,13] A recent meta-analysis[18] concluded that an intervention period of 12 weeks or longer is required to show any measurable intervention effect on LBM preservation. Thus, the current study is most likely of sufficient duration to be able to detect any possible differences between diets.

The present study only focuses on the effect of a dietary intervention on LBM. However, concurrent changes in physical activity level may interact with the impact of dietary intervention. To account for individual variability in physical activity levels, the physical activity status of each subject was measured before and at the end of the intervention period. The measurements indicate that habitual physical activity levels did not change over time in either group. Therefore, we can safely assume that changes in physical activity levels did not impact the outcome of the present study. Studies that included exercise as a cointervention generally show a greater effect of a higher protein intake diet on LBM preservation, which has been confirmed by an RCT and systematic review.[10,21,35,49,50]

The study population of the current work are older men and women (55–70 years), whereas most previous work has been performed in young to middle-aged adults.[7,8,11–16] Although weight loss shows clear health benefits for overweight and obese adults, recent studies indicate that weight loss is not beneficial *per se* in an older population.[51] A key determinant of healthy weight loss in the elderly is the amount of fat and not lean mass loss.[51] The present study confirms that weight loss causes a substantial decline in muscle mass and strength. Therefore, interventions that limit the loss of muscle mass and strength during weight loss are of particular clinical relevance for the older adults.[37]

Our observations imply that it is important to maintain a sufficient amount of protein intake during weight loss. Thus, decreases in energy intake should be derived mainly from decreases in carbohydrates and fat intake. Future studies could aim to optimize protein distribution throughout the day. However, current literature shows that total preservation of LBM is not possible with only a dietary intervention. The combination of exercise and energy intake restriction is generally more effective in preserving LBM.[35,52]

In conclusion, increasing dietary protein intake above habitual intake levels does not preserve LBM, strength or physical performance during prolonged energy intake restriction in overweight older adults.

CONFLICT OF INTEREST

The project is funded by TI Food and Nutrition, a public–private partnership on precompetitive research in food and nutrition. The researchers are responsible for the study design, data collection and analysis, decision to publish and preparation of the manuscript. The industrial partners have contributed to the project through regular discussion. The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

EB, MT, LL and LG designed research; EB, MT, KB and PC conducted research; EB analyzed data; EB, MT, LL and LG wrote the paper; LG had primary responsibility for final content. All authors read and approved the final manuscript.

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