Normal Protein Intake Is Required for Body Weight Loss and Weight Maintenance, and Elevated Protein Intake for Additional Preservation of Resting Energy Expenditure and Fat Free Mass[1,2]

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Abstract

Energy-restricted high-protein diets (HPDs) have shown favorable results for body weight (BW) management, yet studies differ in their outcomes depending on the dietary protein content. Our objective was to determine the effects of dietary protein content on BW loss-related variables during a 6-mo energy restriction with the use of diets containing protein at the level of requirement [normal-protein diet (NPD), $0.8 \, g \cdot kg \, BW^{-1} \cdot d^{-1}$] and above (HPD, $1.2 \, g \cdot kg \, BW^{-1} \cdot d^{-1}$). In overweight and obese participants (24 men and 48 women), BW, body composition, and metabolic responses were assessed before and after subsequent energy intakes of 100, 33, and 67% of the original individual daily energy requirements. Protein intake was consistent in the NPD ($0.8 \pm 0.3 \, g \cdot kg \, BW^{-1} \cdot d^{-1}$) and HPD ($1.2 \pm 0.3 \, g \cdot kg \, BW^{-1} \cdot d^{-1}$) groups throughout the study ($P < 0.001$). BMI and body fat mass similarly decreased in the NPD and HPD groups ($P < 0.01$). Fat free mass (FFM), resting energy expenditure (REE) compared with predicted REE, and diastolic blood pressure (DBP) changed favorably with the HPD compared with the NPD group after BW loss ($P < 0.05$). A NPD of $0.8 \, g \cdot kg \, BW^{-1} \cdot d^{-1}$ is sufficient for BW management, whereas a HPD of $1.2 \, g \cdot kg \, BW^{-1} \cdot d^{-1}$ is necessary for preservation of REE and a stronger initial sparing effect of FFM and lowering of DBP.   J. Nutr. 143: 591–596, 2013.

Introduction

Overweight and obesity are associated with increased risks for several diseases, such as hypertension, hypercholesterolemia, diabetes, and liver disease (1). Obesity results from a positive energy balance over a long period of time, occurring when energy intake exceeds energy expenditure. Usually, achieving body weight (BW) loss is more effective than BW maintenance thereafter (2–11). The beneficial conditions for BW loss are sustained satiety despite negative energy balance (9,12,13) and sustained basal energy expenditure despite BW loss (4,8–10) due to a sparing of fat free mass (FFM)[5] (9,10,14), which is the main determinant of basal energy expenditure. Energy-restricted diets

with a relatively high protein content act on these metabolic targets (2–12). Food intake is reduced under ad libitum conditions, resulting in immediate BW loss. In the long term, BW reaches a new, significantly lower level (2–12). However, increasing the relative protein content of an energy-balanced diet with a normal level of 10–15 energy percent (En%) to an energy-restricted diet with a relative high protein content of 20–30 En% is erroneously perceived as doubling the total protein intake. Due to energy restriction, such a diet is only relatively high in protein, whereas the total amount of protein consumed often does not differ from the amount consumed in the energy balance diet. Weight loss studies comparing relatively high-protein diets (HPDs) and normal-protein diets (NPDs) (7,9–11) often assess the effects of normal- and low-protein intakes; e.g., 30 and 12 En% protein during weight loss yields with an energy restriction of ~30–35% a total protein intake of ~1.1 vs. ~0.5 $g \cdot kg \, BW^{-1} \cdot d^{-1}$ (7). A similar reasoning holds for studies using diets for BW maintenance after BW loss (4,6,9–11), e.g., 18 En% compared with 15 En% protein during weight maintenance yields a total protein intake of ~1.0 vs. ~0.6 $g \cdot kg \, BW^{-1} \cdot d^{-1}$ (4).

[5] Abbreviations used: BF, body fat; BW, body weight; DBP, diastolic blood pressure; DER, daily energy requirement; En%, energy percent; F, factor; FFM, fat free mass; FM, fat mass; GLP-1, glucagon-like peptide-1; HPD, high-protein diet; NPD, normal-protein diet; PYY, peptide-YY; REE, resting energy expenditure; RQ, respiratory quotient; SBP, systolic blood pressure; TFEQ, Three-Factor Eating Questionnaire; VAS, visual analogue scale; W:H ratio, waist:hip ratio.

