

Protein supplements after weight loss do not improve weight maintenance compared with recommended dietary protein intake despite beneficial effects on appetite sensation and energy expenditure: a randomized, controlled, double-blinded trial

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ABSTRACT

Background: High-protein diets increase weight loss (WL) during energy restriction; therefore, it has been suggested that additional protein intake may improve weight maintenance (WM) after WL.

Objective: We investigated the effect of protein supplements from either whey with or without calcium or soy on WM success after WL compared with that of a control.

Design: In a randomized, controlled, double-blinded trial, 220 participants aged 18–60 y with body mass index (in kg/m²) from 27.6 to 40.4 were included. The study was initiated with an 8-wk WL period followed by a 24-wk WM period. During WM, participants consumed the following isocaloric supplements (45–48 g/d): whey and calcium (whey+), whey, soy, or maltodextrin (control). Data were collected at baseline, before WM, and after WM (weeks 0, 8, and 32, respectively) and included body composition, blood biochemistry, and blood pressure. Meal tests were performed to investigate diet-induced-thermogenesis (DIT) and appetite sensation. Compliance was tested by 24-h urinary nitrogen excretion.

Results: A total of 151 participants completed the WM period. The control and 3 protein supplements did not result in different mean \pm SD weight regains (whey+: 2.19 \pm 4.6 kg; whey: 2.01 \pm 4.6 kg; soy: 1.76 \pm 4.7 kg; and control: 2.23 \pm 3.8 kg; $P = 0.96$), fat mass regains (whey+: 0.46 \pm 4.5 kg; whey: 0.11 \pm 4.1 kg; soy: 0.15 \pm 4.1 kg; and control: 0.54 \pm 3.3 kg; $P = 0.96$), or improvements in lean body mass (whey+: 1.87 \pm 1.7 kg; whey: 1.94 \pm 1.3 kg; soy: 1.58 \pm 1.4 kg; and control: 1.74 \pm 1.4 kg; $P = 0.50$) during WM. Changes in blood pressure and blood biochemistry were not different between groups. Compared with the control, protein supplementation resulted in higher DIT (~ 30 kJ/2.5 h) and resting energy expenditure (243 kJ/d) and an anorexigenic appetite-sensation profile.

Conclusion: Protein supplementation does not result in improved WM success, or blood biochemistry after WL compared with the effects of normal dietary protein intake (0.8–1.0 g \cdot kg⁻¹ \cdot d⁻¹). This trial was registered at clinicaltrials.gov as NCT01561131. *Am J Clin Nutr* doi: <https://doi.org/10.3945/ajcn.115.129528>.

Keywords: BMI, calcium, fat mass, lean body mass, protein, soy, weight maintenance, weight loss, whey

INTRODUCTION

For the initial treatment of overweight and obesity, weight loss (WL) by energy restriction is often recommended, and most overweight and obese individuals successfully lose weight (1). However, weight maintenance (WM) after WL is difficult, probably because of a drive to maintain body weight (BW) through a feedback loop between the brain and the periphery that increases appetite and decreases energy expenditure (EE) disproportionately (2). Thus, the lost BW is often regained over time (3, 4). High protein (HP) intake increases the sleeping metabolic rate and diet-induced EE and satiety, reduces hunger, and lowers energy efficiency during a positive energy balance (5, 6). Therefore, diets with an HP content could potentially improve the success of WM after a WL period.

Meta-analyses of energy-restricted diets that included studies with a minimum duration of 4 wk have shown that protein-rich diets [>1.05 g/kg (7) or in the range of 0.95–1.80 g/kg (8)] result in a larger BW reduction while maintaining lean body mass

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Supplemental Tables 1–3 are available from the “Online Supporting Material” link in the online posting of the article and from the same in the online table of contents at <http://ajcn.nutrition.org>.

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Abbreviations used: AOC, area over the curve; BP, blood pressure; BW, body weight; CID, clinical investigation day; DIT, diet-induced thermogenesis; EE, energy expenditure; FFA, free fatty acid; FM, fat mass; HOMA- β , homeostatic model assessment of β cell function; HP, high protein; LBM, lean body mass; LCD, low-calorie diet; PABA, para-amino-benzoic acid; PAL, physical activity level; RCT, randomized controlled trial; REE, resting energy expenditure; VAS, visual analog scale; whey+, whey and calcium; WL, weight loss; WM, weight maintenance.

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(LBM) than do diets with a lower protein content [≤ 1.05 g/kg (7) or in the range of 0.5–1.24 g/kg (8)]. However, meta-analyses and a review of studies with longer durations have shown inconsistent results for weight management (9–11). Lepe et al. (10) reviewed 8 studies with a minimum duration of 6 mo and showed 4 studies that favored HP diets (range: 25–40% of energy) compared with low-protein diets (range: 12–24% of energy), whereas for the studies with a duration ≥ 15 mo, only 1 in 4 studies favored the HP diet. Two meta-analyses were conducted on randomized controlled trials (RCTs) with ≥ 12 mo duration (9, 11). On the basis of 15 RCTs, HP diets ($\geq 25\%$ compared with $\leq 20\%$ of energy from protein with $< 30\%$ of energy from fat) did not improve WM (9). In contrast, 32 RCTs showed that lower carbohydrate intake (i.e., no specific requirements for protein intake or restriction on fat intake; however, the criterion yielded protein intake of 25–60% of energy compared with 10–24% of energy) favored BW and fat mass (FM) loss with no effect on LBM (11). In contrast with meta-analyses of energy-restricted diets, one meta-analysis of 6 studies with HP intake during WM after an initial WL showed that HP diets (range: 18–30% of energy) improved WM with 1.5-kg less BW regain compared with lower protein intake (range: 10–15% of energy) (12).

The meta-analyses do not consider the protein type, but it has been observed that animal protein induces higher 24-h EE than vegetable protein (13), which could affect the WM success. However, most studies that have investigated subtypes of protein have been conducted under WL conditions (14–16), whereas to our knowledge, only one study has investigated effects during WM after an initial WL period without the administration of the WM intervention product during the WL period (17). In addition to an increase in protein content, calcium supplementation was included in the present study because increasing calcium intake may be negatively associated with BMI (18) and may increase WL (19). The primary aim of our study was to investigate the effect of HP intake from either animal (whey) protein with or without calcium or vegetable (soy) protein on WM success after a WL.

METHODS

Study design

The study was a double-blind, randomized intervention that consisted of an 8-wk WL period followed by a 4-armed, parallel 24-wk WM period. The study was conducted at the Department of Nutrition, Exercise and Sports, University of Copenhagen, from January 2012 to December 2013. For practical reasons, participants were recruited in 2 cohorts of ~ 110 participants; the first cohort was recruited between January and May 2012, and the second cohort was recruited between January and April 2013. The initiation of the WL period was commenced continuously in groups of 10–12 participants (i.e., 10 groups were recruited in each cohort). The study was registered at clinicaltrials.gov as NCT01561131 and was conducted in accordance with the ethical standards of the responsible regional committee on biomedical research in Denmark (H-2-2011-145) and the Danish Data Protection Agency (2007-54-0269).

Study participants

Participants were recruited through the web page www.forsogsperson.dk and in the local and free newspapers for the

Copenhagen area. Potential participants received written information and were invited to an information meeting where the informed consent form was signed by the participant. Participants received no financial compensation for their participation; however, a small gift (a dietary calendar to support WM or further WL) was given after completion of the study.

Eligible criteria for the participants were as follows: men or women (nonpregnant, nonlactating, and not planning pregnancy in the next 12 mo), 18–60 y of age and BMI (in kg/m^2) from 28 to 40 at screening. The participants were not allowed to donate blood or to participate in other trials 3 mo before the study initiation or during the study.

Individuals who were dieting or individuals with a temporary change of dietary habits were excluded. Other exclusion criteria were a weight change > 3 kg 2 mo before the study start, sagittal height > 32 cm, and obesity-related surgery. Individuals who were using cholesterol-reducing medicine or other types of medicines that were assessed to influence the primary outcome were excluded, and the use of other medication should have been stable 3 mo before the study start and during the study. Individuals with chronic systemic infections, inflammatory diseases, chronic endocrine diseases, a lack of nutrient absorption, or chronic stomach and liver diseases were excluded. Individuals with cardiovascular diseases, a known heart defect, or brain diseases, and individuals who were diagnosed with cancer in the previous 10 y were excluded. On the basis of a personal assessment by the project workers, individuals with alcohol and drug abuse, who were unable to comply with a low-calorie diet (LCD), or who were physically or mentally unable to comply with the study in general were excluded. In addition, the following individuals were excluded: professional athletes; individuals who planned to participate in an elite sport or who had greater changes in physical activity during the study; smokers, individuals with a known allergy for para-amino-benzoic acid (PABA), and individuals who had a hemoglobin concentration < 7 mmol/L at screening.

Intervention

In the 8-wk WL period, the participants consumed a meal-replacement LCD (800–1000 kcal/d; 15–20% of energy from fat, 35–40% of energy from protein, and 45–50% of energy from carbohydrate) with products from NUPO A/S. The replacement products were given as 7 powder sachets/d, which were consumed as either shakes or soups (dissolved in cold or hot water). In addition, participants were allowed to consume 200 g watery vegetables (tomato, cucumber, radish, celery, and lettuce)/d. During the WL period, the LCD was similar for all participants and was not related to the random assignment during WM. In the WL period, the participants attended 5 group meetings with a department dietitian. The requirement of the WL period was to lose $\geq 8\%$ of the initial BW. If this requirement was not fulfilled at week 8, the participant was excluded from continuing in the WL period.

The 24-wk WM period had a randomized, parallel design in which participants were supplemented with one of the following 4 isocaloric powder supplements: 1) whey protein with a high content (60% of the total protein content) of α -lactalbumin (45 g/d) and calcium (1000 mg/d) [whey and calcium (whey+)] (Arla Foods Ingredients Group P/S); 2) whey protein with a high content (60% of the total protein content) of α -lactalbumin (45 g/d)

(Arla Foods Ingredients Group P/S); 3) soy isolate (45 g/d) (Solbar); or 4) maltodextrin (48 g/d) (Chargill), which was referred to as the control. The supplements were supplied as powder in 3 sachets/d and were expected to constitute 10–15% of energy of daily energy intake. The supplements were dissolved in water and consumed as part of the 3 main meals. In the WM period, there were no restrictions to participants' diets. However, the participants attended dietary counseling in groups by a department dietitian every month where they were advised to follow the official Danish dietary guidelines regardless of which supplement they were randomly assigned to consume. The goal of the WM period was to maintain the WL although additional WL was allowed.

Outcomes

The primary outcomes were changes in FM, BW, and body composition during WM (weeks 8–32). Secondary outcomes were changes in fasting blood lipid profiles (total cholesterol, LDL cholesterol, HDL cholesterol, free fatty acid (FFA), and triglycerides), glucose metabolism (insulin, glucose, and C-peptide), blood pressure (BP) and pulse, reported energy intake and macronutrient composition during WM, and diet-induced thermogenesis (DIT) and subjective appetite sensation during WL and WM (weeks 0–32). In addition, secondary outcomes included changes in BW and body composition, the blood lipid profile, and markers of glucose metabolism during WL (weeks 0–8).

