

# Impact of Protein Intake during Weight Loss on Preservation of Fat-Free Mass, Resting Energy Expenditure, and Physical Function in Overweight Postmenopausal Women: A Randomized Controlled Trial

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## Keywords

Weight loss · Protein · Fat-free mass · Resting energy expenditure · Physical function

## Abstract

**Introduction:** Weight loss in old age increases the risk of sarcopenia caused by the age-related reduction of fat-free mass (FFM). Due to the strong correlation between FFM and resting energy expenditure (REE), the maintenance of this must also be considered. Besides, the physical function (PF) must be maintained. **Objective:** The impact of protein intake on changes in FFM, REE, and PF during weight loss in overweight postmenopausal women was investigated. **Methods:** Fifty-four postmenopausal women (BMI  $30.9 \pm 3.4$ ; age  $59 \pm 7$  years) were randomized into 2 groups receiving energy-restricted diets with either 0.8 g (normal protein; NP) or 1.5 g protein/kg body weight (high protein; HP) for 12 weeks, followed by a 6-month follow-up phase with an ad libitum food intake. FFM, REE, and PF (strength, endurance, and balance) were measured at baseline, after weight loss, and after follow-up. **Results:** Forty-six women completed the weight loss intervention and 29 were followed up. The weight loss was  $-4.6 \pm 3.6$  kg (HP) and  $-5.2 \pm 3.4$  kg (NP; both  $p < 0.001$ ) and the weight regain during follow-up was  $1.3 \pm 2.8$  kg (HP;

$p = 0.03$ ) and  $0.4 \pm 2.5$  kg (NP;  $p = 0.39$ ), with no differences between groups. Similar decreases in FFM ( $-0.9 \pm 1.1$  [HP] vs.  $-1.0 \pm 1.3$  kg [NP]) and REE ( $-862 \pm 569$  [HP] vs.  $-1,000 \pm 561$  kJ [NP]; both  $p < 0.001$ ) were observed in both groups. During follow-up, no changes in FFM were detected in either group, whereas in the NP group the REE increased again ( $+138 \pm 296$ ;  $p = 0.02$ ). The main determinants of FFM loss were the energy deficit and the speed of weight loss. In the NP group, the Short Physical Performance Battery score improved with weight loss ( $+0.6 \pm 0.8$ ;  $p < 0.001$ ) and handgrip strength decreased ( $-1.7 \pm 3.4$  kg;  $p < 0.001$ ), whereas no changes were observed in the HP group. **Conclusions:** An HP weight-loss diet without exercise had no impact on preservation of FFM and REE but may help to maintain muscle strength in postmenopausal women.

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Published by S. Karger AG, Basel

## Introduction

In Germany 61% of women aged 50–59 years are overweight and 27% are obese, and the prevalence increases with age [1]. Obesity can be associated with reduced muscle mass and strength, especially in older people [2]; this is known as “sarcopenic obesity” [3] and it increases the

risk of low physical function (PF) [4]. Weight reduction in particular is also associated with a loss of lean muscle mass [4] and thus increases the risk of sarcopenia in older adults [3]. The combination of a higher prevalence of obesity and sarcopenia in women increases the gender-specific risk [5, 6]. Nonetheless, intentional weight loss can improve the PF [7, 8] and metabolic profile [7, 9, 10]. Therefore, suitable scientifically based dietary recommendations for weight reduction in older people should be generated to minimize the risk of FFM loss. Previous studies have shown that weight loss with a high-protein (HP) diet (1.4 vs. 0.8 g/kg protein/day) may help to preserve FFM in adults aged 20–80 years [9–11]. Two studies in older adults (age 50–70 years) showed a better preservation of FFM with a higher protein intake (30 vs. 15% protein of the total energy intake or 1.11 vs. 0.85 g/kg) during a weight loss of about 9 kg in 20 weeks [12] and 3 kg in 13 weeks, respectively, but the latter with an exercise program [13]. In contrast, some other studies did not show an improved preservation of lean mass with a higher protein content in a weight loss diet (10 weeks to 6 months) in older people (age > 55 years; 0.8–1.0 vs. 1.2–1.7 g/kg protein) [14, 15], one of them with exercise [16]. Also after bariatric surgery, which is accompanied by a significant loss of muscle mass, an HP diet does not prevent muscle mass loss [17]. The designs of the available studies (intervention period, gender, age, and with and without exercise), however, are very heterogeneous and do not allow clear conclusions. In contrast to middle-aged adults, the presence of significant effects in older persons is missing. Furthermore, not all of them were randomized controlled studies (RCT) and only 1 of them had a blinded design. For this issue, no double blinded RCT in postmenopausal women is available.

Due to the association between FFM and resting energy expenditure (REE) [18], the weight loss-induced decrease in FFM also impairs REE and thus increases the risk of weight regain [19]. In addition, Porter Starr et al. [7] showed that a high protein intake (1.2 vs. 0.8 g/kg) can help to improve or maintain PF during weight reduction even without physical exercise in adults aged > 60 years.

The aim of this study was to investigate the effect of an HP diet without exercise during weight loss on changes in FFM, REE, and PF in overweight postmenopausal women. We hypothesized that postmenopausal women on an HP diet experience less of a decrease in muscle mass, REE, and PF during weight reduction compared to the control group. The primary outcome was FFM. Secondary outcomes included REE and PF.