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The risk of a prolonged HPD is that it may promote renal damage via excretion of nitrogenous waste products generated from protein metabolism, thereby increasing glomerular pressure and hyperfiltration. In individuals without renal impairment, changes in dietary protein intake caused adaptive alterations in renal size and function without adverse effects, and these changes may be perceived as a normal adaptive mechanism (15,16). Participants with subclinical renal injury are more susceptible to blood pressure-raising effects of acidifying amino acids in a HPD compared with others (15,16).

The aim of the present study was to determine the effects of dietary protein content on BW loss, BW maintenance thereafter, and body composition during a 6-mo energy restriction with the use of diets containing protein at the level of requirement (NPD; $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$) and above (HPD; $1.2 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$), taking metabolic responses and possible adverse effects into account. The decision for a HPD containing $1.2 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ protein was based on studies showing a clear change in body composition in addition to a change in BW (9), and the choice for an NPD containing $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ protein was based on the protein intake preventing a large loss of FFM (9).

Materials and Methods

The study was conducted in a randomized parallel design including 2 energy-restricted diets differing in protein content, i.e., a NPD and a HPD. The Medical Ethics Committee of the Maastricht University Medical Centre approved of the study. All participants gave written informed consent.

Subjects. Eighty participants were recruited via advertisements in local newspapers. They were instructed orally and a paper version of the study protocol was distributed. Eight of the participants who started dropped out during the first 2 wk, leaving 72 participants, i.e., 36 in the NPD and HPD groups. Dropout was due to personal reasons or inability to fulfill the schedule with visits to the clinic. Participants were overweight or obese (BMI >25 kg/m^2) and aged between 18 and 80 y. Exclusion criteria were smoking, the use of medication except contraceptives in women, underlying malignancy, not being weight stable (BW change >10% during the last 6 mo), and for women, being pregnant or breastfeeding.

After dropout, the mean values of the anthropometrics had not changed; therefore, and because the intervention had hardly started, no intention-to-treat analysis was performed. Based on the study by Nickols-Richardson et al. (17), a power analysis showed that with an α of 0.05 and a β of 0.10, 24 participants/group would be sufficient to measure a difference in BW loss respectively BW regain of 2.2 $\pm$ 1.0 kg during a period of 6 wk.

Dietary intervention. The study followed a standard protocol according to our latest dietary intervention study (18). The duration of the study was 6 mo and consisted of 3 phases. First, the participants underwent a run-in phase of 2 wk at a prescribed energy intake of 100% of the participant-specific daily energy requirements (DERs); the protein source came from meal replacements (18). The prescribed protein intake was $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ in the NPD group and $1.2 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ in the HPD group. During the weight-loss phase of 6 wk, the prescribed energy intake was 33% of DER, with the same total protein intake, again obtained from meal replacements. The diet of 33% of DER was applied to induce BW loss. During the weight-maintenance phase of 17 wk, the prescribed energy intake was 67% of the original DER, again with the same total protein intake obtained from meal replacements. The diet of 67% of the original DER was used to maintain the BW lost during the weight-loss phase, because this level of energy intake corresponds to the DER at that moment in time. During the last 2 mo, different protein sources were also allowed to smooth the transition to a diet possible to maintain during habitual daily life.

The prescribed energy intake was calculated using the equation of Harris-Benedict (19) to estimate the basal metabolic rate and multiplied by a physical activity level of 1.5 for total energy expenditure. Individual daily protein intake was the same during all phases of the study and the carbohydrate content of the NPD and HPD was equal to prevent different effects of carbohydrate on protein metabolism. Due to variations in energy intake in the different phases, the macronutrient composition in En% (protein:carbohydrate:fat) changed in the NPD and HPD groups from 10:50:40 and 20:50:30 in the run-in phase to 30:35:35 and 60:35:5 in the weight-loss phase and 15:45:40 and 30:45:25 in the weight-maintenance phase, respectively. Participants were instructed to eat 4 portions of fruit and vegetables each day and drink at least 1.5 L water. The energy density of their total diet was estimated at 4 kJ/g. Moreover, participants were instructed to maintain their habitual level of physical activity during all phases of the study. Urinary nitrogen, anthropometry, body composition, energy expenditure, eating behavior, physical activity, physiological characteristics, and appetite profile were measured at baseline and after the run-in, weight-loss, and weight-maintenance phases.