Data were collected on the following 3 clinical investigation days (CIDs) during the study: baseline (week 0), before the 24-wk WM period (week 8), and after the 24-wk WM period (week 32). Except for the collection of 24-h urine and 3-d dietary registrations, data collection took place when the participants visited the Department of Nutrition, Exercise and Sports.

Anthropometric measures

BW was measured with the subject in underwear via a digital scale (Lindells). BMI was calculated as BW divided by the square of height. FM and LBM were determined with the subject in underwear via a DXA scan (Lunar iDXA). Waist and hip circumferences were measured twice with a nonelastic tape measure on the skin to the nearest 0.5 cm, and a mean was calculated. Waist circumference was measured halfway between the lowest rib and iliac crest, and the measurement was taken when the participant exhaled. Hip circumference was measured as the largest circumference in the area around the buttock. The sagittal diameter was measured with the subject in a lying position with an abdominal caliper (Holtain-Kahn). Height was measured twice at screening to the nearest 0.5 cm with the use of a wall-mounted stadiometer (Hultafors), and the mean of the 2 measurements was recorded.

BP

After ≥ 10 min of rest in a supine position, BP was measured with an automatically inflated cuff (A&D Instruments Ltd.). BP was measured on the left arm 3 times, and a mean was calculated from the last 2 measurements when the 2 measurements differed ≤ 5 mm Hg. If the 2 last measurements differed > 5 mm Hg, additional measurements were performed until 2 consecutive measurements differed ≤ 5 mm Hg.

Dietary records

At baseline and 2 times during the WM period [6 wk after the initiation of WM (week 14) and at completion of the WM period], participants' food intakes were assessed with the use of 3-d dietary records. Participants registered all ingested foods that were supplied with information on brand names, cooking, and processing. Whenever possible, foods were weighed; otherwise, household measures were applied. Reported daily dietary intakes of energy, macronutrients, and micronutrients were calculated as means from the 3-d dietary records. The dietary records were assessed with the use of a computer database of foods from the National Food Agency of Denmark (Dankost 3000 and Dankost Pro; National Food Agency of Denmark; www.foodcomp.dk/v7/fvdb_search.asp).

Biological material

Compliance with supplement intake was evaluated via an analysis of nitrogen excretion by 24-h urine collection at baseline and 2 times during the WM period (weeks 14 and 32). During the collection of urine, the participants ingested 3 tablets of 80 mg PABA (240 mg/d), which was used as a compliance marker for complete urine collection (PABA recovery between 80% and 120% was accepted). Before the analysis, the weight and density of the urine sample were measured to calculate the volume. Urinary nitrogen excretion was measured via the Dumas combustion method with the use of a VarioMax CN analyzer (Elementar), and the PABA concentration was analyzed with the use of colorimetric spectrophotometry. On the basis of the following formula (20), the actual protein intake was calculated from the nitrogen that was excreted in the 24-h urine collection:

$$\text{protein intake (g/d)} = [\text{urinary nitrogen (g)} + 2\text{g}] \times 6.25 \quad (1)$$

Venous blood samples were drawn at CIDs after an overnight fasting period of ≥ 12 h (at time 2000 h). Blood samples for analyses of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, insulin, and C-peptide were collected in serum tubes and kept at room temperature for 30 min to coagulate. Plasma samples for FFA and glucose analyses were put directly on ice and immediately centrifuged. All samples were centrifuged at $2500 \times g$ for 10 min at 4°C and, thereafter, stored at -80°C until analysis.

Total cholesterol and triglycerides were measured via enzymatic photometric tests; HDL cholesterol, LDL cholesterol, and FFA were measured via enzymatic colorimetric tests, and glucose was measured via the enzymatic hexokinase method on a Pentra 400 Analyzer (HORIBA ABX). C-peptide and insulin were measured via a competitive chemiluminescent enzyme immunoassay (IMMULITE C-Peptide, LKPE1; Siemens Medical Solutions Diagnostics) and a chemiluminescent immunometric assay (IMMULITE/IMMULITE 1000 INSULIN; Siemens Medical Solutions Diagnostics), respectively, with the use of the Immulite 1000 Analyzer (Siemens; Siemens Medical Solutions Diagnostics). Completers with insulin concentrations below the detection limit (5 pmol/L) were excluded from the analysis.

The homeostatic model assessment was used to quantify both the HOMA-IR and the homeostatic model assessment of β cell

function (HOMA- β) from measurements of fasting insulin and glucose. HOMA-IR was calculated as follows (21):

$$[\text{insulin } (\mu\text{U/mL}) \times \text{glucose (mmol/L)}] \div 22.5 \quad (2)$$

and HOMA- β was calculated as follows (21):

$$[20 \times \text{insulin } (\mu\text{U/mL})] \div [\text{glucose (mmol/L)} - 3.5] \quad (3)$$

Physical activity

During WM, the participants were encouraged to follow the official Danish dietary guidelines including physical activity, which recommend physical activity ≥ 30 min/d. Physical activity was assessed with the use of a questionnaire 4 times during the study (weeks 0, 8, 14 and 32). The questionnaire included general assessments of physical work levels and questions about “How often do you sit, stand, walk, sweat, and lift during working hours.” In addition, bicycling to or from work, education, and shopping was evaluated as how often the activity was done as well as the minutes performed and the intensity. Walking and bicycling during spare time were evaluated as well as were sports. A reported physical activity level (PAL) was calculated on the basis of answers from the questionnaire.

Meal test

At baseline and after the WM period (weeks 0 and 32), meal tests were performed with a breakfast test meal that contained the allocated supplement in a smoothie. The meal test was initiated in the morning, and measurements were collected in the fasting state and postprandially after the breakfast test meal (**Figure 1**). The day before the meal tests, participants were provided a dinner (4 MJ; protein: 17% of energy; fat: 33% of energy; carbohydrate: 50% of energy) from the research kitchen at the department. This dinner was the same before both meal test days (weeks 0 and 32). In addition, the participants were not allowed to consume alcohol and should have limited physical activity 48 h before the meal tests.

Appetite and ad libitum energy intake

Subjective appetite sensation was measured in the fasting state and postprandially after consumption of the breakfast test meal (Figure 1) with the use of a visual analog scale (VAS) to assess hunger, satiety, fullness, prospective food consumption, and well-being (22). An ad libitum meal was served 185 min after the initiation of the breakfast test meal to investigate energy intake, and participants were instructed to eat until they were comfortably satisfied. Energy intake from the ad libitum meal was measured by calculating the consumed amount of food and converting this into energy. In addition, the palatability of the

breakfast test meal and the ad libitum meal was assessed right after consumption of the meals with the use of VASs to assess taste, smell, visual appeal, off taste, and overall palatability.

Resting energy expenditure and DIT

EE was measured in a supine position via a ventilated hood system (Jaeger Oxycon PRO; Viasys Healthcare GmbH) with the EE calculation based on the Elia and Livesey formula (23). The precision of the ventilated hood system was validated on a weekly basis with the use of an alcohol-burning test. The first measurement of EE, i.e., the resting energy expenditure (REE), was measured in the fasting state after participants had rested in a supine position ≥ 15 min. The following measurements of EE (after consumption of the breakfast test) (Figure 1) were used to investigate DIT. DIT was calculated as the incremental AUC for EE for 2.5 h after the breakfast test meal with the REE as the baseline measure.

Sample size

The sample-size calculation was based on a minimum FM difference of 1.0 kg with an SD of 1.5 kg and 85% power (24–26). In total, 160 participants were needed to complete the study, and with an expected 20% dropout rate, the aim was to include 200 individuals. However, if the dropout rate during the WL period was $>5\%$, a maximum of an additional 25% of participants were allowed, including up to 250 participants.

Random assignment

Random assignment to the supplement was done in 12 blocks that were generated by sex, BMI with cutoffs at 31 and 35, and habitual calcium intake with a cutoff at 800 mg/d. BMI was calculated at screening (nonfasting weight), and habitual calcium intake was assessed via a self-administered quantitative food-frequency questionnaire (27), which was adjusted to reflect the availability of calcium-rich food products on the market. The computer-based randomization lists were generated at www.randomization.com for the 6 BMI-calcium combinations for each sex, and the allocation ratio to the 4 supplements was 1:1:1:1.

After the inclusion of a participant (before week 0), the project worker, who screened the participant, randomly assigned the participant to a number (I, II, III, or IV), which was coded for the 4 supplements. The coding was performed and concealed by an investigator with no clinical involvement in the trial. Except for the kitchen staff who prepared the breakfast test meal on the CID, the coding was concealed from all participants, project workers, and dietitians. The analyses of the primary outcomes were performed before disclosure. Participants were not assigned to the group meetings with the dietitian on the basis of the supplement they received, which, beyond blinding, eliminated the



FIGURE 1 Meal test design. The meal tests were carried out at baseline (week 0) and after weight maintenance (week 32). The breakfast test meal contained the allocated supplement in a smoothie and was served at time point 0 min. Fasting measurements of subjective appetite (VAS) and EE were done before the test meal, and postprandial measurements continued ~ 3 h after the test meal was served. ad lib., ad libitum; EE, energy expenditure; REE, resting energy expenditure; VAS, visual analog scale.