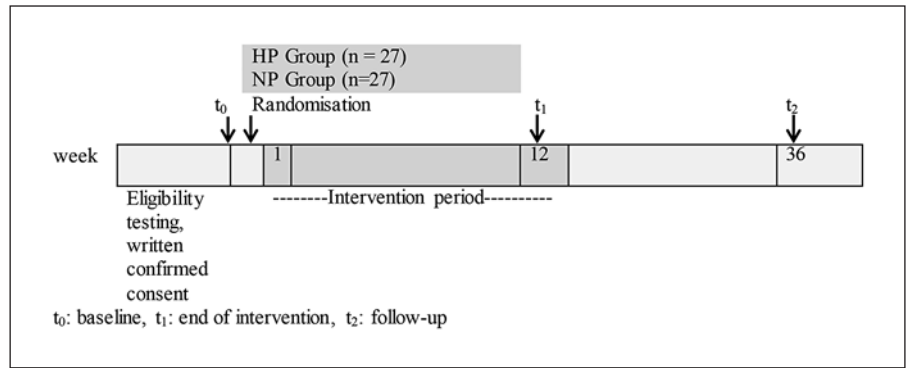
## Subjects and Methods

This study was conducted at the Department Nutritional, Food and Consumer Sciences of the Fulda University of Applied Sciences. Fifty-four women were recruited via local flyers or advertisement and randomized in a 1: 1 ratio to 2 parallel arms, i.e., a normal protein group (NP; 0.8 g/kg body weight/day, based on current recommendations at the time of the start of the study [20]) and a HP group (HP; 1.5 g/kg). In order to be included in this study, participants aged  $\geq 50$  years and with a BMI  $\geq 30$  or  $\geq 27$  and waist circumference > 88 cm had to be able to walk without auxiliaries, climb 10 stairs, and to do their typical daily activities. The estimated physical activity level (PAL) had to be < 1.8 and constant during the last 3 months. All women provided written informed consent before randomization. Participants were excluded for the following reasons: BMI  $\geq 35$ ; creatinine > 2.0 mg/dL; weight change >  $\pm 5\%$  in the last 6 months; type 2 diabetes; thyroid disease; medication (steroids, diuretics, thyroid drugs, statins, weight loss medication, and  $\beta$ -blockers); participation in other weight reduction programs; kidney, heart, or liver failure; protein supplementation during the last 3 months; neurological disorders; electronic implants; active prostheses; life-sustaining electronic devices; aversion or allergy to whey products (self-information).

A 1: 1 randomization with random block sizes between 12 and 16 was done by an independent computer scientist. A computer-generated list with random numbers was used and, relevant to block size, allocated to a ranking. The study participants and study staff were blinded to the allocation into groups. Annulment of blinding was done after completion of the study.

The intervention period of 12 weeks included 4 nutrition training sessions for both groups separately and telephone interviews to enhance compliance. The weight loss period was followed by an ad libitum food intake for 6 months without intervention (follow-up phase). The primary outcome was the change in FFM after the 12-week weight loss period, and secondary outcome parameters were changes in REE and PF after 12 weeks. An outline of the study protocol is given in Figure 1.

The individual total energy expenditure was calculated as the measured REE multiplied by the PAL (estimated based on a 7-day activity diary) and reduced by about 3,139 kJ (750 kcal) per day for both groups to derive the individual prescription of energy intake. At baseline, dietary history gave information about the preferences and aversions toward food. The individually prescribed energy intake was translated into a meal plan as follows: the participants received a meal replacement (Precon shake; Darmstadt, Germany; 1,565 kJ [374 kcal], 12.2 g fat, 8.7 g carbohydrates, and 46.6 g protein per 100 g) 2 times a day prepared with 300 mL milk (1.5% fat). The third meal and/or snack should be chosen from the individual diet plan. The shakes of the HP group were fortified with whey powder (Primal State, Berlin, Germany; 1,686 kJ [403 kcal], 8.5 g fat, 5.7 g carbohydrates, 76 g protein per 100 g) to achieve the target amount of protein. The differences in protein intake were compensated for by the caloric load on carbohydrate intake in the control group. There were no restrictions of timing for meal intake. For the first 3 weeks, women obtained individual daily meal plans and at each following session they received 10 new plans. Two dietitians were trained in advance to ensure the same content and methods were used during training in all of the groups. Participants had to keep a food diary (after the first and third training sessions for 7 consecutive days) and food checklists (on the remaining days).



**Fig. 1.** Schematic representation of this study.

Anthropometry, REE, and PF measurements were done by a trained nutritionist at baseline ( $t_0$ ), after the intervention time ( $t_1$ ), and after the ad libitum phase ( $t_2$ ).

REE was measured under standardized conditions after an overnight fast in the morning using indirect calorimetry (Quark RMR; Cosmed, Germany). Before REE was recorded for at least 20 min, the subjects lay supine on a patient couch for 30 min. The first 10 min of data were discarded. The calorimeter was calibrated each morning according to the manufacturer's guidelines.

Body height was determined barefoot in a standing position using a stadiometer (seca 274; seca GmbH, Hamburg, Germany). Body weight was recorded after an overnight fast barefoot in light, everyday clothing using a bioelectrical impedance analysis (seca medical body composition analyzer; seca mBCA 515/514). FFM and fat mass were measured with the patient in a standing position and barefoot, with 8 electrodes for both hands and feet (seca mBCA 515/514), under standardized conditions, with an empty bladder, minimal fasting time, and abstinence from strenuous physical exercise of 8 h, respectively. Waist circumference was measured in an upright position between the lower rib and the iliac crest using a seca tapeline.

The Short Physical Performance Battery (SPPB) [21] was used to measure 3 categories, i.e., balance, gait speed, and strength. Women had to walk a distance of 400 m as fast as they could without running. A Jamar hand dynamometer was used to measure isometric hand grip strength using the standardized Southampton protocol [22].

Fasting blood samples were analyzed at the Fulda clinic for concentrations of serum urea nitrogen in order to assess differences in protein intake among study groups. To assess kidney and liver function, the glomerular filtration rate and creatinine and albumin levels were measured.

Target weight loss was calculated using a body weight planner [23] based on weight, sex, age, height, PAL, % fat mass, % calories from carbohydrates and REE and compared with the actual weight loss for evaluation of compliance.

