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Relatively high-protein or 'low-carb' energy-restricted diets for body weight loss and body weight maintenance?☆,☆☆

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HIGHLIGHTS

- ► The research unmasks the success of 'low-carb' diets for body weight management.
- ► Similar protein contents, similar body-weight management irrespective of carbohydrate content.
- ► High- vs. normal-protein diets show the favorable effects on body-weight management.
- ► A high-protein normal-carbohydrate diet reduces diastolic blood pressure more.

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ABSTRACT

Background: 'Low-carb' diets have been suggested to be effective in body weight (BW) management. However, these diets are relatively high in protein as well.

Objective: To unravel whether body-weight loss and weight-maintenance depends on the high-protein or the 'low-carb' component of the diet.

Design: Body-weight (BW), fat mass (FM), blood- and urine-parameters of 132 participants (age = 50 ± 12 yr; BW = 107 ± 20 kg; BMI = 37 ± 6 kg/m^2; FM = 47.5 ± 11.9 kg) were compared after 3 and 12 months between four energy-restricted diets with 33% of energy requirement for the first 3 months, and 67% for the last 9 months: normal-protein normal-carbohydrate (NPNC), normal-protein low-carbohydrate (NPLC); high-protein normal-carbohydrate (HPNC), high-protein low-carbohydrate (HPLC); 24 h N-analyses confirmed daily protein intakes for the normal-protein diets of 0.7 ± 0.1 and for the high-protein diets of 1.1 ± 0.2 g/kg BW ($p<0.01$).

Results: BW and FM decreased over 3 months ($p<0.001$): HP (−14.1 ± 4 kg; −11.9 ± 1.7 kg) vs. NP (−11.5 ± 4 kg; −9.3 ± 0.7 kg) ($p<0.001$); LC (−13.5 ± 4 kg; −11.0 ± 1.2 kg) vs. NC (−12.3 ± 3 kg; −10.3 ± 1.1 kg) (ns). Diet × time interaction showed HPLC (−14.7 ± 5 kg; −11.9 ± 1.6 kg) vs. HPNC (−13.8 ± 3 kg; −11.9 ± 1.8 kg) (ns); NPLC (−12.2 ± 4 kg; −10.0 ± 0.8 kg) vs. NPNC (−10.7 ± 4 kg; −8.6 ± 0.7 kg) (ns); HPLC vs. NPLC ($p<0.001$); HPNC vs. NPNC ($p<0.001$). Decreases over 12 months ($p<0.001$) showed HP (−12.8 ± 4 kg; −9.1 ± 0.8 kg) vs. NP (−8.9 ± 3 kg; −7.7 ± 0.6 kg) ($p<0.001$); LC (−10.6 ± 4 kg; −8.3 ± 0.7 kg) vs. NC (11.1 ± 3 kg; 9.3 ± 0.7 kg) (ns). Diet × time interaction showed HPLC (−11.6 ± 5 kg ; −8.2 ± 0.7 kg) vs. HPNC (−14.1 ± 4 kg; −10.0 ± 0.9 kg) (ns); NPNC (−8.2 ± 3 kg; −6.7 ± 0.6 kg) vs. NPLC (−9.7 ± 3 kg; −8.5 ± 0.7 kg) (ns); HPLC vs. NPLC ($p<0.01$); HPNC vs. NPNC ($p<0.01$). HPNC vs. all other diets reduced diastolic blood pressure more. Relationships between changes in BW, FM, FFM or metabolic parameters and energy percentage of fat in the diet were not statistically significant. Metabolic profile and fat-free-mass were improved following weight-loss.

Conclusion: Body-weight loss and weight-maintenance depends on the high-protein, but not on the 'low-carb' component of the diet, while it is unrelated to the concomitant fat-content of the diet.

Abbreviations: HP, high-protein; HPLC, high-protein low-carbohydrate; HPNC, high-protein normal-carbohydrate; LC, low-carbohydrate; NC, normal-carbohydrate; NP, normal protein; NPLC, normal-protein low-carbohydrate; NPNC, normal-protein normal-carbohydrate.