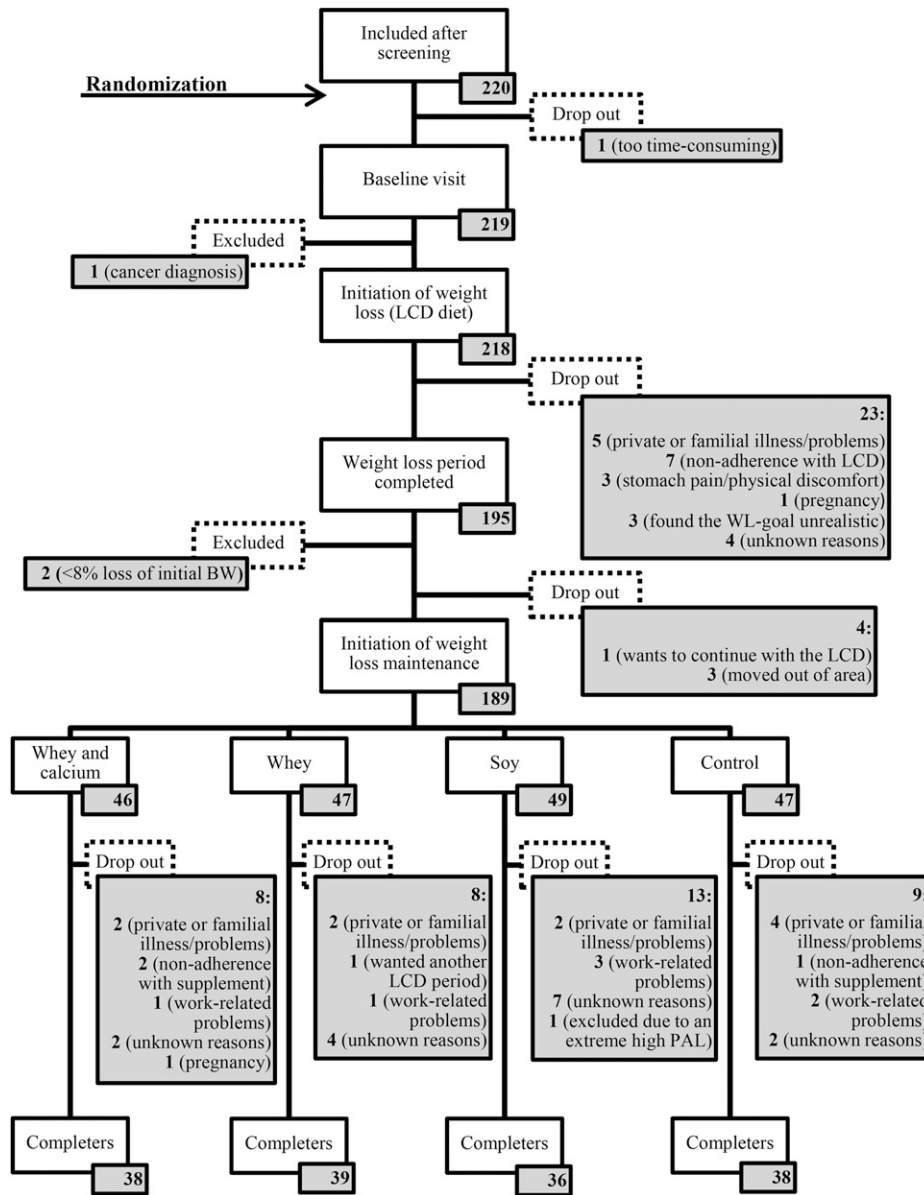


FIGURE 2 Flowchart. A total of 220 participants were included and were randomly assigned in the study. A total of 195 participants completed the weight-loss period, and the weight-maintenance period was completed by 151 participants (whey and calcium: $n = 38$; whey: $n = 39$; soy: $n = 36$; and control: $n = 38$). BW, body weight; LCD, low-calorie diet; PAL, physical activity level, WL, weight loss.

possibility that the dietitian could give different dietary advice to different supplement groups.

Statistical analyses

Data are presented as means \pm SDs unless stated otherwise. Categorical data are presented as percentages. Statistical analyses on primary and secondary outcomes were performed with the use of STATA software (version 10.1; StataCorp LP). The level of significance was set at $P < 0.05$. All presented data are from completers. Baseline data for all included participants are available in Supplemental Material (**Supplemental Table 1**). Outcomes on baseline data for completers (week 0) or at the initiation of WM (week 8) were tested for group differences with the use of an ANOVA and adjusted for age and sex. The same approach was used to test for group differences in reported

dietary intake and PALs at different time points (weeks 0, 8, 14, and 32). Age and PAL were analyzed with a Kruskal-Wallis test, which, thus, was not adjusted for covariates (age and sex).

Changes over time for all participants as an entire group (weeks 0–8 and 8–32) were analyzed with the use of a paired t test or paired-sample Wilcoxon’s Signed Rank test. Changes during WL (weeks 0–8) were tested for group differences with an ANOVA and adjusted for age and sex.

Results from the WM period (change during WM) were analyzed with an ANCOVA and adjusted for the change of the outcome during the WL period as well as for age and sex. A second ANCOVA model, which was further adjusted for changes in BW during the WL period and the reported PAL, was used to reanalyze the primary outcomes.

For comparison of protein intakes, intragroup comparisons were analyzed with a paired t test, and for nonnormally distributed data,

TABLE 1For completers, characteristics at baseline (week 0) and before the initiation of WM (week 8) ($n = 151$)¹

| | Week 8 | | | | | P^2 |
|---------------------------------|------------------------------|--|---------------------------|--------------------------|--------------------------|---------------------|
| | Week 0, all ($n = 151$) | Whey protein and calcium ($n = 38$) | Whey protein ($n = 39$) | Soy protein ($n = 36$) | Control ($n = 38$) | |
| Anthropometric measure | | | | | | |
| BW, kg | 96.3 ± 13.4 | 83.8 ± 13.0 | 82.7 ± 11.9 | 84.0 ± 11.7 | 83.6 ± 10.9 | 0.94 [§] |
| BMI, kg/m ² | 33.2 ± 3.31 | 28.8 ± 3.24 | 28.5 ± 3.16 | 28.9 ± 3.27 | 28.9 ± 2.72 | 0.85 [§] |
| FM, kg | 41.3 ± 8.75 | 32.7 ± 9.66 | 31.8 ± 8.10 | 32.2 ± 9.57 | 33.2 ± 6.98 | 0.82 [§] |
| LBM, kg | 51.7 ± 7.91 | 48.1 ± 8.09 | 48.1 ± 7.39 | 48.9 ± 6.71 | 47.5 ± 7.83 | 0.99 |
| Fat, % | | | | | | |
| Whole body | 42.9 ± 5.50 | 38.8 ± 7.86 | 38.1 ± 6.49 | 37.9 ± 7.51 | 39.6 ± 5.98 | 0.73 |
| Android | 50.7 ± 6.00 | 43.2 ± 10.0 | 43.2 ± 8.07 | 44.0 ± 9.32 | 44.6 ± 8.02 | 0.84 |
| Gynoid | 45.7 ± 6.91 | 42.9 ± 8.73 | 41.5 ± 7.24 | 39.9 ± 8.04 | 43.6 ± 6.71 | 0.10 |
| WC, cm | 103.0 ± 10.3 | 89.9 ± 8.78 | 89.8 ± 9.96 | 92.2 ± 9.33 | 90.1 ± 8.29 | 0.59 [§] |
| HC, cm | 116.5 ± 7.77 ³ | 109.1 ± 8.53 | 108.4 ± 7.23 | 107.3 ± 7.51 | 108.6 ± 6.78 | 0.79 [§] |
| Sagittal height, cm | 23.9 ± 2.45 ⁴ | 20.1 ± 2.23 | 19.9 ± 2.22 | 20.3 ± 2.23 | 20.2 ± 1.81 | 0.91 |
| Blood pressure | | | | | | |
| Systolic, mm Hg | 118 ± 10.8 | 122 ± 14.0 | 118 ± 12.5 | 120 ± 12.4 | 118 ± 10.1 | 0.32 |
| Medicated excluded ⁵ | 117 ± 9.27 | 121 ± 11.4 | 116 ± 8.83 | 119 ± 9.07 | 117 ± 9.75 | 0.18 [§] |
| Diastolic, mm Hg | 76.1 ± 9.15 | 76.9 ± 9.83 | 73.9 ± 8.27 | 75.1 ± 9.10 | 73.4 ± 7.87 | 0.51 [§] |
| Medicated excluded ⁵ | 75.4 ± 8.69 | 75.3 ± 8.91 | 73.1 ± 7.30 | 73.8 ± 8.51 | 73.2 ± 7.79 | 0.70 |
| Pulse, beats/min | 69.2 ± 8.16 | 60.0 ± 8.70 | 60.0 ± 8.36 | 58.6 ± 7.02 | 58.1 ± 8.10 | 0.65 |
| Medicated excluded ⁵ | 68.9 ± 8.21 | 58.9 ± 8.63 | 60.2 ± 8.10 | 58.7 ± 7.17 | 57.7 ± 8.13 | 0.58 |
| Lipid profile | | | | | | |
| Total cholesterol, mmol/L | 5.34 ± 0.92 ⁴ | 4.21 ± 0.63 | 3.95 ± 0.71 | 4.12 ± 0.95 | 4.14 ± 0.74 | 0.39 |
| HDL cholesterol, mmol/L | 1.37 ± 0.33 ⁴ | 1.27 ± 0.29 | 1.20 ± 0.22 | 1.15 ± 0.20 | 1.21 ± 0.26 | 0.32 [§] |
| LDL cholesterol, mmol/L | 3.20 ± 0.79 ⁴ | 2.35 ± 0.56 | 2.21 ± 0.59 | 2.39 ± 0.83 | 2.32 ± 0.62 | 0.58 |
| Triglycerides, mmol/L | 1.42 ± 0.79 ⁴ | 0.89 ± 0.20 | 0.79 ± 0.23 | 0.91 ± 0.29 | 0.89 ± 0.22 | 0.08 [§] |
| FFA, μ mol/L | 448 ± 145 ⁴ | 816 ± 282 | 835 ± 217 | 788 ± 278 | 865 ± 335 | 0.60 [§] |
| Glucose metabolism | | | | | | |
| Glucose, mmol/L | 5.70 ± 0.70 ⁴ | 5.36 ± 0.59 | 5.28 ± 0.47 | 5.44 ± 0.54 | 5.29 ± 0.36 | 0.56 [§] |
| Insulin, pmol/L | 71.1 ± 38.3 ⁴ | 48.0 ± 26.9 ⁶ | 49.9 ± 40.0 ⁶ | 56.9 ± 29.2 ⁶ | 52.2 ± 28.2 ⁶ | 0.24 ^{§,6} |
| C-peptide, pmol/L | 651 ± 236 ⁴ | 524 ± 217 | 512 ± 218 | 564 ± 199 | 542 ± 199 | 0.39 [§] |
| HOMA-IR | 2.69 ± 1.88 ⁴ | 1.68 ± 1.02 ⁶ | 1.76 ± 1.50 ⁶ | 2.02 ± 1.14 ⁶ | 1.79 ± 1.01 ⁶ | 0.24 ^{§,6} |
| HOMA- β | 93.5 ± 43.2 ⁴ | 75.5 ± 42.5 ⁶ | 75.5 ± 48.4 ⁶ | 85.0 ± 40.8 ⁶ | 83.2 ± 40.5 ⁶ | 0.35 ^{§,6} |

¹ All values are means \pm SDs. Data are given for baseline (at week 0 before weight loss) for all completers and for the initiation of the WM period (week 8) according to supplements provided in the WM period. BW, body weight; FFA, free fatty acid; FM, fat mass; HC, hip circumference; HOMA- β , homeostatic model assessment of β cell function; LBM, lean body mass; WC, waist circumference; WM, weight maintenance.

² P values are for tests of group differences at week 8. Differences between groups were analyzed with the use of an ANOVA and adjusted for age and sex. [§]Variables were log-transformed before the analysis. Systolic blood pressure (all) was inverse transformed before the ANOVA. No differences between groups were observed for any of the outcomes (overall supplement effect: $P > 0.05$).

³ $n = 150$.

⁴ $n = 149$.

⁵ $n = 135$ (whey protein and calcium: $n = 32$; whey protein: $n = 36$; soy protein: $n = 31$; and control: $n = 36$).

⁶ Whey protein and calcium: $n = 36$; whey protein: $n = 37$; soy protein: $n = 36$; and control: $n = 38$.

Wilcoxon's Signed Rank test was used. For intergroup comparison, differences between all protein groups compared with the control group or between the protein groups were analyzed with the use of an ANOVA and adjusted for age and sex.