Biomarker of protein intake. The 24-h urine samples were collected in urine bottles with 10 mL H$_2$SO$_4$ to prevent nitrogen loss through evaporation. The volume and nitrogen concentration were measured, the latter with a nitrogen analyzer (CHN-O-Rapid; Heraeus) to confirm the 24-h protein intake.

Anthropometry and body composition. Height was measured by using a wall-mounted stadiometer (Seca-stadiometer, model 220) and BW was measured with participants wearing underwear after an overnight fast by using a calibrated scale of the Bod Pod (Life Measurement). BMI was calculated as BW divided by height squared (kg/m^2). Waist and hip circumferences were determined in the standing position by using a tape measure. Waist circumference (cm) was measured at the site of the smallest circumference between the ribcage and ileac crest and hip circumference (cm) was measured at the site of the largest circumference between the waist and thighs (18). Accordingly, the waist:hip ratio (W:H ratio) was calculated by dividing waist by hip circumference.

Body composition was calculated from body volume, measured by means of the BodPod (20), and total body water measured by means of the ^{2}H$_2$O dilution technique using Siri's (21–23) 3-compartment model. The dilution of the ^{2}H$_2$O isotope is a measure for total body water. FFM was calculated by dividing total body water by the hydration factor of 0.73 (21). Participants wore tightly fitting bathing suits and a swim cap during of the BodPod measurements and had not engaged in exercise at least 1 h before the measurements.

Energy expenditure. After resting on a bed for 30 min, resting energy expenditure (REE) was measured for 30 min by using an open circuit ventilated hood system (24). Participants were lying in the supine position. Gas analysis was performed by a paramagnetic oxygen analyzer (OmniCal type 1155B, Crowborough) and an infrared carbon dioxide analyzer (OmniCal type 1520/1507). The REE was calculated following Brouwer's formula (25) and the respiratory quotient (RQ) by dividing CO$_2$ production by O$_2$ consumption.

Eating behavior and physical activity. The validated Dutch translation of the Baecke Activity Questionnaire was used to measure habitual physical activity (26). The questionnaire consists of 3 indices of physical activity: work, sport, and leisure time (27). Attitudes toward food intake were assessed by using a validated Dutch translation of the Three Factor Eating Questionnaire (TFEQ), which measures the 3 factors (F) involved in eating behavior: F1, cognitive restraint; F2, disinhibition of eating or emotional eating; and F3, general hunger.

Physiological characteristics. Plasma concentrations of glucose, insulin, FFA, TG, LDL cholesterol, HDL cholesterol, total cholesterol, glucagon-like peptide-1 (GLP-1), peptide-YY (PYY) 3–36, and creatinine were determined following a standard protocol according to our latest dietary intervention study (18).

Systolic and diastolic blood pressures (SBP and DBP) were recorded using an automatic blood pressure monitor with participants in a sitting position (OMRON M6).

Appetite profile. Appetite profile was assessed using visual analogue scales (VASs) consisting of 100-mm lines anchored with "not at all" and "extremely," where the participants had to indicate by a cross-line their perceived amount of hunger, fullness, desire to eat, and amount they estimated they could eat. These VAS ratings were collected in the fasted state at every visit.

Statistics. Data are presented as means ± SDs unless stated otherwise. Differences in baseline variables between the diet groups were evaluated using independent samples *t* tests. Factorial ANOVA with repeated measures, with baseline values as covariate, were used to test changes over time within the diet groups and whether changes differed between the NPD and HPD groups. Bonferroni corrections for multiple comparisons and post-hoc analyses were applied with the ANOVA tests. Simple linear regression analyses were used to determine relations between variables. Differences were regarded as significant if $P < 0.05$. All analyses were performed with SPSS version 16.0.2 for Macintosh OS X.