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1. Introduction

Obesity, one of the major health problems of today, develops due to an imbalance between energy intake and energy expenditure. Both energy intake and energy expenditure have been targeted to combat the development of increasing body weight (BW) and fat mass (FM). Reducing this excess of BW and FM can successfully be reached by restricting energy intake, supported by a balanced macronutrient composition of the diet. In this aspect, 'low' carbohydrate energy-restricted diets have suggested to be successful compared to normal or higher carbohydrate intakes [1–5], because they seem to work indirectly by dramatic food restriction [1–5]. However, these energy-restricted diets did not only decrease the energy percentage (En%) from carbohydrate but also increased the En% from protein and from fat of the diet compared to the control diet [1–5]. Diets with 20 to 30 En% of protein during energy restrictions of about 50%, named 'relatively high' protein energy-restricted diets, actually sustain the daily absolute amount of required protein of at least 0.8 g/kg BW per day. These 'relatively high' protein diets resulted in relatively larger reduction of BW and FM, and supported BW maintenance thereafter, since they promote a sustained level of satiety, sustained energy expenditure, increased fat oxidation and sparing of fat-free mass (FFM) [6–8]. Regarding the macronutrient composition of these energy-restricted diets, the question remains whether it is the decrease in carbohydrate or an increase in daily absolute-protein intake, which has a stimulating effect on reducing BW and FM. Therefore, the aim of this study was to compare possible differences in reduction of BW and FM, and of BW maintenance thereafter, as a result of energy intake restriction of four diets offered parallel in a 2×2 factorial design, contrasting low vs. normal carbohydrate intake (LC vs. NC) and high vs. normal protein intake (HP vs. NP) at the same time. We analyzed which combination of the carbohydrate and protein proportions in the diet would show the greatest efficacy for BW loss and for BW maintenance thereafter, and whether it is the HP or LC factor that is crucial for the effect.

2. Material and methods

The Medical Ethics committee of the University Medical Centre Groningen approved the study. The study is registered in www.clinicaltrials.gov, number NCT00862953. All participants gave their written informed consent.

2.1. Procedure

The study was conducted following a randomized 2×2 factorial design, including four parallel energy-restricted diets differing in protein and/or carbohydrate content. The dietary intervention included a run-in period of two weeks with energy intakes of 100%, followed by an energy intake restriction to 33% of the subjects' original energy requirements for three months to achieve significant reduction in BW, and a BW maintenance period of nine months thereafter with an energy intake restriction to 67% of the subjects' original energy requirements. Energy intake was calculated for each participant individually based upon the equation of Harris-Benedict [9] for estimation of basal metabolic rate (BMR), and multiplied by a Physical Activity Index of 1.5 for total energy expenditure (BMR×1.5 = energy intake at 100%). Thus, the four dietary interventions consisted of iso-energetic diets at the level of the individual. The four diets had the following macronutrient compositions: protein/carbohydrate/fat of 20/25/55 En% for the high-protein low-carbohydrate (HPLC) diet, of 20/50/30 En% for the high-protein normal-carbohydrate (HPNC) diet, of 10/25/65 En% for the normal-protein low-carbohydrate (NPLC) diet, and of 10/50/40 En% for the normal-protein normal-carbohydrate (NPNC) diet during the run-in period of energy intakes of 100%. Daily absolute protein intakes of 0.8 g/kg BW (NP) and 1.2 g/kg BW (HP) were intended to keep constant during the run-in period and during the total energy restriction period of one year to ensure sufficient protein availability for the preservation of FFM during weight loss. This resulted in the following macronutrient compositions during the weight loss period of time when 33% of the original energy requirement was provided: protein/carbohydrate/fat of 60/5/35 En% for HPLC, of 60/35/5 En% for HPNC, of 30/5/65 En% for NPLC, and of 30/35/35 En% for NPNC. During the weight maintenance period thereafter energy intakes were 67% of the individuals' energy requirement, resulting in macronutrient compositions of 30/25/45 En% for HPLC; of 30/45/25 En% for HPNC; of 15/25/60 En% for NPLC and of 15/45/40 En% for NPNC. The implications of the macronutrient compositions for the actual diets were thoroughly explained to the subjects individually by dieticians by means of daily menus, including food items that fitted the daily macronutrient intake.