ANOVA and ANCOVA models with a nonnormal distribution of residuals were (log) transformed before reanalysis. Conclusions were based on P values from the reanalyses; however, nonadjusted data are presented in the tables. When an overall supplement effect from the ANOVA or ANCOVA was observed ($P < 0.05$), post hoc pairwise comparisons between groups (with Bonferroni correction) were conducted.

The effect of supplements on VAS scores was tested with the use of a repeated-measures ANCOVA with the MIXED procedure in the Statistical Analysis System (SAS) software package

(version 9.4; SAS Institute) with appropriate covariates (fasting VAS score, sex, age, BMI, and BW change during WL). An ANCOVA was used to test the effect of supplements on the appetite VAS AUC or area over the curve (AOC), DIT, and ad libitum intake at the meal tests. In the model, an interaction between supplements and visits was included, and sex was used as covariate. The VAS AUC and AOC were further adjusted for age, BMI, and baseline VAS scores. DIT was further adjusted for age, hood instrument (I, II, or III), FM, and LBM.

A per-protocol analysis was performed to eliminate bias from lack of compliance, whereby 1 of 3 participants from the protein groups with the lowest protein intakes were excluded from the analysis along with 1 of 3 participants from the control group who had the highest protein intakes. Protein intake was calculated as grams per kilogram per day from the excretion of nitrogen, and

TABLE 2
For completers, characteristics after WM (week 32) and changes during WM according to supplement intake ($n = 151$)¹

| Anthropometric measure | Protein ($n = 113$) ² | | | | | | | | | | | Protein compared with control ⁴ | All ⁵ | |
|---------------------------------|------------------------------------|----------------------------|---------------------------|----------------------------|---------------------------|----------------------------|---------------------------|----------------------------|---------------------------|----------------------------|----------------------|--|------------------|-----------------------------|
| | Wheny protein ($n = 39$) | | | Soy protein ($n = 36$) | | | Control ($n = 38$) | | | ΔWM | | | | Between groups ³ |
| | Week 32 | ΔWM | Week 32 | ΔWM | Week 32 | ΔWM | Week 32 | ΔWM | Week 32 | ΔWM | | | | |
| BW, kg | 86.0 ± 13.0 | 2.19 ± 4.60 | 84.7 ± 12.4 | 2.01 ± 4.62 | 85.8 ± 12.3 | 2.23 ± 3.78 | 85.5 ± 12.4 | 1.99 ± 4.59 | 0.96 | 0.81 | <0.0001 | | | |
| BMI, kg/m ² | 29.5 ± 3.32 | 0.77 ± 1.62 | 29.2 ± 3.43 | 0.71 ± 1.62 | 29.7 ± 3.14 | 0.76 ± 1.28 | 29.4 ± 3.34 | 0.69 ± 1.61 | 0.96 | 0.86 | <0.0001 | | | |
| FM, kg | 33.2 ± 9.00 | 0.46 ± 4.52 | 31.9 ± 8.40 | 0.11 ± 4.07 | 33.7 ± 7.89 | 0.54 ± 3.28 | 32.5 ± 9.02 | 0.24 ± 4.21 | 0.96 | 0.64 | 0.054 [†] | | | |
| LBM, kg | 49.9 ± 8.25 | 1.87 ± 1.70 | 50.0 ± 7.49 | 1.94 ± 1.34 | 49.2 ± 8.08 | 1.74 ± 1.42 | 50.1 ± 7.63 | 1.80 ± 1.49 | 0.50 | 0.40 | <0.0001 | | | |
| Fat, % | 38.3 ± 6.77 | -0.41 ± 3.66 | 37.3 ± 6.59 | -0.86 ± 3.23 | 39.1 ± 6.20 | -0.54 ± 2.45 | 37.7 ± 7.01 | -0.63 ± 3.33 | 0.93 | 0.79 | 0.09 [†] | | | |
| Body | 43.1 ± 8.37 | -0.11 ± 5.92 | 42.6 ± 8.78 | -0.56 ± 5.22 | 44.4 ± 8.51 | -0.25 ± 3.78 | 43.1 ± 8.99 | -0.37 ± 5.34 | 0.95 | 0.68 | 0.81 [†] | | | |
| Android | 42.6 ± 7.76 | -0.31 ± 3.49 | 40.5 ± 7.36 | -0.98 ± 3.12 | 39.1 ± 10.0 | -0.81 ± 2.97 | 40.7 ± 9.90 | -0.70 ± 3.19 | 0.73 | 0.61 | 0.06 [†] | | | |
| Gynoid | 92.5 ± 9.38 | 2.63 ± 4.19 | 91.0 ± 10.3 | 1.23 ± 3.47 | 92.0 ± 10.2 | 1.84 ± 4.22 | 92.0 ± 9.94 | 1.39 ± 4.14 | 0.08 | 0.63 | <0.0001 | | | |
| WC, cm | 110.7 ± 8.00 | 1.53 ± 4.62 | 109.2 ± 7.08 | 0.79 ± 4.26 | 110.1 ± 6.71 | 1.49 ± 4.21 | 109.3 ± 7.56 | 0.98 ± 4.15 | 0.73 ⁶ | 0.82 ⁶ | 0.0002 [†] | | | |
| HC, cm | 20.9 ± 2.26 | 0.74 ± 1.27 | 20.6 ± 2.25 | 0.67 ± 1.49 | 20.7 ± 2.43 | 0.57 ± 0.97 | 20.7 ± 2.31 | 0.57 ± 1.44 | 0.48 ⁷ | 0.86 ⁷ | <0.0001 [†] | | | |
| Sagittal height, cm | | | | | | | | | | | | | | |
| Blood pressure | | | | | | | | | | | | | | |
| Systolic, mm Hg | 118 ± 12.4 | -4.53 ± 10.4 | 115 ± 9.63 | -2.54 ± 12.2 | 114 ± 10.6 | -6.06 ± 10.6 | 114 ± 7.83 | -3.97 ± 7.99 | 116 ± 10.9 | 0.97 | 0.65 | <0.0001 [†] | | |
| Diastolic, mm Hg | 117 ± 10.9 | -4.16 ± 8.95 | 115 ± 9.74 | -0.28 ± 8.46 | 114 ± 11.0 | -4.32 ± 8.40 | 114 ± 7.86 | -3.39 ± 7.69 | 115 ± 10.4 | 0.78 | 0.0001 | | | |
| Medicated excluded ⁸ | 72.3 ± 8.42 | -4.68 ± 7.54 | 71.0 ± 9.37 | -2.92 ± 8.87 | 70.9 ± 8.06 | -4.17 ± 7.10 | 70.3 ± 7.36 | -3.08 ± 7.62 | 71.4 ± 8.60 | 0.41 | 0.10 | <0.0001 | | |
| Pulse, beats/min | 70.8 ± 7.51 | -4.47 ± 7.79 | 71.4 ± 9.43 | -1.67 ± 7.88 | 70.2 ± 8.02 | -3.55 ± 7.29 | 70.4 ± 7.37 | -2.75 ± 7.68 | 70.8 ± 8.34 | 0.41 | 0.12 | <0.0001 | | |
| Medicated excluded ⁸ | 66.1 ± 8.45 | 6.11 ± 6.53 | 67.6 ± 6.35 | 7.62 ± 8.31 | 68.0 ± 8.53 | 9.42 ± 6.78 | 66.1 ± 9.11 | 8.05 ± 7.06 | 67.2 ± 7.79 | 0.55 | 0.19 | <0.0001 | | |
| Lipid profile | 64.9 ± 7.27 | 6.03 ± 6.36 | 68.1 ± 6.18 | 7.94 ± 7.83 | 68.3 ± 8.77 | 9.52 ± 7.19 | 66.0 ± 9.33 | 8.25 ± 7.25 | 67.1 ± 7.50 | 0.45 | 0.28 | <0.0001 | | |
| Total cholesterol, mmol/L | 5.07 ± 0.87 | 0.85 ± 0.79 | 4.78 ± 0.77 | 0.83 ± 0.60 | 4.97 ± 1.08 | 0.85 ± 0.68 | 4.90 ± 0.99 | 0.77 ± 0.62 | 4.94 ± 0.91 | 1.00 ⁹ | 0.98 ⁹ | <0.0001 [†] | | |
| HDL cholesterol, mmol/L | 1.43 ± 0.31 | 0.15 ± 0.27 | 1.45 ± 0.33 | 0.25 ± 0.28 | 1.31 ± 0.28 | 0.16 ± 0.19 | 1.43 ± 0.33 | 0.22 ± 0.20 | 1.39 ± 0.31 | 0.34 ⁹ | 0.37 ⁹ | <0.0001 [†] | | |
| LDL cholesterol, mmol/L | 2.96 ± 0.69 | 0.61 ± 0.57 | 2.74 ± 0.61 | 0.53 ± 0.44 | 3.00 ± 0.88 | 0.61 ± 0.56 | 2.84 ± 0.76 | 0.51 ± 0.50 | 2.90 ± 0.73 | 0.90 ⁹ | 0.90 ⁹ | <0.0001 | | |
| Triglycerides, mmol/L | 1.22 ± 0.69 | 0.33 ± 0.61 | 0.87 ± 0.37 | 0.08 ± 0.34 | 1.16 ± 0.72 | 0.25 ± 0.59 | 1.00 ± 0.50 | 0.11 ± 0.37 | 1.08 ± 0.63 | 0.10 ^{9,9} | 0.33 ^{9,9} | <0.0001 | | |
| FFA, μmol/L | 373 ± 155 | -442 ± 290 | 386 ± 150 | -449 ± 247 | 390 ± 185 | -398 ± 314 | 418 ± 191 | -446 ± 393 | 383 ± 163 | 0.63 ⁹ | 0.26 ⁹ | <0.0001 | | |
| Glucose metabolism | | | | | | | | | | | | | | |
| Glucose, mmol/L | 5.50 ± 0.47 | 0.14 ± 0.42 | 5.52 ± 0.37 | 0.24 ± 0.48 | 5.61 ± 0.56 | 0.17 ± 0.47 | 5.41 ± 0.49 | 0.11 ± 0.035 | 5.54 ± 0.47 | 0.74 ⁹ | 0.81 ⁹ | <0.0001 | | |
| Insulin, pmol/L | 50.4 ± 23.2 ¹⁰ | 3.21 ± 18.9 ¹¹ | 48.4 ± 24.6 ¹⁰ | -1.50 ± 33.9 ¹¹ | 56.3 ± 35.4 ¹⁰ | -0.64 ± 27.7 ¹¹ | 54.6 ± 43.6 ¹⁰ | 2.42 ± 37.6 ¹¹ | 51.6 ± 28.1 ¹⁰ | 0.34 ⁹ | 0.15 ⁹ | 0.37 ^{8,11} | | |
| C-peptide, ¹² pmol/L | 539 ± 160 | 15.5 ± 135 | 504 ± 173 | -16.8 ± 185 | 541 ± 174 | -23.3 ± 158 | 531 ± 190 | -10.5 ± 177 | 528 ± 168 | 0.19 ¹³ | 0.52 ¹³ | 0.92 [§] | | |
| HOMA-IR | 1.80 ± 0.87 ¹⁰ | 0.15 ± 0.69 ¹¹ | 1.73 ± 0.94 ¹⁰ | -0.02 ± 1.32 ¹¹ | 2.07 ± 1.48 ¹⁰ | 0.05 ± 1.16 ¹¹ | 1.94 ± 1.75 ¹⁰ | 0.15 ± 1.12 ¹⁰ | 1.87 ± 1.12 ¹⁰ | 0.06 ± 1.08 ¹¹ | 0.46 ^{§,14} | 0.75 ^{§,14} | | |
| HOMA-β | 72.8 ± 33.0 ¹⁰ | -2.03 ± 34.5 ¹¹ | 68.5 ± 33.4 ¹⁰ | -7.01 ± 37.4 ¹¹ | 77.3 ± 41.1 ¹⁰ | -7.82 ± 31.9 ¹¹ | 81.7 ± 50.3 ¹⁰ | -1.56 ± 40.6 ¹¹ | 72.8 ± 35.8 ¹⁰ | -5.65 ± 34.5 ¹¹ | 0.78 ^{§,14} | 0.16 ^{§,11} | | |

¹ All values are means ± SDs. Data are given for completion of the WM period (week 32) and the change during WM according to supplement intake. [§]Variables were log transformed before analysis.