To detect differences in changes in FFM with weight loss between groups, 27 women per group were needed at a significance level of  $\alpha = 0.05$  and a power of 80%. This calculation was based on an effect size of 0.69 and an SD of 1.5 [10]. Data are presented as means  $\pm$  SD. An intention-to-treat analysis with the last observation carried forward for all of the participants and a per protocol analysis for the participants who completed the study were conducted. Between-subject analyses served to compare outcomes be-

**Table 1.** Baseline characteristics of the study participants by treatment group

	HP group (n = 27)	NP group (n = 27)	p value <sup>a</sup>
Age, years	59.0 $\pm$ 6	58.7 $\pm$ 6	0.566
PAL	1.4 $\pm$ 0.1	1.4 $\pm$ 0.1	0.377
Body weight, kg	85.1 $\pm$ 8.6	84.9 $\pm$ 9.8	0.929
BMI	30.5 $\pm$ 2.8	31.3 $\pm$ 4.0	0.439
FFM, kg	46.8 $\pm$ 6.9	46.7 $\pm$ 5.0	0.940
Fat mass, kg	38.3 $\pm$ 5.6	38.2 $\pm$ 6.9	0.947
Waist circumference, cm	98.2 $\pm$ 6.9	98.4 $\pm$ 10.8	0.878
REE, kJ (kcal)	7,174 $\pm$ 707 (1,714 $\pm$ 169)	7,082 $\pm$ 837 (1,692 $\pm$ 200)	0.665
SPPB score	9.4 $\pm$ 1.1	9.9 $\pm$ 1.0	0.119
Handgrip strength, kg	28.7 $\pm$ 7.2	29.0 $\pm$ 4.9	0.652
400-m walk, min:s	4:10 $\pm$ 0:33	04:11 $\pm$ 0:31	0.692
Urea, mg/dL	33.2 $\pm$ 7.6	27.5 $\pm$ 5.6	0.003

Data are presented as means  $\pm$  SD. <sup>a</sup> Significance level for an independent *t* test.

tween groups over time (independent *t* test), and within-subject analyses were used to measure effects over time (dependent *t* test,  $t_0 - t_1$ ,  $t_1 - t_2$ ). An analysis of variance (ANOVA), if applicable with a Greenhouse-Geisser correction, was also done with Bonferroni-corrected pairwise comparisons to detect specific time differences. The significance level was set at  $\alpha = 0.05$ .

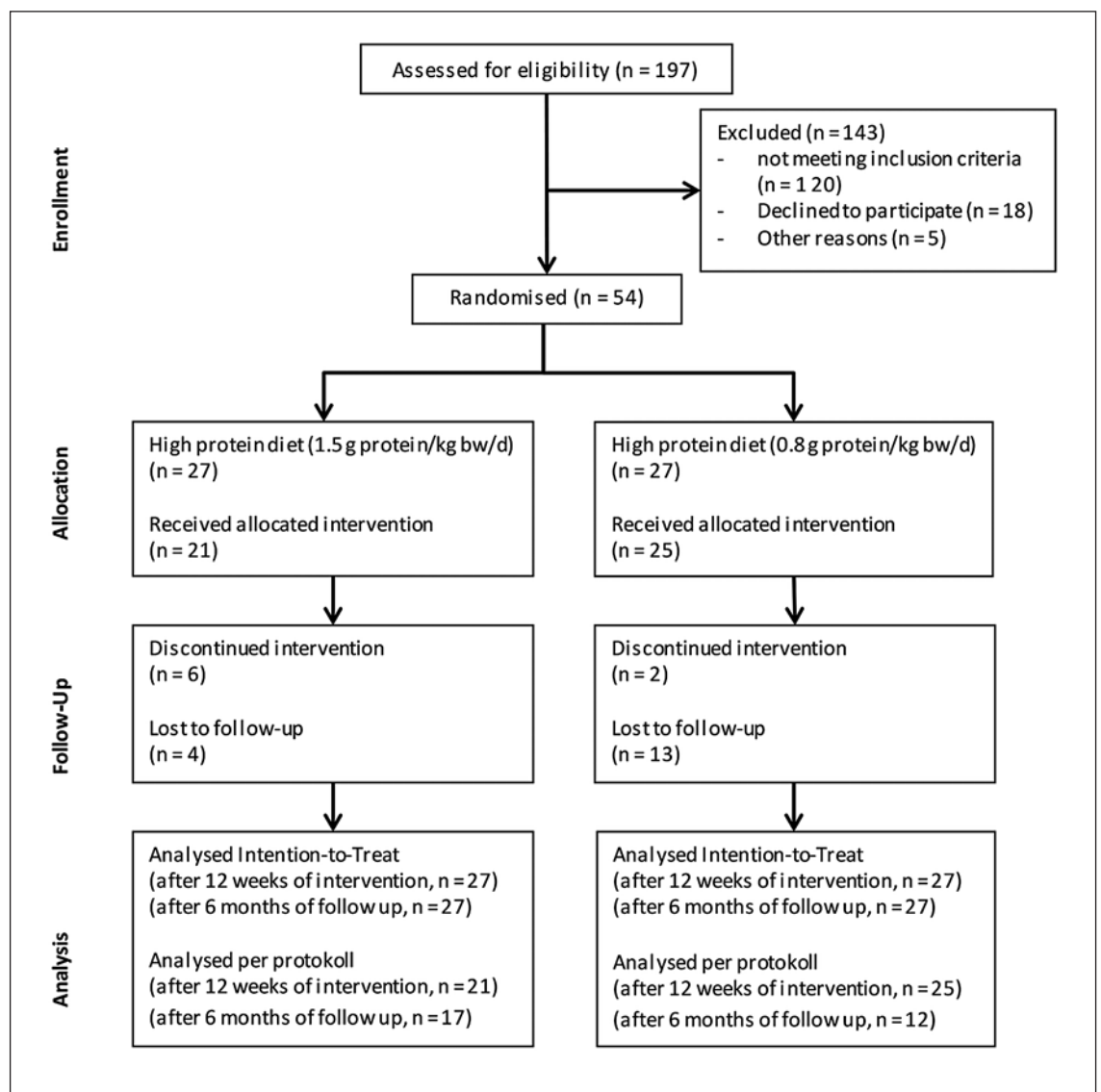
## Results

After screening 197 subjects for eligibility (October 2016 to April 2017), 54 women were randomized into 2 groups (Fig. 2). Eight women dropped out during the intervention because of adverse events not related to this study ( $n = 5$ ) and nonacceptance of the shakes ( $n = 3$ ). Twenty-nine women completed this study, including the