Results

Biomarker of protein intake. At baseline, nitrogen excretion did not differ between the HPD (11.2 ± 1.1 g/d) and NPD (11.2 ± 1.1 g/d) groups, indicating a comparable protein intake corresponding to 0.8 ± 0.3 g · kg $BW^{-1} · d^{-1}$ in both diet groups at baseline. During the weight-loss and -maintenance phases, nitrogen excretion significantly increased in the HPD group (16.3 ± 1.8 g/d) but remained the same in the NPD group (11.1 ± 1.1 g/d), indicating that the protein intake increased in the HPD group (102 ± 27 g/d or 1.2 ± 0.3 g · kg $BW^{-1} · d^{-1}$) ($P < 0.001$) but did not change in the NPD group during the intervention (70 ± 20 g/d or 0.8 ± 0.3 g · kg $BW^{-1} · d^{-1}$) (treatment × time, $P < 0.001$).

BW and body composition. Baseline values of BW, BMI, % body fat (BF), fat mass (FM), FFM, waist and hip circumference, and W:H ratio did not differ between groups. BW, BMI, FM, % BF, and W:H ratio significantly decreased over time ($P < 0.01$ for W:H ratio) (**Table 1**) in both diet groups without significant differences between the groups. FFM significantly decreased in

the NPD and HPD groups after the weight-loss phase but did not differ from baseline after the weight-maintenance phase. A treatment × time interaction for loss of FFM after the weight-loss phase was observed between the NPD (−1.3 ± 0.2 kg) and HPD groups (−0.6 ± 0.2 kg) ($P < 0.05$).

Energy expenditure. Because in general FFM is the main determinant of REE and because REE expressed as a function of FFM shows an intercept (28), we checked in the whole study population at baseline whether REE was indeed linearly related to FFM. This appeared to be the case; the REE was 0.08 FFM + 2.7 ($R^2 = 0.8$; $P < 0.001$) and the mean REE was 7.02 ± 1.1 MJ/d (**Table 2**).

The REE during the weight-loss and -maintenance phases was measured using indirect calorimetry and was calculated based on this regression equation, with FFM obtained from the body composition measurements during the weight-loss and -maintenance phases. By filling in the FFM values from the respective phases, this resulted in a calculated mean REE of 6.86 MJ/d for the NPD group after the weight-loss phase and 7.02 MJ/d after the weight-maintenance phase; for the HPD group, the calculated mean REE was 6.94 MJ/d after the weight-loss phase and 7.02 MJ/d after the weight-maintenance phase.

Measuring REE during the weight-loss and -maintenance phases yielded the following equations of the regression lines. For the NPD group, the REE was 0.08 FFM + 2.6 ($R^2 = 0.7$; $P < 0.001$) after weight loss and 0.07 FFM + 2.7 ($R^2 = 0.5$; $P < 0.001$) after weight maintenance. The mean REE was 6.76 ± 0.9 MJ/d after weight loss and 6.48 ± 0.9 MJ/d after weight maintenance. For the HPD group, the REE was 0.08 FFM + 2.5 ($R^2 = 0.7$; $P < 0.001$) and 0.08 FFM + 2.5 ($R^2 = 0.7$; $P < 0.001$), respectively. The mean REE values were 6.74 ± 0.9 and 6.82 ± 0.9 MJ/d.

The slope of the regression line based on the measured REE of the NPD group decreased during the weight-maintenance phase compared with baseline, indicating that the REE slightly decreased for a given FFM. The slope of the regression line for

TABLE 1 Anthropometry and behavioral scores in the NPD and HPD groups during a 6-mo intervention[1]