Since usual Dutch breakfasts and lunches are bread-based, breakfasts and lunches consisted of whole-meal and multi-grain bread (low glycemic index-CHO) and butter (fat) cheese, cold sliced meat, cold fish (P and fat), marmalade, and honey (CHO), and a dairy-based drink (P and fat). Dinners consisted of boiled potatoes (CHO), vegetables and meat or fish (P and fat), with a sauce (fat), and a dairy-based dessert (P and fat). Water and a limited amount of coffee and tea (in total of 3 cups a day without sugar) were allowed to be drunk. By adapting the relative amounts of the food-items to the necessary macronutrient compositions, the necessary percentages of energy from the different macronutrients, as well as the different absolute amounts were obtained. Each individual received their unique menu to achieve weight loss over three months, and weight maintenance over nine months thereafter, based upon 33% respectively 67% of their subject-specific energy need for energy balance and specific macronutrient composition of one out of the four interventions. If necessary, if the subjects could not achieve the required protein contents using the recipes, the diets were supplemented with whey-protein milkshakes (Ollyxa, Alwit B.V., Hengelo, The Netherlands) to reach the prescribed protein content of the diet. Twenty-four-hour urine nitrogen was used to quantify the protein content, as a compliance marker for the level of daily absolute protein intake. In addition, all subjects followed a standardized group-organized program, guided by the dietitians and each group consisted of subjects from all four diet groups. This group-organized program focused on eating behavior and healthy diet in general, and not with respect to the individual diets; questions on how to cope with the personal diet were answered individually. Subjects visited the clinic every week in the first month, every two weeks during the following three months and once a month during the last eight months, resulting in 19 sessions over the 12 months.

2.2. Subjects

The subjects were recruited from potential clients of a weight management program of an outpatient-clinic in the city of Hengelo, The Netherlands. These subjects were referred by their general practitioner or specialist. A dietitian explained the study protocol orally and handed out a paper version. Overweight and obese subjects were included. The subjects ranged in BMI from 27 to 60 kg/m^2, in BW from 77 to 183 kg and in age from 23 to 71 yrs. Exclusion criteria were cancer, HIV infection, psychiatric disease, more than 10% reduction of BW during the last six months; moreover, women who were pregnant or breastfeeding were excluded. The subjects were stratified into four groups following age, BMI, body-weight, Baecke's score of physical activity, and Three Factor Eating Questionnaire (TFEQ) score for attitude toward eating. Although, smoking and alcohol intake did not belong to in- or exclusion criteria, there were no heavy smokers or alcohol-consumers participating in the study. Of the 139 subjects who started, seven dropped out; two in the HPLC group, two in the HPNC group, two in the NPLC group, and one in the NPNC group, all during the first weeks. The subjects stopped due to several reasons, such as personal reasons and inability to fulfill the schedule with visits to the clinic.

2.3. Measurements

At the clinic, BW was measured at all visits. The subjects were weighed in their underwear after an overnight fast, using a calibrated hospital scale to the nearest 0.1 kg (Tanita TBF-310). Height was measured at screening to the nearest 0.1 cm (Seca-stadiometer). Waist circumference was measured each month at the site of the smallest circumference between rib cage and iliac crest and hip circumference was measured at the site of the largest circumference between waist and thighs. Systolic and diastolic blood pressures were recorded each month using an automatic blood pressure monitor with subjects in sitting position (Microlife BP 3AC1-2).

At baseline, and after three and 12 months of intervention, the following measurements were performed. Body composition was assessed with the deuterium (^2H_2O) dilution technique [10]. The dilution of the deuterium isotope is a measure for total body water. FFM was calculated by dividing total body water by the hydrating factor 0.73.

Physical activity was assessed using the validated Baecke questionnaire [11]. This validated questionnaire explains 48% of the variation in Physical Activity Level (PAL) as measured with doubly labeled water [12]. The Baecke questionnaire consists of three components: work activity, sports activity, and leisure activity. Attitude toward food intake was determined using a validated Dutch translation of the Three Factor Eating Questionnaire (TFEQ) [13].

Fasting venous blood samples were taken to analyze concentrations of plasma insulin, glucose, creatinine, triacylglycerol (TAG), and high-density lipoprotein (HDL), low-density lipoprotein (LDL) and total cholesterol, and concentrations of albumin and creatinine in urine samples. Twenty-four-hour urine nitrogen content was determined in order to calculate real protein intake. The subjects were thoroughly instructed to collect all urine excreted after the first volume voided in the morning until and including the first morning urine voided the following day. Urine samples were collected in containers with 10 mL H_2SO_4 to prevent nitrogen loss through evaporation. Volume and nitrogen concentration were measured, the latter with a nitrogen analyzer (CHN-O-Rapid; Heraeus, Hanau, Germany). Plasma was obtained by centrifugation (1500⁎g for 10 min at 4 °C), frozen in liquid nitrogen and stored at −80 °C until analysis.