**Table 2.** Dietary energy and macronutrient intake data in the first and third quarters of the intervention

		Energy, kJ/day (kcal/day)	Protein, g/day	Carbohydrates, g/day	Fat, g/day
Intervention weeks 1–3	HP (n = 21)	6,048±695 (1,445±166)	114±16	127±22	45±8
	NP (n = 25)	5,249±917 (1,254±219)*	61±9*	134±37	46±14
Intervention weeks 7–9	HP (n = 20)	6,082±992 (1,453±237)	112±22	126±20	48±12
	NP (n = 25)	5,584±1101 (1,334±263)	65±11*	138±33	51±12
Mean	HP (n = 20)	6,065±737 (1,449±176)	113±17	127±18	46±9
	NP (n = 25)	5,417±904 (1,294±216)*	63±9*	136±29	48±11

Data are presented as means ± SD. \* Significant difference between groups ( $p < 0.05$ , independent  $t$  test).



**Fig. 2.** CONSORT flow chart of the study design.

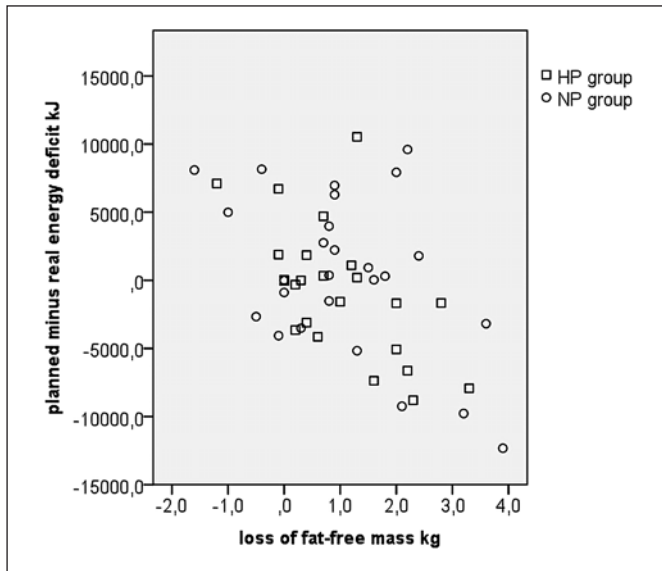
**Table 3.** Absolute changes in body weight, FFM, fat mass, waist circumference, REE, SPPB, 400-m walk, and handgrip with weight loss and follow-up by treatment group

	HP group		NP group		<i>p</i> value (HP × NP) <sup>b</sup>
	mean ± SD	<i>p</i> value <sup>a</sup>	mean ± SD	<i>p</i> value <sup>a</sup>	
Body weight, kg					
Baseline	85.1±8.6		84.9±9.8		
Change at t <sub>1</sub>	-4.6±3.6	<0.001	-5.2±3.4	<0.001	0.538
Change at t <sub>2</sub>	+1.3±2.8	0.028	+0.4±2.5	0.392	0.253
FFM, kg					
Baseline	46.8±6.9		46.7±5.0		
Change at t <sub>1</sub>	-0.9±1.1	<0.001	-1.0±1.3	<0.001	0.575
Change at t <sub>2</sub>	+0.4±1.4	0.146	-0.0±1.6	0.890	0.181
FM, kg					
Baseline	38.3± 5.6		38.2±6.9		
Change at t <sub>1</sub>	-3.8±2.8	<0.001	-4.0±2.7	<0.001	0.718
Change at t <sub>2</sub>	+0.9±2.1	0.040	+0.3±2.1	0.478	0.315
Waist circumference, cm					
Baseline	98.2±6.9		98.4±10.8		
Change at t <sub>1</sub>	-6.5±4.2	<0.001	-7.2±4.0	<0.001	0.669
Change at t <sub>2</sub>	-0.6±4.6	0.525	-0.3±2.4	0.522	0.756
REE, kJ (kcal)					
Baseline	7,175±707 (1,714±169)		7,083±837 (1,692±200)		
Change at t <sub>1</sub>	-862±569 (-206±136)	<0.001	-1,000±561 (-239±134)	<0.001	0.369
Change at t <sub>2</sub>	+25±276 (+ 6±66)	0.650	+138±297 (+33±71)	0.022	0.148
SPPB, score					
Baseline	9.4±1.1		9.9 ±1.0		
Change at t <sub>1</sub>	+0.4±0.9	0.051	+0.6±0.8	<0.001	0.463
Change at t <sub>2</sub>	+0.4±0.7	0.015	+0.1±0.4	0.327	0.073
400-m walk, min:s					
Baseline	4:10±0:33		04:11±0:31		
Change at t <sub>1</sub>	-0:00±0:07	0.876	-0:05±0:12	0.045	0.281
Change at t <sub>2</sub>	+0:01±0:19	0.630	+0:02±0:26	0.636	0.919
Handgrip, kg					
Baseline	28.7±7.2		29.0±4.9		
Change at t <sub>1</sub>	+0.1±2.6	0.798	-1.6±3.3	<0.001	0.041
Change at t <sub>2</sub>	+0.1±1.9	0.864	-0.2±1.5	0.493	0.577