[†] Analyzed with the use of a paired-sample Wilcoxon's Signed Rank test. BW, body weight; FFA, free fatty acid; FM, fat mass; HC, hip circumference; HOMA-β, homeostatic model assessment of β cell function; LBM, lean body mass; WC, waist circumference; WM, weight maintenance.

² Values are means of the 3 protein groups (wheny protein and calcium, wheny protein, and soy protein).

³ P values for test of group differences in the ΔWM were analyzed with the use of an ANCOVA and adjusted for the change of the outcome during weight loss, sex, and age. No overall supplement effects were observed for any of the outcomes (all $P > 0.05$).

⁴ P values for the test of differences in the ΔWM between protein groups (wheny protein and calcium, wheny protein, and soy protein) compared with the control group were analyzed with the use of an ANCOVA and adjusted for the change of the outcome during weight loss, sex, and age. No differences were observed for any of the outcomes ($P > 0.05$).

⁵ P values for comparisons of before and after WM results (week 8 compared with week 32) were analyzed with the use of a paired t test for all completers.

⁶ $n = 150$ (wheny protein and calcium: $n = 38$; wheny protein: $n = 39$; soy protein: $n = 35$; and control: $n = 38$). All protein groups: $n = 111$.

⁷ $n = 149$ (wheny protein and calcium: $n = 37$; wheny protein: $n = 36$; and control: $n = 38$). All protein groups: $n = 111$.

⁸ $n = 135$ (wheny protein and calcium: $n = 32$; wheny protein: $n = 31$; and control: $n = 36$). All protein groups: $n = 99$.

⁹ $n = 149$ (wheny protein and calcium: $n = 36$; wheny protein: $n = 39$; soy protein: $n = 36$; and control: $n = 38$). All protein groups: $n = 111$.

¹⁰ $n = 147$ (wheny protein and calcium: $n = 37$; wheny protein: $n = 35$; and control: $n = 38$). All protein groups: $n = 109$.

¹¹ $n = 145$ (wheny protein and calcium: $n = 37$; wheny protein: $n = 35$; and control: $n = 38$). All protein groups: $n = 107$.

¹² $n = 150$ (wheny protein and calcium: $n = 38$; wheny protein: $n = 36$; and control: $n = 38$). All protein groups: $n = 112$.

¹³ $n = 148$ (wheny protein and calcium: $n = 36$; wheny protein: $n = 38$; and control: $n = 38$). All protein groups: $n = 110$.

¹⁴ $n = 143$ (wheny protein and calcium: $n = 33$; wheny protein: $n = 35$; and control: $n = 38$). All protein groups: $n = 105$.

cutoffs were determined from participants who had PABA recovery between 80% and 120% and a urinary volume >500 mL at week 32.

RESULTS

In total, 220 participants were included in the study (**Figure 2**). The dropout rate during the WL period was 10.6%. The main reasons for dropout were a lack of adherence to the LCD or private or familial illnesses or problems. After WL, 2 participants were excluded because of WL <8% of the initial BW, and 4 participants dropped out before the WM period was initiated. Hence, the WM period was initiated by 189 participants of whom 152 completed the period (dropout rate: 19.6%). One participant was excluded from all analyses because the participant increased the reported PAL from 2.1 (after WL) to 2.6 and 2.93 (extremely active) for weeks 14 and 32, respectively. Therefore, the completer analyses include 151 participants. The dropout rate in the soy group was 26.5%, whereas the dropout rates in the 3 remaining groups were from 17.0% to 19.2%, with no difference in the number of dropouts between all groups ($P > 0.62$). The main reasons for dropout during WM were private or familial illnesses or problems, job-related problems, or unknown reasons (**Figure 2**).

The 219 participants who completed the baseline CID were 171 women and 48 men with a mean age of 40.0 ± 10.7 y and a mean weight of 96.6 ± 13.8 kg (men: 108.9 ± 13.7 kg; women: 93.2 ± 11.7 kg) (**Supplemental Table 1**). Of 219 participants, 20% were overweight, 48% were obese category I (BMI ≥ 30 to <35), 29% were obese category II (BMI ≥ 35 to <40), and 3% were obese category III (BMI ≥ 40). Overall baseline characteristics of completers are presented in **Table 1**, and characteristics according to random assignment to supplementation that was provided during WM are presented in **Supplemental Table 2**.

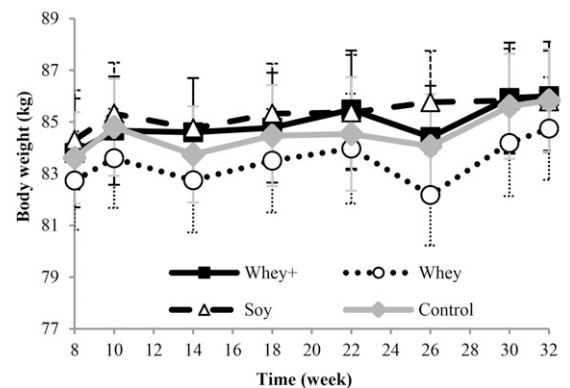
Overall, completers lost 12.7 ± 2.95 kg of BW during the 8-wk WL period, which corresponded to $13.2\% \pm 2.34\%$ of their initial BW. Waist and hip circumference as well as sagittal height decreased during this period (**Supplemental Table 3**). Except for an increase in FFA concentrations, all other markers of blood lipid profiles and glucose metabolism decreased with no differences between groups (**Supplemental Table 3**). Overall, systolic BP and diastolic BP did not change during WL. However, for completers who did not use BP-reducing medicine, diastolic BP decreased, and the change in systolic BP differed between groups (overall supplement effect: $P = 0.042$). After adjustment for multiple testing with the use of Bonferroni correction, none of the pairwise tests remained significant (**Supplemental Table 3**). There were no differences in the characteristics between the supplement groups before the initiation of the WM period (**Table 1**).

Overall, completers gained 2.05 ± 4.39 kg BW, FM tended to increase 0.31 ± 3.99 kg, and LBM increased 1.78 ± 1.47 kg during WM (**Table 2**). However, there were no differences in the changes in BW (**Figure 3**), LBM, or FM between the groups or between all protein groups compared with the control group (**Table 2**). Waist and hip circumferences as well as sagittal height all increased during WM. No differences between groups were observed for waist or hip circumference and sagittal height or between all protein groups compared with the control group

(**Table 2**). An analysis of anthropometric variables for men and women separately did not change the overall results. A minor difference was observed for FM whereby an increase was observed for men (1.56 ± 3.54 kg; $P = 0.0273$) but not for women (0.03 ± 4.04 kg; $P = 0.93$). However, no differences in the change in FM between groups were observed (men: $P = 0.46$; women: $P = 0.96$).

The reported PAL increased 0.029 during WL ($P = 0.016$) but did not change during WM, and no differences were observed between the groups (**Table 3**). The inclusion of the reported PAL during WM and the change in BW during WL as covariates in the analyses did not change the effect of supplementation on BW, FM, and LBM (all $P \geq 0.52$).

Per-protocol analyses with cutoffs for protein intake in the protein groups ($>1.27 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) and control group ($<1.28 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) showed that there were no differences in changes in BW, FM, hip circumference, and sagittal height between the 4 groups or between protein groups compared with the control group during the WM period (all $P > 0.23$). An overall supplement effect ($P = 0.03$) was observed for the change in waist circumference between groups, and after post hoc tests with adjustment for multiple testing, the change in waist circumference during WM differed between the whey+ and soy groups ($P = 0.024$) when the analysis was based on compliance at week 14, and the PABA requirements were fulfilled, but the overall supplement effect disappeared when the analysis was based on compliance at week 32 ($P > 0.52$) or at both weeks 32 and 14 ($P > 0.22$). No difference in the change in waist circumference was observed between the protein groups compared with the control group (all $P \geq 0.67$). The change in LBM did not differ between groups when the analyses were based on compliance at week 14 or 32 with or without fulfilling the PABA requirement (all $P \geq 0.18$), but if compliance both at weeks 14 and 32 were fulfilled, the



| Time (week) | 8 | 10 | 14 | 18 | 22 | 26 | 30 | 32 |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Whey+ n: | 38 | 38 | 38 | 38 | 33 | 35 | 37 | 38 |
| Whey n: | 39 | 39 | 37 | 34 | 35 | 34 | 38 | 39 |
| Soy n: | 36 | 36 | 35 | 36 | 28 | 35 | 36 | 36 |
| Control n: | 38 | 37 | 37 | 36 | 31 | 34 | 37 | 38 |
| N: | 151 | 150 | 147 | 143 | 127 | 128 | 148 | 151 |

FIGURE 3 Mean \pm SEM BW during 24 wk of WM. BWs are shown during the 24-wk WM period in the 4 groups of completers ($n = 151$). BWs at weeks 8 and 32 were measured in the fasting state, whereas all other time points denote nonfasting measurements. Analysis with an ANCOVA and adjusted for the change in BW during weight loss, sex, and age showed no differences for the change in BW during WM (week 32 – week 8) between groups ($P = 0.96$) or for protein groups compared with the control group ($P = 0.81$). BW, body weight; whey+, whey and calcium; WM, weight maintenance.

protein groups ($n = 47$) had a 0.80-kg larger increase in LBM than that of the control group ($n = 16$) ($P = 0.038$).

Except for FFA concentrations that decreased, all other blood lipid profile markers increased during the WM period (Table 2). No differences were observed between groups or between all protein groups compared with the control group for total-cholesterol, LDL-cholesterol, HDL-cholesterol, FFA, and triglyceride concentrations. In the WM period, glucose concentrations increased, whereas insulin and C-peptide concentrations, HOMA-IR, and HOMA- β did not change, but there were no differences between groups or between all protein groups compared with the control group (Table 2).