Phase	NPD			HPD		
	Run-in	WL	WM	Run-in	WL	WM
Participants, M/F (*n*)	12/24			12/24		
Age, *y*	44 ± 4			44 ± 4		
BW, *kg*	90.0 ± 14	84.1 ± 12[a]	82.8 ± 11[a]	90.0 ± 14	85.0 ± 13[a]	83.0 ± 12[a]
BMI, *kg/m²*	32.0 ± 0.5	29.9 ± 0.4[a]	29.5 ± 0.4[a]	32.0 ± 0.5	30.2 ± 0.5[a]	29.8 ± 0.4[a]
FFM, *kg*	54.0 ± 9.8	52.7 ± 8.5[b]	53.2 ± 8.3[cd]	54.0 ± 9.6	53.4 ± 8.1[b]	53.9 ± 8.0[cd]
FM, *kg*	36.0 ± 9.2	31.4 ± 8.2[a]	29.6 ± 7[ac]	36.0 ± 9.3	31.2 ± 8.1[a]	29.1 ± 8.4[ac]
BF, *%*	40 ± 7	37.3 ± 6[a]	35.7 ± 6[ac]	40 ± 7	37.2 ± 6[a]	35.0 ± 6[ac]
W:H ratio	0.92 ± 0.03	0.91 ± 0.03[a]	0.90 ± 0.03[a]	0.92 ± 0.03	0.90 ± 0.03[a]	0.89 ± 0.03[a]
TFEQ F1	8.2 ± 4.3	10.3 ± 4.1[a]	10.4 ± 4.2[a]	8.2 ± 4.2	10.2 ± 4.0[a]	10.5 ± 4.1[a]
TFEQ F2	5.9 ± 2.5	4.8 ± 2.2[a]	4.8 ± 2.3[a]	5.8 ± 2.3	4.8 ± 2.1[a]	4.8 ± 2.1[a]
TFEQ F3	4.5 ± 2.8	3.2 ± 2.4[a]	2.6 ± 2.1[a]	4.5 ± 2.6	3.0 ± 2.0[a]	2.5 ± 1.9[a]
BAQ: total	8.3 ± 1.4	8.5 ± 1.6	8.4 ± 1.5	8.3 ± 1.3	8.4 ± 1.4	8.5 ± 1.5
BAQ: work	2.7 ± 0.6	2.7 ± 0.7	2.7 ± 0.7	2.7 ± 0.5	2.7 ± 0.5	2.7 ± 0.5
BAQ: sport	2.6 ± 0.8	2.7 ± 0.8	2.6 ± 0.8	2.6 ± 0.7	2.7 ± 0.8	2.6 ± 0.9
BAQ: leisure	3.0 ± 0.6	3.1 ± 0.7	3.1 ± 0.8	3.0 ± 0.9	3.0 ± 0.8	3.2 ± 0.8

[1] Values are means ± SDs. [a]Different from baseline or run-in phase, $P < 0.01$. [b]Treatment × time, weight-loss phase, $P < 0.05$. [c]Different from weight-loss phase, $P < 0.01$. [d]Treatment × time, weight-maintenance phase, $P < 0.05$. BAQ, Baecke Activity Questionnaire; BF, body fat; BW, body weight; F, factor; FFM, fat free mass; FM, fat mass; HPD, high-protein diet; NPD, normal-protein diet; TFEQ, Three Factor Eating Questionnaire; W:H ratio, waist:hip ratio; WL, weight loss; WM, weight maintenance.

TABLE 2 Metabolic responses in the NPD and HPD groups during a 6-mo intervention[1]