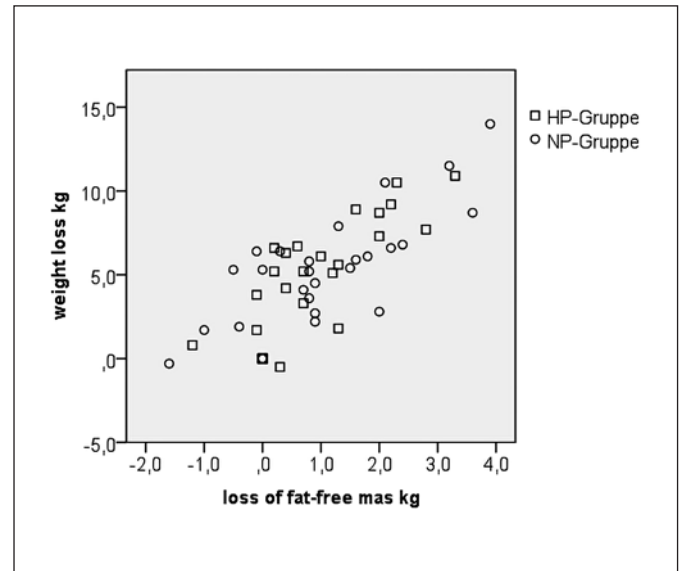
<sup>a</sup> Significance level for dependent *t* test within-group changes (t<sub>0</sub>-t<sub>1</sub>; t<sub>1</sub>-t<sub>2</sub>). <sup>b</sup> Significance level for independent *t* test between-group changes.

follow-up. Baseline characteristics are shown in Table 1. There were no significant differences in baseline characteristics between the groups, except for a higher serum urea in the HP group (*p* = 0.003). During the intervention, the HP group consumed on average 113 ± 17 g protein (1.4 ± 0.1 g/kg) and the NP group 63 ± 9 g protein per day (0.8 ± 0.1 g/kg; Table 2). The results of the per protocol analysis were not different from the results of the intention-to-treat analysis. So, only the results of the intention-to-treat analysis are presented.

No differences were found between the predicted and real energy deficits calculated by the body weight planner [23]; the values were: -179 ± 1,117 kJ/day (-43 ± 267 kcal/day; *p* = 0.468) in the HP group and 105 ± 1,326 kJ/day (25 ± 317 kcal/day; *p* = 0.686) in the NP group. However, there was a difference between the real and predicted changes in FFM in both groups (1.7 ± 1.1 kg; *p* < 0.001 [HP] and 1.4 ± 1.4 kg; *p* < 0.001 [NP]). The recorded total energy intake was higher in the HP group than in the NP group. The deviation of the recorded energy intake from the planned one was smaller in the HP



**Fig. 3.** Loss of lean mass vs. the planned minus real energy deficit. Correlation coefficient of the HP group:  $r = -0.58$ ;  $p = 0.002$ . Correlation coefficient of the NP group:  $r = -0.45$ ;  $p = 0.019$ . Correlation coefficient overall:  $r = -0.49$ ;  $p < 0.001$ .



**Fig. 4.** Loss of lean mass vs. weight loss. Correlation coefficient of the HP group:  $r = -0.80$ ;  $p < 0.001$ . Correlation coefficient of the NP group:  $r = -0.78$ ;  $p < 0.001$ . Correlation coefficient overall:  $r = -0.78$ ;  $p < 0.001$ .

group than in the NP group, but this was not significant ( $p = 0.44$ ).

The intervention led to a similar weight loss in both groups (Table 3), which was still significant at the end of the follow-up phase. ANOVA showed no within-group differences during follow-up ( $t_1$  vs.  $t_2$ ;  $p = 0.074$ ) and no between-group differences ( $t_1$ :  $p = 0.538$ ,  $t_2$ :  $p = 0.264$ , and over time:  $p = 0.343$ ). A higher energy deficit and a higher weight loss, respectively, were associated with a higher loss of FFM ( $r = -0.49$ ;  $p < 0.001$ ,  $r = 0.78$ ;  $p < 0.001$ ; Fig. 3, 4). No significant between-group differences were observed for changes in FFM, fat mass, and waist circumference at  $t_1$  and  $t_2$  (all  $p > 0.05$ ). The decrease in FFM did not correlate with protein consumption (in g/kg FFM). Thirty-nine percent of the variance in FFM loss was explained by the speed of weight loss during the first 3 weeks and the energy deficit.

REE showed a similar decrease in weight loss in both groups ( $p = 0.369$ ). At  $t_2$  significant reductions remained without a group effect ( $p = 0.871$ ). After adjusting REE for FFM using regression analysis, REE remained reduced with weight loss ( $p < 0.001$ ).

The SPPB revealed improvements with weight loss (HP:  $p = 0.051$ ; NP:  $p < 0.001$ ) and follow-up (both groups:  $p < 0.001$ ), with no differences between groups. Results from the 400-m test remained unchanged in both groups

with weight loss and follow-up. Handgrip strength remained unchanged with weight loss ( $p = 0.798$ ) in the HP group but decreased in the NP group ( $p = 0.019$ ). This group effect was significant ( $p = 0.041$ ).

## Discussion

The hypothesis that an increased protein intake without exercise during weight loss in postmenopausal women promotes the preservation of FFM was not confirmed. Reasons for the lack of group effects in the weight loss phase may be the large difference between the predicted and real FFM changes. However, compliance was the same in both groups, so there is no systematic bias. Limitations in measurement precision and the validity of the bioelectrical impedance analysis during the instable phase of weight loss, i.e., because of a decrease in FFM hydration [24], would be another reason, though there needs to be at least a minimal difference, which could not be shown. Furthermore, our results are consistent with studies that used dual X-ray absorptiometry or air displacement plethysmography (Table 4); Backx et al. [15] used 1.7 vs. 0.9 g/kg protein in their 12-week RCT and achieved a weight loss of about 9 kg in 61 subjects aged  $> 55$  years, without differences in FFM loss between the groups. Similarly, in