Metabolic parameters were assessed. Insulin concentrations were measured using an immuno-metrical chemo-luminescention assay on an Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics); the coefficient of variation (cv) was 2.1±1.9%. Albumin-, glucose-, TAG-, and HDL- and total cholesterol concentrations were measured using a standardized enzymatic colorimetric method on the Cobas 6000 analyzer (Roche Diagnostics), with coefficients of variation (cv) of respectively 3.2±2.9%, 2.3±1.8%, 1.6±1.1%, 0.8±0.4%, and 2.6±2.0%. LDL cholesterol was calculated according to the Friedewald formula. The homeostatic model assessment (HOMA)-index was calculated by glucose (mmol/L)⁎insulin (mUnits/L)/22.5.

2.4. Statistics

Data are presented as means with standard deviations unless stated otherwise. Differences between groups were tested by ANCOVA with the baseline value, i.e. the value of phase 1, of the tested parameter of interest as covariate. Changes over time, i.e. 3 months and 12 months between and within groups were tested by factorial repeated measures ANOVA with Bonferroni's post hoc corrections. Repeated measures were performed with a 4-factor ANOVA repeated measures, treating the four levels of a single grouping factor. The interaction of protein×carbohydrate on the changes in the variables over time was tested. In order to test for the different fat-contents of the diets, regression analyses were performed to test the possible relationship between changes in body-weight, body-fat, and metabolic parameters and energy percentage from fat in the diet. Differences and results from the regression analyses were regarded as statistically significant if $p<0.05$. Analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 16.0.2 for Macintosh OS X.

3. Results

3.1. Protein intake

Daily absolute protein intake was significantly higher in the relatively HP diet groups compared with the NP diet groups during the three months of weight loss as well as the nine months of weight maintenance thereafter (mean HP vs. NP; 1.1±0.2 vs. 0.7±0.2 g/kg BW, $p<0.01$). For 9 subjects, 4 in the HPNC group and 5 in the HPLC group it was necessary to provide the subjects with the protein supplement, the whey-protein milkshakes, to reach the prescribed protein content of the diet. According to the N-content of the urine samples, there was no statistically significant difference in protein intake between the supplemented subjects and the remaining subjects.

3.2. Body weight and body composition

BW and FM decreased over 3 months ($p<0.001$): HP (−14.1±4 kg; −11.9±1.7 kg) vs. NP (−11.5±4 kg; −9.3±0.7 kg) ($p<0.001$); LC (−13.5±4 kg; −11.0±1.2 kg) vs. NC (−12.3±3 kg; −10.3±1.1 kg) (ns). Diet×time interaction showed HPLC (−14.7±5 kg; −11.9±1.6 kg) vs. HPNC (−13.8±3 kg; −11.9±1.8 kg) (ns); NPLC (−12.2±4 kg; −10.0±0.8 kg) vs. NPNC (−10.7±4 kg; −8.6±0.7 kg) (ns); HPLC vs. NPLC ($p<0.001$); HPNC vs. NPNC ($p<0.001$). Decreases over 12 months ($p<0.001$) showed HP (−12.8±4 kg; −9.1±0.8 kg) vs. NP (−8.9±3 kg; −7.7±0.6 kg) ($p<0.001$); LC (−10.6±4 kg; −8.3±0.7 kg) vs. NC (11.1±3 kg; 9.3±0.7 kg) (ns). Diet×time interaction showed HPLC (−11.6±5 kg; −8.2±0.7 kg) vs. HPNC (−14.1±4 kg; −10.0±0.9 kg) (ns); NPNC (−8.2±3 kg; −6.7±0.6 kg) vs. NPLC (−9.7±3 kg; −8.5±0.7 kg) (ns); HPLC vs. NPLC ($p<0.01$), and ns for FM; HPNC vs. NPNC ($p<0.01$).

FFM decreased slightly in all diet groups, without differences between diets (Table 1).

Relationships between body-weight changes, or changes in FM, or in FFM and energy percentage of fat in the diet were not statistically significant.

3.3. Metabolic profile

The metabolic profile parameters were decreased similarly in all groups after three months of energy restriction ($p<0.01$, Table 2). Diastolic blood pressure was reduced significantly more with the HPNC diet, compared with all other diets ($p<0.01$, Table 2). No significant interactions were observed. Relationships between changes in any of the metabolic parameters and energy percentage of fat in the diet were not statistically significant.