Phase	NPD			HPD		
	Run-in	WL	WM	Run-in	WL	WM
REE measured, *MJ/d*	7.02 ± 1.1	6.76 ± 0.9	6.48 ± 0.9[b]	7.02 ± 1.0	6.74 ± 0.9	6.82 ± 0.09[b]
REE calculated, *MJ/d*		6.86[2]	7.02		6.94	7.02
RQ	0.84 ± 0.04	0.81 ± 0.04[a]	0.84 ± 0.04	0.84 ± 0.03	0.81 ± 0.03[a]	0.84 ± 0.04
SBP, *mm Hg*	129 ± 14	117 ± 12[a]	117 ± 13[a]	128 ± 15	116 ± 13[a]	116 ± 14[a]
DBP, *mm Hg*	81 ± 9	76 ± 12[ab]	74 ± 9[a]	81 ± 9	72 ± 9[ab]	72 ± 9[a]
Glucose, *mmol/L*	5.1 ± 0.4	5.1 ± 0.4	5.0 ± 0.3	5.1 ± 0.3	5.1 ± 0.4	4.9 ± 0.4
Insulin,[3] *mIU/mL*	15.1 ± 5.9	14.2 ± 5.8[a]	4.1 ± 5.7[a]	15.0 ± 5.8	14.1 ± 5.8[a]	14.0 ± 5.7[a]
HOMA-IR index	3.5 ± 1.5	2.6 ± 1.2[a]	2.5 ± 1.2[a]	3.4 ± 1.6	2.5 ± 1.2[a]	2.4 ± 1.2[a]
FFA, *mmol/L*	470 ± 157	433 ± 126[a]	437 ± 132[a]	472 ± 159	428 ± 134[a]	431 ± 129[a]
TG, *mmol/L*	1.3 ± 0.6	1.2 ± 0.5[a]	1.2 ± 0.6[a]	1.2 ± 0.7	1.0 ± 0.6[a]	1.0 ± 0.6[a]
LDL cholesterol, *mmol/L*	3.7 ± 0.8	3.1 ± 0.6[a]	3.1 ± 0.7[a]	3.7 ± 0.8	3.1 ± 0.6[a]	3.1 ± 0.6[a]
HDL cholesterol, *mmol/L*	1.4 ± 0.4	1.3 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	1.3 ± 0.3	1.4 ± 0.4
Total cholesterol, *mmol/L*	5.4 ± 0.8	4.6 ± 0.7[a]	4.7 ± 0.7[a]	5.4 ± 0.8	4.6 ± 0.7[a]	4.7 ± 0.7[a]
GLP-1, *pmol/L*	2.0 ± 2.0	1.6 ± 1.5[a]	1.5 ± 1.5[a]	2.0 ± 1.9	1.6 ± 1.4[a]	1.5 ± 1.5[a]
PYY, *pmol/L*	72 ± 34	38 ± 27[a]	40 ± 32[a]	74 ± 38	39 ± 23[a]	46 ± 29[a]
Creatinine, *μmol/L*	76 ± 13	80 ± 15	82 ± 15	77 ± 14	76 ± 12	75 ± 12

[1] Values are means ± SDs, n = 36/group. [a]Different from baseline or run-in phase, $P < 0.01$. [b]Treatment × time, compared with baseline or run-in phase, $P < 0.01$. No significant differences were observed from the weight-loss phase to the weight-maintenance phase. DBP, diastolic blood pressure; GLP-1, glucagon-like peptide-1; HPD, high-protein diet; NPD, normal-protein diet; PYY, peptide-YY; REE, resting energy expenditure; RQ, respiratory quotient; SBP, systolic blood pressure, WL; weight loss, WM; weight maintenance.
[2] Values are means, n = 36/group.
[3] Conversion to SI units:1 mIU/mL = 6.945 nmol/L.

the HPD group did not differ from the slope at baseline. The measured REE decreased more in the NPD group (-0.5 ± 0.02 MJ/d) than in the HPD group (-0.2 ± 0.01 MJ/d) compared with the predicted REE values after the weight-maintenance phase (treatment × time, $P < 0.01$).

The baseline RQ did not differ between groups. In both groups, the RQ significantly decreased after 2 mo compared with baseline ($P < 0.01$) (Table 2). Changes in RQ over time did not significantly differ between groups.

Eating behavior and physical activity. Baseline values and significant changes over time in all TFEQ scores did not differ between the groups. Both groups increased in F1 and decreased in F2 and F3 after 6 mo compared with baseline ($P < 0.01$) (Table 2). Decreases in F3 were related to decreases in BW ($r = 0.34$; $P < 0.05$), FM ($r = 0.4$; $P < 0.05$), and % BF ($r = 0.4$; $P < 0.05$) and decreases in F2 were related to decreases in BW ($r = 0.3$; $P < 0.05$).

Baseline Baecke Activity Questionnaire scores were similar and changes in total physical activities over time during the 6 mo of the interventions were absent in both diet groups (Table 1).