At baseline, the completers had a reported energy intake of 9878 kJ/d with a macronutrient distribution of 35.7% of energy from fat, 45.6% of energy from carbohydrate, and 17.1% of energy from protein ($\sim 1.02 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) and a reported calcium intake of 1195 mg/d as well as a reported PAL of 1.62 ± 0.24 . Except for a difference in reported fat intake, no baseline differences were observed between groups (Table 3). Protein intakes that were calculated from the 24-h urinary nitrogen excretion did not differ between groups at baseline (Table 4). At week 32, the completers' reported energy intake was reduced to 8146 kJ/d (without supplements). The diet (without supplements) consisted of 32.8% of energy from fat, 46.2% of

TABLE 3
For completers, reported habitual diet (without supplement) and PAL ($n = 151$)¹

| | Whey protein and calcium ($n = 38$) | Whey protein ($n = 39$) | Soy protein ($n = 36$) | Control ($n = 38$) |
|---|--|--------------------------------|------------------------------|--------------------------------|
| Baseline (week 0) | | | | |
| Energy, ² kJ/d | 9531 \pm 2692 | 9969 \pm 2659 | 10,509 \pm 3612 | 9552 \pm 2069 |
| Fat, ² E% | 33.8 \pm 4.47 ^b | 36.4 \pm 5.96 ^{a,b} | 37.5 \pm 6.06 ^a | 35.3 \pm 6.02 ^{a,b} |
| Carbohydrate, ² E% | 47.1 \pm 5.88 | 44.7 \pm 7.83 | 45.5 \pm 5.24 | 45.0 \pm 6.23 |
| Protein ² | | | | |
| E% | 17.2 \pm 2.69 | 16.9 \pm 3.51 | 16.3 \pm 3.53 | 18.0 \pm 3.81 |
| $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ | 1.01 \pm 0.29 | 1.02 \pm 0.27 | 1.00 \pm 0.26 | 1.03 \pm 0.20 |
| Calcium, ² mg/d | 1190 \pm 422 | 1193 \pm 400 | 1210 \pm 544 | 1191 \pm 423 |
| PAL ³ | 1.58 \pm 0.21 | 1.65 \pm 0.26 | 1.64 \pm 0.24 | 1.61 \pm 0.23 |
| Before WM (week 8), ⁴ PAL | 1.64 \pm 0.26 | 1.69 \pm 0.28 | 1.62 \pm 0.22 | 1.63 \pm 0.24 |
| WM (week 14) | | | | |
| Energy, ⁵ kJ/d | 7209 \pm 2074 | 7283 \pm 2157 | 7379 \pm 2316 | 6760 \pm 1720 |
| Fat, ⁵ E% | 31.0 \pm 6.25 | 32.7 \pm 7.24 | 31.7 \pm 6.17 | 34.3 \pm 7.14 |
| Carbohydrate, ⁵ E% | 47.6 \pm 8.18 ^a | 44.9 \pm 9.70 ^{a,b} | 48.0 \pm 7.51 ^a | 41.4 \pm 7.26 ^b |
| Protein ⁵ | | | | |
| E% | 19.3 \pm 4.12 | 20.3 \pm 5.46 | 18.5 \pm 4.40 | 20.7 \pm 4.86 |
| $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ | 0.97 \pm 0.31 | 1.03 \pm 0.35 | 0.94 \pm 0.29 | 0.97 \pm 0.24 |
| Calcium, ⁵ mg/d | 1054 \pm 404 | 1039 \pm 405 | 1028 \pm 381 | 981 \pm 278 |
| PAL ⁶ | 1.62 \pm 0.22 | 1.74 \pm 0.30 | 1.66 \pm 0.27 | 1.63 \pm 0.22 |
| After WM (week 32) | | | | |
| Energy, ^{7,*} kJ/d | 8040 \pm 2197 | 8515 \pm 2361 | 8411 \pm 3821 | 7576 \pm 2209 |
| Fat, ⁷ E% | 31.4 \pm 5.79 | 34.1 \pm 7.31 | 32.5 \pm 6.91 | 33.0 \pm 7.88 |
| Carbohydrate, ⁷ E% | 46.9 \pm 6.38 | 44.5 \pm 10.2 | 48.5 \pm 7.50 | 44.9 \pm 9.14 |
| Protein, ⁷ | | | | |
| E% | 19.1 \pm 3.72 | 19.2 \pm 4.98 | 18.4 \pm 3.74 | 19.9 \pm 5.18 |
| $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ * | 1.03 \pm 0.25 | 1.13 \pm 0.33 | 1.03 \pm 0.36 | 1.01 \pm 0.28 |
| Calcium, ⁷ mg/d | 1053 \pm 369 | 1080 \pm 453 | 1105 \pm 542 | 987 \pm 379 |
| PAL ⁸ | 1.68 \pm 0.25 | 1.70 \pm 0.29 | 1.62 \pm 0.23 | 1.63 \pm 0.21 |

¹ All values are means \pm SDs. Data are given for baseline (week 0), the initiation of WM (week 8), during WM (week 14), and at the completion of WM (week 32) according to supplement intake. At each time point, differences between groups were analyzed with the use of an ANOVA and adjusted for sex and age. Energy intake, protein (E%), and calcium at week 0, 14 and 32, protein ($\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) at week 14 and fat at week 32 were log-transformed before analysis. The reported PAL was analyzed with the use of a Kruskal-Wallis test. Except for fat intake (E%) at week 0 and carbohydrate intake (E%) at week 14, no differences between groups were observed (all $P > 0.05$). For fat intake (E%) (overall supplement effect: $P = 0.0426$) and carbohydrate intake (E%) (overall supplement effect: $P = 0.0027$), group differences remained significant after adjustment for multiple testing with the use of Bonferroni correction, and the significant differences are marked with different superscript letters. *During WM, differences were analyzed with the use of paired t tests. Overall, energy intake ($P < 0.0001$) and log-transformed protein intake ($\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) ($P < 0.0001$) increased from weeks 14 to 32; however, changes (week 32 minus week 14) did not differ between groups (ANOVA adjusted for sex and age). E%, percentage of energy; PAL, physical activity level; WM, weight maintenance.

² $n = 146$ (whey protein and calcium: $n = 38$; whey protein: $n = 38$; soy protein: $n = 34$; and control: $n = 36$).

³ $n = 147$ (whey protein and calcium: $n = 37$; whey protein: $n = 38$; soy protein: $n = 35$; and control: $n = 37$).

⁴ $n = 142$ (whey protein and calcium: $n = 36$; whey protein: $n = 37$; soy protein: $n = 34$; and control: $n = 35$).

⁵ $n = 141$ (whey protein and calcium: $n = 36$; whey protein: $n = 36$; soy protein: $n = 34$; and control: $n = 35$).

⁶ $n = 138$ (whey protein and calcium: $n = 35$; whey protein: $n = 38$; soy protein: $n = 32$; and control: $n = 33$).

⁷ $n = 141$ (whey protein and calcium: $n = 37$; whey protein: $n = 37$; soy protein: $n = 34$; and control: $n = 33$).

⁸ $n = 148$ (whey protein and calcium: $n = 38$; whey protein: $n = 39$; soy protein: $n = 36$; and control: $n = 35$).

energy from carbohydrate, and 19.1% of energy from protein ($\sim 1.05 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) and a reported calcium intake of 1057 mg/d with no differences between the groups (Table 3). During WM (weeks 14–32), the reported dietary intake (without supplements) showed an overall increase in energy and protein intakes of 825 kJ/d ($P < 0.0001$) and $0.070 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ($P < 0.0001$), respectively. However, increased energy and protein intakes did not differ between groups (Table 3). After the addition of intake of protein from supplements (45 g/d) to the reported habitual diet, protein intake differed between control and protein groups both at weeks 14 and at 32, and urinary nitrogen excretion confirmed the differences in protein intake (Table 4). Protein intake, which was calculated from urinary nitrogen excretion and evaluated via PABA, differed at week 32 between the protein groups (overall protein effect: $P = 0.017$), and post hoc tests with correction for multiple testing showed that the whey group had higher protein intake than that of the whey+ group ($P = 0.018$) (Table 4).

The REE and respiratory quotient decreased from week 0 to 32 by 467 kJ/d ($P < 0.0001$) and 0.022 ($P < 0.0001$), respectively. Adjusted for age and sex, the control group decreased the REE by 243 kJ/d more than did the protein groups ($P = 0.025$), but no

difference was observed between all groups ($P = 0.09$). The change in the respiratory quotient did not differ between groups (protein groups compared with control group: $P = 0.54$; between groups: $P = 0.52$). Further adjustment for LBM did not change the supplement effect on the REE or respiratory quotient, but LBM as a covariate had a significant influence ($P < 0.05$) on the models. At baseline (week 0), the REE was linearly associated with LBM [$0.097 \text{ LBM} + 1.726$ ($R^2 = 0.61$, $P < 0.001$)], and the mean REE was $6.75 \pm 0.98 \text{ MJ/d}$. On the basis of this regression equation, we calculated the predicted REE after WM from the LBM that was measured at week 32 (all completers: 6.58 MJ/d; whey+: 6.58 MJ/d; whey: 6.59 MJ/d; soy: 6.63 MJ/d; and control: 6.51 MJ/d). At week 32, the measured REE was linearly associated with LBM in all groups (all $P < 0.001$), and except for the whey+ group, the slope of the regression lines decreased after the intervention compared with at baseline [i.e., predicted REE (Figure 4)]. The mean measured REE was $6.31 \pm 0.89 \text{ MJ/d}$ for all completers, and for each group, the REE was as follows: whey+: $6.37 \pm 1.03 \text{ MJ/d}$; whey: $6.28 \pm 0.83 \text{ MJ/d}$; soy: $6.32 \pm 0.85 \text{ MJ/d}$; and control: $6.26 \pm 0.88 \text{ MJ/d}$. A paired t test showed that the overall measured REE was 0.278 MJ/d lower than the predicted REE (all completers: $P < 0.0001$), and