**Table 4.** Intervention studies on the effects of protein intake during weight loss on FFM

Study	Proband characteristics	Protein intake	Energy restriction	Weight reduction	FFM change
Johnston et al. [27]	21 subjects Male and female Age 20–75 years BMI 20–42	125 g/day 80 g/day	–500 kcal/day from the TEE (calculated using a formula) 6 weeks	–2.3±2.9 kg (P) –2.0±1.8 kg (C) Group difference n.s.	+1.5±3.8 kg (P) –0.5±1.5 kg (C) Group difference $p = 0.008$
Verreijen et al. [13]	88 subjects Male and female Age >55 years BMI >30 (men) or >28 (women) and waist circumference >88 cm (women) or >102 cm (men)	1.11 g/kg BW/day 0.85 g/kg BW/day	–600 kcal/day from the TEE (REE measured by indirect calorimetry + PAL measured using a 3-day movement diary) 13 weeks	–3.4±3.6 kg –2.8±2.8 kg Group difference n.s.	Appendicular muscle mass +0.4±1.2 kg (P) –0.5±2.1 kg (C) Group difference $p = 0.03$ Leg muscle mass +0.3±1.2 kg (P) –0.6±1.8 kg (C) Group difference $p = 0.01$
Backx et al. [15]	61 subjects Male and female Age 55–70 years BMI 27–40 and waist circumference >88 cm (women) or >102 cm (men)	1.7 g/kg BW/day 0.9 g/kg BW/day	–25% of the baseline energy intake calculated from a validated 177-item food frequency questionnaire 12 weeks	From 92.8±11.0 to 83.9±10.1 kg (P) From 90.5±10.0 to 81.5±9.7 kg (C) Group difference n.s.	From 54.8±12.2 to 53.1±11.4 kg (P) From 54.5±9.3 to 52.4±9.1 kg (C) Group difference n.s.
Bales et al. [8]	80 women Age ≥45 years BMI ≥30	1.2 g/kg BW/day 0.8 g/kg BW/day	–500 kcal/day from the TEE (calculated using a formula) 6 months	–6.2±5.9 kg (P) –6.4±4.9 kg (C) Group difference n.s.	–0.6±1.1 kg (P) –1.0±1.1 kg (C) Group difference n.s.
Porter Starr et al. [7]	67 subjects Male and female Age >60 years BMI ≥30	1.2 g/kg BW/day 0.8 g/kg BW/day	–500 kcal/day 6 months	–8.7±7.4 kg (P) –7.5±6.2 kg (C) Group difference n.s.	–1.1±1.5 kg (P) –1.8±2.9 kg (C) Group difference n.s.
Layman et al. [25]	130 subjects Male and female Age 40–56 years BMI >27	1.6 g/kg BW/day 0.8 g/kg BW/day	–500 kcal/day 4-month weight reduction 8 months with a stable weight	After 4 months: –8.2±0.5 kg (P) –7.0±0.5 kg (C) Group difference n.s.	–2.6±0.2 kg (P) –2.4±0.2 kg (C) Group difference n.s.
Flechtner-Mors et al. [26]	110 subjects Male and female Age 25–70 years BMI 27–45	1.34 g/kg BW/day 0.8 g/kg BW/day	–500 kcal from the REE (formula) 3-month weight reduction 9 months with a stable weight	After 3 months: –7.4±4.6 kg (P) –4.8±4.0 kg (C) Group difference $p < 0.01$	After 3 months –2.8±3.6 kg (P) –3.2±2.7 kg (C) Group difference n.s.

TEE, total energy expenditure; n.s., nonsignificant; EN%, percentage of energy; P, protein group; C, control group; BW, body weight.

**Table 5.** Intervention studies on the effects of protein intake during weight loss on REE

Study	Proband characteristics	Protein intake	Energy restriction	Weight reduction, kg	REE change
Tang et al. [10]	43 men Age >21 years BMI 25–39.9	1.4 g/kg BW/day 0.8 g/kg BW/day	–750 kcal/day from REE × 1.5 estimated by the Harris-Benedict formula 12 weeks	–9.1±0.7 (P) –10.6±0.6 (C) Group difference n.s.	–104±77 kcal/day (P) –171±71 kcal/day (C) Group difference n.s.
Luscombe et al. [37]	36 subjects Male and female Age 34–65 years Hyperinsulinemia BMI 27–43	27 EN% 16 EN%	–30% from TEE (measured via the bicarbonate-urea method) 16 weeks, thereof 4 weeks of balanced energy	–7.9±1.1 (P) –8.0±0.7 (C) Group difference n.s.	–650±171 kJ/day (P) –780±132 kJ/day (C) Group difference n.s.
Baba et al. [38]	13 men Hyperinsulinemia BMI >31	45% 12%	80 E% from REE (measured by indirect calorimetry) 4 weeks	–8.3±0.7 (P) –6.0±0.6 (C) Group difference $p < 0.05$	–132±51 kcal/day (P) –384±85 kcal/day (C) Group difference $p < 0.05$

TEE, total energy expenditure; n.s., nonsignificant; EN%, percentage of energy; P, protein group; C, control group; BW, body weight.