Physiological characteristics. Baseline values of variables related to blood pressure, insulin sensitivity, and lipid did not differ between groups. Both groups similarly decreased in SBP and DBP after 6 mo ($P < 0.05$). After 2 mo, DBP decreased more in the HPD group compared with the NPD group ($P < 0.05$, treatment × time) (Table 2); the decreases in SBP and DBP were related to the decreases in FM ($r = 0.32$; $P < 0.05$, and $r = 0.38$; $P < 0.05$, respectively).

Similarly, plasma concentrations of insulin and HOMA-IR index significantly decreased over time, but these changes did not differ between the groups ($P < 0.01$) (Table 2). Decreases in plasma concentrations of insulin and HOMA-IR index ($r = 0.35$; $P < 0.05$) were related to decreases in BW. Decreases in plasma concentrations of LDL, total cholesterol, TG, and FFA did not

differ between groups ($P < 0.01$) (Table 2); the decrease in TG was related to the decrease in BW ($r = 0.33$; $P < 0.05$).

Appetite profile and related blood hormones. Baseline values of VAS of hunger and satiety ratings and plasma concentrations of GLP-1 and PYY did not differ between groups. Similarly, in both groups, no changes over time in VAS ratings of the appetite profile were observed. Decreases in plasma GLP-1 and PYY concentrations did not differ between groups ($P < 0.01$) (Table 2).

Adverse events and kidney function. The baseline creatinine concentrations did not differ between groups and creatinine concentrations did not significantly change over time in either group (Table 2). No adverse events occurred.

Discussion

The NPD and HPD groups, consisting of 33% energy intake relative to baseline DER during BW loss and 67% energy intake relative to baseline DER during BW maintenance thereafter, respectively, were equally effective for BW loss, loss of BF, and BW maintenance. Using 24-h urine nitrogen measurements repeatedly, the contrast in protein intake between the NPD group and the HPD group of 0.8 vs. 1.2 g · kg BW^{-1} · d^{-1} was confirmed. Both diets showed a FFM-sparing effect, which was initially stronger in the HPD group. The REE as a function of FFM did not decrease in the HPD group, thereby facilitating maintenance of reduced BW, as was previously shown (2–5,8–11,14).

Reduction of BW was equally facilitated by the satiety capacity of protein (2,4,7–10). Scores on the TFEQ indicate that adherence to both diets was very good, i.e., overall hunger (TFEQ F3) decreased and related to decreases in BW and in % BF. Also, the increase in control, indicated by increases in cognitively dietary restraint (TFEQ F1), and decreases in the

disinhibition of control (TFEQ F2) were similarly underscored by BW loss and maintenance thereafter in both groups.

The concentrations of the peptides GLP-1 and PYY similarly decreased over time, probably due to fewer nutrients being present in the gut. The metabolic profile including insulin sensitivity and lipid metabolism was remarkably improved in both groups, mainly as an effect of BW loss, indicating that the originally worse metabolic profile probably was the effect and not the cause of overweight (29–32). Blood pressure similarly decreased after 6 mo, although initially DBP decreased more in the HPD group compared with the NPD group. Decreases in SBP and DBP were related to decreases in FM. This also indicates that kidney function related to blood pressure was not affected by these diets. Creatinine clearance of the kidney remained between normal values of 60 and 120 μmol/L urinary creatinine for 24 h.

The observation in the present study that both diets were similarly effective for BW loss, FM loss, and BW maintenance thereafter confirms earlier observations (30,31,33–35), which reported no difference between 2 levels of protein in the diet. In those studies, NPDs containing 15–16 En% of protein still provided sufficient protein (~60 g) to sustain satiety, FFM, and energy expenditure and the diet thus shows a similar effect on BW loss compared with a diet higher in protein content. The availability of a sufficient amount of protein in the control diet obviously caused this result (30,31,33–35). However, when the control diet provided an insufficient amount of protein, a rapid BW regain was shown, similar to the effect of most diets that due to the limited energy content do not provide a sufficient amount of protein (2,4,7–12). The FFM-sparing effect of HPDs has not only been shown during BW loss and maintenance thereafter (2,4,7–12,30,31,33–35) but also in longer term studies in energy balance (36,37).