TABLE 4

For completers, compliance evaluation on the basis of protein intake reported in dietary records and calculated from urinary nitrogen excretion ($n = 151$)¹

| | Baseline (week 0) | | | | WM (week 14) | | | | WM (week 32) | | | |
|-------------------------------------|-------------------|---|-------------------------|--------------------|--------------|---|-------------------------|---------------------|--------------|---|-------------------------|---------------------|
| | <i>n</i> | Protein intake, $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ | | P^2 | <i>n</i> | Protein intake, $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ | | P^2 | <i>n</i> | Protein intake, $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ | | P^2 |
| | | From dietary records | From nitrogen excretion | | | From dietary records ³ | From nitrogen excretion | | | From dietary records ³ | From nitrogen excretion | |
| All samples | | | | | | | | | | | | |
| Protein groups ⁴ | 108 | 1.01 ± 0.27 | 1.05 ± 0.32 | 0.035 [†] | 104 | 1.53 ± 0.34 | 1.39 ± 0.47 | 0.0002 [†] | 106 | 1.61 ± 0.34 | 1.35 ± 0.46 | <0.0001 |
| Control | 36 | 1.03 ± 0.20 | 1.07 ± 0.30 | 0.41 | 34 | 0.97 ± 0.24 | 1.05 ± 0.25 | 0.15 | 33 | 1.01 ± 0.28 | 1.17 ± 0.31 | 0.0052 [†] |
| P^5 | — | 0.90 | 0.89 | — | — | <0.0001 [§] | 0.0001 | — | — | <0.0001 | 0.045 | — |
| Whey protein and calcium | 36 | 1.00 ± 0.29 | 1.09 ± 0.37 | 0.051 [†] | 35 | 1.51 ± 0.35 | 1.45 ± 0.61 | 0.062 [†] | 36 | 1.58 ± 0.29 | 1.28 ± 0.42 | 0.0002 |
| Whey protein | 38 | 1.02 ± 0.27 | 1.06 ± 0.27 | 0.12 [†] | 35 | 1.59 ± 0.37 | 1.36 ± 0.37 | 0.0024 | 36 | 1.66 ± 0.36 | 1.49 ± 0.50 | 0.055 |
| Soy protein | 34 | 1.00 ± 0.26 | 1.00 ± 0.31 | 0.90 | 34 | 1.49 ± 0.31 | 1.37 ± 0.042 | 0.051 | 34 | 1.57 ± 0.36 | 1.29 ± 0.43 | 0.001 |
| P^6 | — | 0.88 | 0.42 | — | — | 0.39 | 0.67 | — | — | 0.46 | 0.067 | — |
| PABA requirement⁷ | | | | | | | | | | | | |
| Protein groups ⁴ | 81 | 1.02 ± 0.27 | 1.10 ± 0.26 | 0.007 | 68 | 1.52 ± 0.35 | 1.45 ± 0.41 | 0.035 [§] | 53 | 1.60 ± 0.30 | 1.51 ± 0.43 | 0.14 |
| Control | 24 | 1.01 ± 0.19 | 1.07 ± 0.23 | 0.27 | 15 | 0.98 ± 0.28 | 1.16 ± 0.24 | 0.079 | 15 | 1.03 ± 0.29 | 1.12 ± 0.29 | 0.26 |
| P^5 | — | 0.84 | 0.52 | — | — | <0.0001 [§] | 0.016 | — | — | <0.0001 | 0.0013 [§] | — |
| Whey protein and calcium | 30 | 1.03 ± 0.30 | 1.11 ± 0.27 | 0.049 | 24 | 1.58 ± 0.35 | 1.55 ± 0.41 | 0.53 | 22 | 1.58 ± 0.27 | 1.39 ± 0.39^a | 0.045 |
| Whey protein | 28 | 1.01 ± 0.24 | 1.10 ± 0.24 | 0.093 | 24 | 1.51 ± 0.35 | 1.38 ± 0.36 | 0.10 [§] | 16 | 1.69 ± 0.30 | 1.76 ± 0.43^b | 0.53 |
| Soy protein | 23 | 1.02 ± 0.28 | 1.09 ± 0.29 | 0.30 | 20 | 1.47 ± 0.34 | 1.42 ± 0.45 | 0.49 | 15 | 1.53 ± 0.35 | $1.44 \pm 0.40^{a,b}$ | 0.36 |
| P^6 | — | 0.91 | 0.89 | — | — | 0.56 | 0.32 | — | — | 0.34 | 0.017 | — |

¹ All values are means \pm SDs. Data is given for baseline (week 0), during WM (week 14) and at the completion of WM (week 32). [§]Variables were log transformed before analysis. [†]Analyzed with the use of a paired-sample Wilcoxon's Signed Rank test. PABA, para-amino-benzoic acid; WM, weight maintenance.

² Intragroup comparison of reported protein intake from dietary records with that calculated from nitrogen excretion. Data were analyzed with the use of a paired t test.

³ Protein intake of 45 g/d from the supplement was added to the dietary records in all the protein groups.

⁴ Values are means of the 3 protein groups (whey protein and calcium, whey protein, and soy protein).

⁵ Intergroup comparison of protein intake between protein groups with that of the control group. Data were analyzed with the use of an ANOVA and adjusted for age and sex.

⁶ Inter-protein-group comparison of protein intake between the 3 protein groups. Data were analyzed with the use of an ANOVA and adjusted for age and sex. Differences between groups after adjustment for multiple testing with the use of Bonferroni correction are marked by different superscript letters.

⁷ Only samples from completers with PABA recovery between 80% and 120% and a urine volume $\geq 500 \text{ mL}$ were included in the analyses.

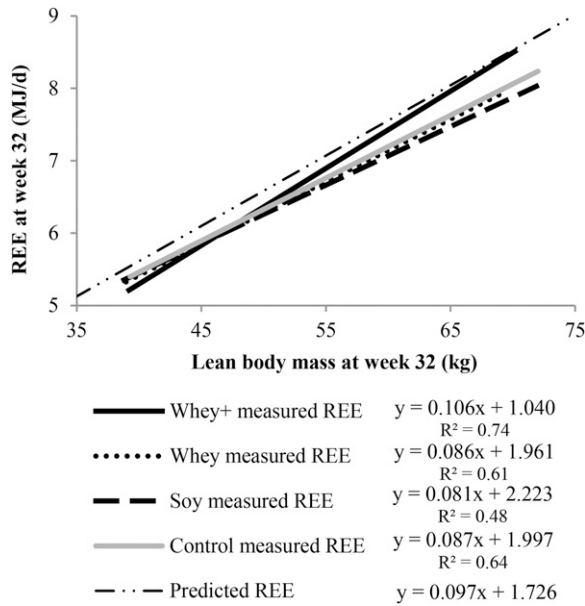


FIGURE 4 REE as a function of LBM. At baseline (week 0), REE was linear associated with lean body mass [0.097 LBM + 1.726 ($R^2 = 0.61$)]. At week 32, this regression equation was used to predict the REE for comparison with the measured REE. Except for the whey+ group, the slope of the regression lines for measured REE decreased after the intervention compared with that of the predicted REE. REE, resting energy expenditure; whey+, whey and calcium.

this difference was also observed within each supplement group (all $P < 0.02$). However, the measured REE minus the predicted REE when adjusted for age and sex did not differ between groups (all groups: $P = 0.83$; protein groups compared with control group: $P = 0.75$).

At both meal tests, DIT differed between the groups; adjusting for LBM did not change the results, but because the covariate was significant in the model, the DIT results presented were from the adjusted model (overall supplement effects: week 0: $P = 0.0015$; week 32: $P = 0.040$). Post hoc tests showed that the consumption of the control supplement in week 0 resulted in a lower DIT (least-squares mean: 125.4 kJ/2.5 h; 95% CI: 113.6, 137.1 kJ/2.5 h) than with the consumption of the whey+ (least-squares mean difference: 26.4 kJ/2.5 h; 95% CI: 4.2, 48.5 kJ/2.5 h), whey (29.6 kJ/2.5 h; 95% CI: 7.1, 52.0 kJ/2.5 h), and soy (29.1 kJ/2.5 h; 95% CI: 6.5, 51.6 kJ/2.5 h) supplements. In week 32, the post hoc tests showed the same numerical pattern, although DIT was higher only after soy consumption compared with the control group (29.1 kJ/2.5 h; 95% CI: 2.3, 56.0 kJ/2.5 h). There was no interaction between groups and visits ($P = 0.76$).

At both meal tests, there were differences in the VAS scores for satiety (overall supplement effects: week 0: $P = 0.013$; week 32: $P = 0.0012$) (Figure 5), fullness (overall supplement effects: week 0: $P = 0.0033$; week 32: $P = 0.013$), and prospective consumption (overall supplement effects: week 0: $P = 0.0057$; week 32: $P = 0.0052$) between groups. The post hoc tests showed that, at week 0, the VAS scores of satiety were higher in the whey+ group than in the soy group ($P = 0.026$), and fullness scores were higher in the whey+ group ($P = 0.017$) and the whey group ($P = 0.042$) than in the control group. The VAS scores of prospective consumption were lower in the whey+ group ($P = 0.019$) and in the whey group ($P = 0.037$) than in the control group. At

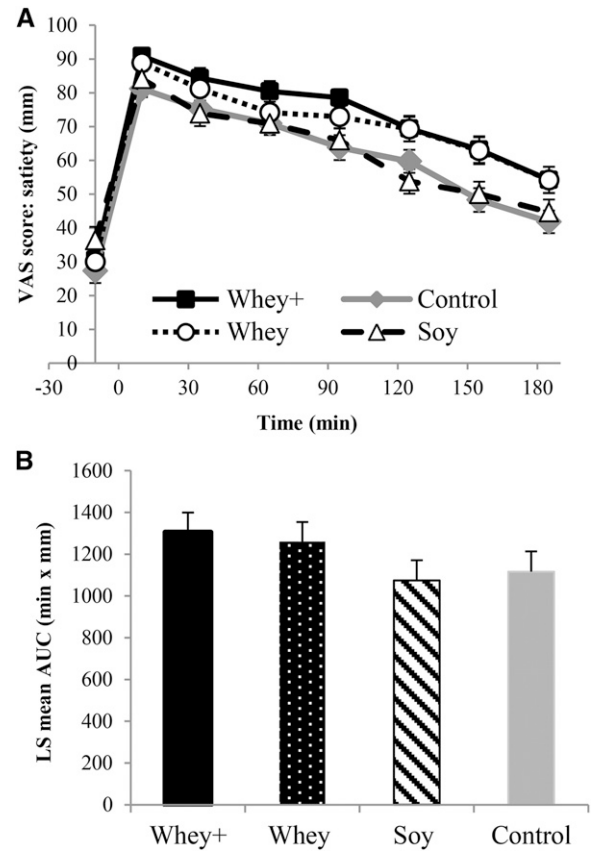


FIGURE 5 The breakfast test meal was served at time 0 min, and the postprandial satiety VAS was collected until 185 min (whey+: $n = 38$; whey: $n = 38$; soy: $n = 36$; and control: $n = 36$). On the VAS, 100 mm corresponds to “I cannot eat another bite.” (A) Mean \pm SEM satiety scores during the meal test at week 32 (after weight maintenance). A repeated-measures ANCOVA was used to test differences between postprandial scores (overall supplement effect: $P = 0.0012$). After adjustment for multiple testing, the post hoc tests showed differences between whey+ compared with the control: $P = 0.025$; whey+ compared with soy: $P = 0.0059$; and whey compared with soy: $P = 0.0033$. There were no differences between baseline values [$P > 0.05$ (ANOVA)] and no time \times supplement interaction ($P > 0.05$). (B) LS means (95% CIs) of satiety AUC. An ANCOVA was used to test the effect of supplements (overall supplement effect: $P = 0.0017$). After adjustment for multiple testing, the post hoc tests showed differences between whey+ compared with the control: $P = 0.032$; whey+ compared with soy: $P = 0.0050$; and whey compared with soy: $P = 0.036$. LS, least squares; VAS, visual analog scale; whey+, whey and calcium.