**Table 6.** Intervention studies on the effects of protein intake during weight loss on PF

Study	Proband characteristics	Protein intake	Energy restriction	Weight reduction, kg	PF change
Porter Starr et al. [7]	67 subjects Male and female Age >60 years BMI ≥30	1.2 g/kg BW/day 0.8 g/kg BW/day	-500 kcal/day 6 months	-8.7±7.4 (P) -7.5±6.2 (C) Group difference n.s.	SPPB: +2.4±1.7 (P) +0.9±1.7 (C) <i>p</i> = 0.02 group difference Hand grip strength was unchanged in both groups
Wycherley et al. [43]	43 men Age 20–45 years BMI 27–39.9	35% (1.3 g/kg BW/day) 17% (0.8 g/kg BW/day)	-7,000 kJ/day 12 weeks	-10.7±5.3 (P) -8.7±3.5 (C) Group difference n.s.	Hand grip strength: -1.9±4.2 kg (P) -1.4±4.1 kg (C) Group differences n.s. Isometric knee strength: +16.8 ± 35.2 (P) +11.5 ± 36.8 (C) Group difference n.s.
Verreijen et al. [13]	88 probands Male and female Age >55 years BMI >30 (men) or >28 (women) and waist circumference >88 cm (women) or >102 cm (men)	1.11 g/kg BW/day 0.85 g/kg BW/day	-600 kcal/day from TEE (REE measured by indirect calorimetry + PAL measured by a 3-day movement diary) 13 weeks	-3.4±3.6 -2.8±2.8 Group difference n.s.	Hand grip strength: +2.0 ± 4.6 kg (P) +2.2 ± 4.1 kg (C) Group difference n.s. 4-m walking speed: +0.11±0.25 m/s (P) +1.04±0.22 m/s (C) Group difference n.s. 400-m walking speed: +0.04±0.1 m/s (P) +0.05±0.11 m/s (C) Group difference n.s. Chair rising test: -2.4±4.0 s (P) -1.4±3.1 s (C) Group difference n.s.
Backx et al. [15]	61 subjects Male and female Age 55–70 years BMI 27–40 and waist circumference >88 cm (women) or >102 cm (men)	1.7 g/kg BW/day 0.9 g/kg BW/day	-25% from the baseline energy intake calculated from a validated 177-item food frequency questionnaire 12 weeks	From 92.8±11.0 to 83.9±10.1 (P) From 90.5±10.0 to 81.5±9.7 (C) Group difference n.s.	Hand grip strength: -3±6 kg (P) -1±5 kg (C) Group difference n.s. SPPB score: +0.1±0.7 (P) +0.1±0.7 (C) Group difference n.s. 400-m walking speed: +0.05±0.09 m/s (P) +0.02±0.08 m/s (C) Group difference n.s. Leg press: -9±14 kg (P) -9±13 kg (C) Group difference n.s.
Beavers et al. [39]	69 subjects Male and female Age 65–79 years BMI 30–40	1.2 g/kg BW/day (weight loss) 0.8 g/kg BW/day (weight stable)	-500 kcal/day Weight loss group Stable-weight group	-6.6±0.4 (weight loss) -0.2±0.5 (stable weight)	Gait speed: +0.01 (-0.02 to 0.04) m/s (weight loss) -0.02 (-0.05 to 0.01) m/s (weight stable) Group difference n.s.



**Table 6** (continued)

Study	Proband characteristics	Protein intake	Energy restriction	Weight reduction, kg	PF change
Verreijen et al. [16]	100 subjects Male and female Age 55–80 years BMI ≥28 (men) or >25 (women) and waist circumference >88 cm (women) or >102 cm (men)	1.3 g/kg BW/day 0.8 g/kg BW/day	–600 kcal/day from TEE (REE measured by indirect calorimetry + PAL measured by a 3-day movement diary) 10 weeks	–2.1±3.6 (P) –1.7±1.8 (C) –2.6±2.9 (exercise) –2.0±2.2 (exercise + P)	Hand grip strength: –1.7±6.5 kg (P) +1.8±6.6 kg (C) –1.8±11.6 kg (exercise) –2.0±6.0 kg (exercise + P) Protein × exercise interaction <i>p</i> = 0.03 4-m walking speed: +0.08±0.26 m/s (P) +0.13±0.24 m/s (C) +0.08±0.13 m/s (exercise) +0.20±0.24 m/s (exercise + P) Protein × exercise interaction <i>p</i> = 0.045 400-m walking speed: +0.07±0.1 m/s (P) +0.04±0.15 m/s (C) +0.07±0.07 m/s (exercise) +0.08±0.15 m/s (exercise + P) Group differences n.s. Chair rising test: –1.6±1.7 s (P) –1.6±2.1 s (C) –1.0±2.7 s (exercise) –1.4±2.7 s (exercise + P) Group differences n.s.

EE, total energy expenditure; n.s., nonsignificant; EN%, percentage of energy; P, protein group, C, control group; BW, body weight.

the POWR-UP study, 60 women aged > 45 years were randomized to either an HP group (1.3 g/kg) or an NP group (0.8 g/kg) and achieved a weight loss of 6% within 6 months, without group effects on FFM loss [8]. In addition, in the MEASURE-UP study, equal losses of FFM were observed in 67 older subjects (> 60 years) over a 6-month period of weight loss regardless of the protein amount (0.8 vs. 1.2 g/kg) [7]. Layman et al. [25] also achieved similar FFM losses after 4 months of weight loss in 130 subjects (age 40–56 years) with either 0.8 or 1.6 g/kg protein. In a study with 110 adults (age 25–70 years) a significant greater weight loss but without differences in FFM loss was observed after 3 months of weight loss and after 9 months of weight maintenance in the HP group (0.8 vs. 1.34 g/kg) [26]. Also a study on bariatric patients (age 18–65 years) showed no better preservation of lean mass after a 6-month weight loss follow-up with a high protein intake (25.4 ± 3.7 vs. 15.8 ± 4.4% of the energy in-

take; *p* < 0.001) [17]. Other studies, however, have reported contradictory findings (Table 4). Verreijen et al. [13] reported preservation of appendicular muscle mass in 88 obese adults aged > 55 years on a 13-week high-whey protein hypocaloric diet (1.1 vs. 0.85 g/kg), which was confirmed in a 6-week study on adults (age 20–75 years) with insulin resistance (15 vs. 23% of energy intake) [27]. A recent meta-analysis concluded improved maintenance of FFM during weight loss without exercise in older adults (mean > 50 years) consuming an HP diet (≥25% of the total energy or ≥1.0 g/kg) for a time of 8–104 weeks (mean 25 weeks) [28]. Nevertheless, the included studies had different primary outcomes (e.g., bone density, IGF level, and cardiovascular risk factors) and were very heterogeneous. Only 4 or 2 of 20 articles provided details on randomization or blinding procedures. In addition, certain studies were considered as 2 separate studies, which may have artificially increased the protein effect. Only 3 of 20