The following studies on protein diet-induced BW loss qualify as good examples to compare the present results with, because in those studies, the amount of protein intake was confirmed by the biomarker urinary nitrogen, a sufficient number of participants was included, and a sufficiently long duration was applied. The study by Skov et al. (7) showed that an elevated compared with a lowered protein energy restriction diet for 26 wk promotes BW loss and loss of FM, while sparing FFM without increasing appetite. A difference in BW loss of 8.9 compared with 5.1 kg between 2 diets containing 30 En% and 12 En% from protein was observed in negative energy balance. However, due to the 35% energy restriction, the given dosages of protein would have been 20 En% compared with 8 En% in an energy balance situation. In a study by Leidy et al. (38), the effect of protein diets on body composition was assessed. Participants followed an energy-deficit diet for 12 wk containing elevated protein or normal protein (30 vs. 18 En% from protein). With an energy restriction of ~35%, protein intake was comparable to 20 vs. 12 En% in energy balance. With comparable BW and FM loss, the loss of FFM was less in the elevated-protein diet. The difference between both studies is that Leidy et al. (38) found no differences in BW loss, whereas Skov et al. (7) did. This was obviously due to the differences in the protein contents of the diets. Skov et al. (7) compared a HPD with a diet containing a low-protein content, whereas Leidy et al. (38) compared it with a diet containing normal, sufficiently high protein. Nevertheless, both elevated-protein diets observed a larger FFM-sparing effect and no increases in hunger or appetite. The present study assessed the effects of a high- compared with a NPD with a stronger energy restriction (67%). The comparison of these 3 studies shows that with a modest to strong energy restriction, sustaining original protein intake at a sufficiently high level of 0.8 g · kg BW^{-1} · d^{-1} promotes BW loss. Elevating the protein content more does not show a greater BW loss yet may show a stronger initial FFM-sparing effect. Comparing a HPD or NPD with a lower protein diet shows a difference in BW loss and body composition due to the decreased protein intake in the control diet below the level that FFM is preserved. Therefore, in a situation of energy restriction, it is important to provide protein at the level of the requirement. Providing additional protein does not have a main effect.

Similarly, in studies assessing the effects of relatively HPDs compared with NPDs on BW maintenance after BW loss, often diets with a normal to slightly elevated total protein content were compared with diets containing a low to normal total protein content due to the lower energy intake compared with the initial energy balance situation (4,8). A protein intake of 18 En% compared with 15 En% resulted in improved BW maintenance in overweight individuals after BW loss and was related to beneficial changes in body composition, substrate oxidation, and satiety (4,8). Furthermore, Larsen et al. (11) observed in a multi-center study that a modest increase in protein content led to an improvement in maintenance of BW loss over a period of 6 mo. In fact, diets differing in protein content (25 vs. 13 En% from protein) were compared. The protein content in this BW maintenance situation is comparable to 18 vs. 9 En% from protein in an energy balance situation. The difference in BW maintenance may again have been due to the comparison with a low-protein diet as control. Different from these results are those reported by Keogh et al. (39) comparing BW maintenance following BW loss between a diet containing 40 En% protein and a diet with a protein content of 20 En%. No differences were observed between the diets regarding BW loss. Similar to the results of weight loss studies when the control group ingests a sufficient amount of protein, which in this case was 67 g/d, differences in BW maintenance are unlikely to occur.

Taken together, the results of this study may contribute to the understanding of whether the until-now supposed differences in outcomes of protein diet studies can be attributed to the phenomenon that the control diets contained protein at least at the level of requirement or just insufficient amounts, the latter causing the regain effect. Therefore, we suggest that an inadequate protein content in the diet greatly contributes to the risk of BW regain. Based on the comparison of a number of reliable studies, we conclude that a NPD of 0.8 g · kg BW^{-1} · d^{-1} is sufficient for BW management, whereas a HPD of 1.2 g · kg BW^{-1} · d^{-1} is necessary for preservation of REE and a stronger initial sparing effect of FFM and lowering of DBP.

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