week 32, post hoc tests showed that satiety and fullness scores were higher in the whey+ group than in the control group (satiety: $P = 0.025$; fullness: $P = 0.015$) and the soy groups (satiety: $P = 0.0059$; fullness: $P = 0.010$), and the satiety scores were higher in the whey group than in the soy group ($P = 0.033$). The prospective consumption scores were higher in the whey+ group than in the control group ($P = 0.043$) and soy groups ($P = 0.029$). VAS AUC results yielded similar results to those of the repeated measurement ANOVA (Figure 5). There were no differences in baseline VAS scores between the 2 visits for any of the appetite variables. Analyses of the appetite VAS AUC and AOC showed that there was no interaction between supplements and visits ($P > 0.50$). At week 32, there was a time \times supplement interaction ($P = 0.0006$) in the well-being VAS scores. Post hoc tests showed that the completers in the whey+ group felt less well at time 10 min (right after

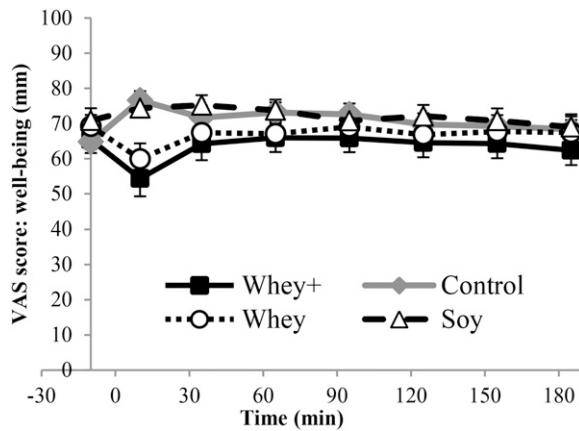


FIGURE 6 Mean \pm SEM well-being scores during the meal test at week 32 (after weight maintenance). The breakfast test meal was served at time 0 min, and the postprandial well-being VAS was collected until 185 min (whey+: $n = 38$; whey: $n = 38$; soy: $n = 36$; and control: $n = 36$). On the VAS, 100 mm corresponds to “I feel really good.” A repeated-measures ANCOVA was used to test differences between postprandial scores (supplement \times time interaction: $P = 0.0006$). Results of post hoc tests at time 10 min were as follows: whey+ compared with control: $P < 0.0001$; whey+ compared with soy: $P = 0.042$; and whey compared with control: $P = 0.0031$. VAS, visual analog scale; whey+, whey and calcium.

consumption of the breakfast test meal) than in the control and soy groups and in the whey group compared with the control group (Figure 6). There was no difference in ad libitum energy intake between the 4 supplement groups either at week 0 ($P = 0.41$) or week 32 ($P = 0.43$), and there was no interaction between supplements and visits ($P = 0.89$). No differences in palatability in the ad libitum test meal were observed.

DISCUSSION

Contrary to our hypothesis, we did not show that the consumption of protein supplements, which resulted in a daily protein intake of 1.45 g/kg compared with 1.16 g/kg in controls, improved body composition over a 24-wk WM period. However, the acute effect of the protein supplements during the meal test resulted in a subjective appetite sensation that was expected to reduce energy intake compared with the effect of the control, but this change was not reflected by a reduction in ad libitum energy intake. Likewise, the changes in REE and DIT did not improve WM for protein-supplemented groups compared with the control group.

The effect of protein during WL and WM may differ, and although several studies have reported results on an HP diet during WL, only a very few studies, to our knowledge, have reported results on HP only during WM and are thus comparable with the current study. In contrast to the current study, 3 short-term studies with a WL period of 4–5 wk and a WM period of 12–13 wk all concluded that adding protein to the diet improved WM (17, 24, 28). In 2 of the 3 studies with reported measurements of urinary nitrogen excretion, the urinary nitrogen excretion [8.6 g/d (28) and 9.8 g/d (17)] in the control groups was lower than in the present study (12.8–13.1 g/d). Unfortunately, the studies did not report the absolute dose in grams per kilogram per day and, therefore, it can only be speculated if such protein intakes are representative of a normal protein intake ($>0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) (29). In the current study, the control group consumed protein doses $>1.0 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (confirmed

by urinary nitrogen excretion) throughout the WM period as their habitual protein intake, thereby suggesting that these previous studies of short duration could have overestimated the effect of increasing protein intake during WM because the control groups had a relatively low protein intake and not a normal (habitual) protein intake. Results from long-term studies with WL periods of 4–12 wk followed by WM periods of 24–48 wk have been inconsistent. Two studies (25, 30) showed that an increase in protein intake improved WM, whereas 2 other studies (31, 32) did not. Except for one study (32), all of the long-term studies evaluated urinary nitrogen excretion and showed that there was a difference in protein intake between the control and protein groups, but absolute protein intake in grams per kilogram per day was not reported in any of the studies. A plausible reason for the different results could have been related to the difference between protein intakes in the protein compared with control groups. To evaluate the effect of protein intake, “the protein spread theory” has been suggested by Bosse and Dixon (33), who calculated the average percentage of energy that was spread during the study as

$$\left[\left(\text{g} \times \text{kg}^{-1} \times \text{d}^{-1} \text{ intake in HP group} - \text{g} \times \text{kg}^{-1} \times \text{d}^{-1} \text{ intake in control group} \right) \div \left(\text{g} \times \text{kg}^{-1} \times \text{d}^{-1} \text{ intake in control group} \right) \right] \times 100 \quad (4)$$

The results of the 51 studies included in the analysis had an average spread of 52.0–62.7% for the studies that reported anthropometric benefits for the HP group, whereas the studies that reported no benefits had an average spread of 30.3–41.6% (33). According to the protein-spread theory, the obtained difference in the protein intake between the protein groups and the control group in the current study was not effective because the average spread was close to 30%. The low average spread was due to high habitual protein intake in the control group during WM (protein intake: $1.12\text{--}1.16 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$; 19.9–20.7% of energy; 93–95 g/d).

According to Bosse and Dixon (33), the protein spread that was needed to obtain beneficial effects in the current study would have required the protein groups to have had protein intake of $\sim 1.85 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ or a doubling in current intake (90 g protein supplement/d), which corresponded to a caloric load of 1530 kJ/d from protein supplements. In line with the protein-spread theory, we observed a beneficial effect on LBM in the protein groups compared with in the control group in the per-protocol analysis. The stringent compliance control that was applied in the per-protocol analysis resulted in a mean protein intake (calculated from the urinary nitrogen excretion) of $1.66\text{--}1.70 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in the protein groups and of $0.91\text{--}0.96 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in the control group with an average spread of 73–87%.

A limitation of our study design is that it excluded the possibility for conducting an ITT analysis because random assignment took place after the inclusion of participants (week 0) and not after the WL period (week 8). This time point for random assignment was chosen to ensure that participants were in energy balance and, therefore, not influenced by their WL in the investigation of the baseline effect of the supplements on appetite and energy metabolism. Potentially, the dropout during WL could have introduced bias in the baseline homogeneity. However, we

did not show differences between groups before the initiation of the WM period (week 8). We cannot exclude that there may have been an imbalance in unmeasured or unknown characteristics between groups at week 8, but we assess that the benefit of random assignment was obtained, and risk of bias from extraneous factors or confounding variables that influenced our results was low. In the study, we aimed for 160 completers. However, only 151 of the 220 randomly assigned participants completed the WM period and were included in the analyses. On the basis of the obtained results, it is unlikely that the addition of 1 to 4 participants/supplement group would have changed the results significantly. In addition, analyses of the large numbers of outcomes and the use of several statistical methods could have led to an inflation of type I error.

Relatively high habitual protein intake in our study was probably due to an increased public awareness of the satiating effects of protein, which has been generally accepted over the past few years. However, not all studies have provided support that protein intake above the recommended intake induced increased satiety (34, 35). Soenen et al. (34) showed that protein intake of $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ was as effective as protein intake of $1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ to reduce and maintain weight. Long et al. (36) showed that the response to a protein-rich test meal (2.2 g/kg) depends on habitual protein intake, and Martens et al. (37) showed that an HP diet, when BW is stable, increases satiety and fullness in the short term but not persistently. All of these studies indicate that there is an adaptation to HP over time and that an HP diet, compared with normal protein intake, would have no effect or could even result in weight gain or an increase in waist circumference as was observed in observational studies (38–40). In our study, subjective VAS scores on appetite, fullness, and prospective consumption differed mainly between the whey and the whey+ groups compared with the control or soy group. The meal tests did not suggest that there was an adaptation to HP intake, because the trend in the subjective appetite sensation was the same before and after 24 wk of HP intake. For DIT, the same numerical pattern (the control group having a lower DIT than that of the protein groups) was observed at week 32, but only the difference between the soy group compared with control group remained significant. This result indicates that DIT was sustained in the long term for the soy group but not for the whey groups. However, this sustained effect did not improve WM in the soy group compared with the control group. After WM, the REE decreased more in the control group than in the protein groups. However, there was no difference between groups in the measured REE minus the predicted REE when REE was investigated as a function of LBM. The results on subjective appetite sensation did not reduce ad libitum energy intake in the protein groups or improved WM. In addition, the increased REE in the protein groups, the increased DIT in the soy group, and the more sustained REE for a given LBM in the whey+ group did not improve WM success in our study. One explanation of the differential effects on appetite could have been due to the immediate effects of each treatment that were reflected in a lower well-being in the whey+ and whey groups right after consumption of the supplements, which carried over to the rest of the test day and resulted in an overall subjective appetite-sensation difference between the groups. The lack of effects of the HP diet on WM success could have been due to the relatively small differences in DIT and REE ($<30 \text{ kJ}/2.5 \text{ h}$ and 243 kJ/d , respectively) between the control and

protein groups, thereby indicating an estimated WL of 10–15 g/d. Another explanation could be a diminished compliance as the duration of a study increases. However, we did not observe a lower compliance in the current study because the HP groups still had significantly higher protein intake than that of the control group at week 32 (confirmed by the excretion of urinary nitrogen). Except for the effect on LBM, the exclusion of less compliant completers in the per-protocol analysis did not change the overall results, thereby suggesting that compliance was not a significant factor for the lack of effect of HP diets in the present study.

In conclusion, individuals who are overweight or obese with a habitual protein intake of $0.8\text{--}1.0 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ may not improve their WM success by supplementing their habitual diet with 45 g whey or soy protein/d compared with maltodextrin.

The authors' responsibilities were as follows—AA, AS, JKL, and LHL: designed the study; LK, JKL, NBS, and CKR: conducted the research; LK, LBS, and LHL: analyzed the data and wrote the manuscript; LK and LHL: had primary responsibility for the final content of the manuscript; and all authors: critically reviewed the manuscript and read and approved the final manuscript. Arla Foods Ingredients Group P/S (represented by AS) contributed to the design of the research and approved the manuscript but had no role in the conduct of the study or in the collection, management, analysis, or interpretation of the data. NUPO A/S had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript. LK, LBS, JKL, AA, and LHL have received funding for research from Arla Foods A/S, Denmark, and the Danish Dairy Research Foundation. AS is employed by Arla Foods a.m.b.a. The remaining authors reported no conflicts of interest related to the study.

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