studies, however, showed a significant difference in FFM loss between groups and only 1 of them included adults aged > 50 years. Furthermore, the studies also included adults aged < 50 years. Of major importance for the evaluation of FFM loss is weight loss over time [29–31]. Because energy deficit showed a positive association with FFM loss, the results also support that energy deficit is an important predictor and therefore a confounder in studies analyzing the effect of protein on weight loss. Improved satiety and an increased thermic effect of food on an HP diet [32, 33] facilitate weight loss on an HP diet and might therefore contribute to a higher energy deficit which prevents/or acts against the FFM-preserving effects of an HP diet. Weight loss was, however, not significantly higher in the HP diet group ( $p = 0.54$ ).

Our assumption that the REE is better maintained following an increased protein intake during weight loss could not be confirmed. This is due to the lack of an effect of the HP diet on preservation of FFM. In addition, weight reduction may lead to an adaptive thermogenesis, indicating that the REE decline is independent of the loss of FFM [29, 34–36]. Here, there was a difference after adjustment of REE for FFM, and therefore an adaptive thermogenesis was present and the risk of weight regain was higher. Tang et al. [10] showed a significant better preservation of FFM after 12 weeks of weight loss in the HP group (1.4 vs. 0.8 g/kg), and macronutrients did not affect the reduction in REE in 43 overweight middle aged men. Luscombe et al. [37] also did not find an effect of diet composition (27 vs. 16% protein, 12-week intervention) on REE in adults (age 35–65 years). By contrast, Baba et al. [38] found a significant lower decrease in REE in the HP group (45 vs. 12%) in 13 men ( $-132 \pm 51$  vs.  $-384 \pm 85$  kcal) after a 4-week energy-restricted diet. Data of FFM changes, however, were not available (Table 5).

Similar to the present study, improvements in PF (SPPB score:  $+2.4 \pm 1.7$  [HP] vs.  $+0.9 \pm 1.7$  [NP];  $p < 0.01$ ), but with group effects in favor of the HP group (1.2 vs. 0.8 g/kg) in adults aged > 60 years, were shown after 6 months of weight reduction by 8 kg [7]. Verreijen et al. [13] also observed a significant improvement in walking speed and hand grip strength in 88 older subjects (age > 55 years) after a weight loss of 3.5 kg within 13 weeks, without an impact of protein intake (1.11 and 0.85 g/kg). An HP diet (1.3 g/kg) in combination with physical training led to greater improvements of these parameters within 10 weeks of weight reduction compared to an HP diet or training alone in older adults (age > 55 years) [16]. In contrast, other studies found no improvement in walking speed in a weight loss group with 1.2 g/kg protein versus

a stable-weight group with 0.8 g/kg in older adults (age > 65 years, 6-month intervention) [39]. Also muscle strength or PF changes remained unaffected after 12 weeks of energy restriction ( $-25\%$ ) by protein intake (1.7 vs. 1.0 g/kg) in 61 adults aged  $\geq 55$  years [15] (Table 6).

### *Strengths and Limitations*

The present study protocol was based on the recommendations of Bellg et al. [40] for enhancement of treatment fidelity. We recorded a dropout rate of 15% during the intervention phase, which is quite similar to the rate of 15–20% in other studies [13, 39, 41] and below 20%, a value above which Schulz and Grimes [42] postulate a limited validity of study results. However, during follow-up the loss of subjects was 46%. Therefore, these results should be interpreted with caution and further studies with a long-term follow-up period are needed. Nevertheless, this study was characterized by good compliance, demonstrated by the protein-induced increase in serum urea levels in the HP compared to the NP group as well as the low difference between the measured and the predicted weight loss. Dietary records indicated that the target protein intakes of 1.5 and 0.8 g/kg were almost achieved, with  $1.4 \pm 0.1$  and  $0.8 \pm 0.1$  g/kg of protein per day in both groups. The differences in the recorded energy intake illustrate the problem of under- or overreporting, as there was no difference in weight loss. This double-blind RCT is characterized by an intensive assessment of dietary history and individual nutritional plans. The a priori measurement of REE by indirect calorimetry and the assessment of PAL allowed estimation of individual energy requirements and a personalized prescription for energy intake.

In conclusion, this study indicates that an energy-reduced HP diet in combination with 2 meal replacements and high-quality whey protein without physical exercise was more effective for maintaining PF measured by hand grip strength compared to an isocaloric weight loss diet with a normal protein intake in overweight, postmenopausal women. The energy deficit and the speed of weight loss have an important influence on preservation of FFM and should be more considered as a confounder in further studies. Physical exercise should be integrated into weight loss interventions whenever feasible for the subjects.

### **Acknowledgement**

We thank all of the women for their participation. We would also like to thank the students and dieticians for their active support in conduction of this study.

## Statement of Ethics

This study complies with the guidelines for human studies and was conducted in accordance with the World Medical Association Declaration of Helsinki. The Ethics Committee of the Fulda University of Applied Sciences endorsed the study protocol, and written informed consent was obtained from all of the subjects. This study is registered in the German Clinical Trial Register (DRKS00011238).

## Conflict of Interest Statement

The authors have no conflict of interests to declare.

## Funding Sources

We acknowledge support from the Open Access Publishing Fund of the Fulda University of Applied Sciences. The shake powder was sponsored by the company Precon, and the whey protein powder was sponsored by the company Primal State. The preparation of data and this paper was carried out independently of the sponsors.

## Author Contributions

I.E. created the study design, conducted this study, performed the statistical analysis, and wrote this paper. K.K.-M. supervised this study. A.B.-W., S.C.B., and K.K.-M. critically revised this paper. All of the authors read and approved the final version of this work.

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