Total and LDL cholesterol, and TAG reversed in all diet groups during the nine months of weight maintenance after an initial decrease during three months of energy restriction ($p<0.05$). Decreases in systolic ($r=0.3$, $p<0.01$) and diastolic blood pressures ($r=0.2$, $p<0.05$), and heart rate ($r=0.3$, $p<0.005$) were related to the decrease in BMI. Decreases in insulin concentrations ($r=0.3$, $p<0.01$), HOMA-index ($r=0.3$, $p<0.01$) and TAG ($r=0.2$, $p<0.05$) were related to the decrease in FM and BMI.

Table 1
Anthropometry (mean ± SD) of the subjects (n = 132), 33 in each group: HPNC, HPLC, NPNC, NPLC; age 50 ± 12 yrs. Measurements after phase 1: two weeks run-in; phase 2: three months weight loss; phase 3: nine months weight maintenance.

	Phase	HPNC	HPLC	NPNC	NPLC
BMI (kg/m^2)	1	37.5 ± 5.6	36.6 ± 4.6	36.2 ± 4.7	37.0 ± 5.4
	2	32.0 ± 3.6^{*,a,c}	31.6 ± 3.3^{*,b,d}	32.5 ± 3.4^{*,a,b}	32.8 ± 4.0^{*,c,d}
	3	33.5 ± 3.8^{*,a,c}	32.7 ± 3.9^{*,b,d}	33.3 ± 3.7^{*,a,b}	33.7 ± 4.2^{*,c,d}
BW (kg)	1	106.1 ± 18.5	108.1 ± 21.7	105.3 ± 18.6	107.2 ± 17.7
	2	92.3 ± 17.4^{*,a,c}	93.4 ± 17.2^{*,b,d}	94.6 ± 17.9^{*,a,b}	95.0 ± 17.2^{*,c,d}
	3	94.0 ± 16.2^{*,a,c}	96.5 ± 17.1^{*,b,d}	97.1 ± 16.6^{*,a,c}	97.5 ± 17.1^{*,c,d}
FFM (kg)	1	57.4 ± 10.3	63.2 ± 14.3	57.7 ± 11.1	58.5 ± 9.8
	2	55.5 ± 7.7*	60.4 ± 12.2*	55.6 ± 10.8*	56.3 ± 9.1*
	3	55.3 ± 7.6*	59.8 ± 11.9*	56.2 ± 10.7*	57.3 ± 9.9*
FM (kg)	1	48.7 ± 12.1	44.9 ± 11.4	47.6 ± 12.2	48.7 ± 11.6
	2	36.8 ± 8.1^{*,a,c}	33.0 ± 7.0^{*,b,d}	39.0 ± 8.6^{*,a,b}	38.7 ± 7.9^{*,c,d}
	3	38.7 ± 8.4^{*,a,c}	36.7 ± 7.3^{*,b,d}	40.9 ± 8.9^{*,a,b}	40.2 ± 8.4^{*,c,d}
%BF	1	45.9 ± 5.2	41.5 ± 5.4	45.2 ± 5.3	45.4 ± 5.2
	2	39.9 ± 4.3^{*,a,c}	35.3 ± 4.1^{*,b,d}	41.2 ± 4.6^{*,a,b}	40.7 ± 4.8^{*,c,d}
	3	40.7 ± 4.7^{*,a,c}	36.6 ± 4.8^{*,b,d}	42.1 ± 4.7^{*,a,b}	41.7 ± 4.9^{*,c,d}
W/H ratio	1	0.95 ± 0.04	0.95 ± 0.05	0.95 ± 0.05	0.95 ± 0.04
	2	0.93 ± 0.04*	0.93 ± 0.05*	0.93 ± 0.04*	0.95 ± 0.04
	3	0.93 ± 0.04*	0.93 ± 0.05*	0.93 ± 0.04*	0.94 ± 0.04

NPNC: normal-protein normal-carbohydrate; NPLC: normal-protein low-carbohydrate; HPNC: high-protein normal-carbohydrate; HPLC: high-protein low-carbohydrate; BMI: body mass index; BW: body weight; FFM: fat-free mass; FM: fat-mass, %BF: percentage body fat: W/H: waist/hip.
$^{*}p<0.001$; changes over time compared to phase 1; ANOVA repeated measures.
$^{a,b,c,d}p<0.01$; treatment × time interaction; same types indicate significant differences post-hoc; factorial ANOVA with Bonferroni's corrections.

3.4. Physical activity and eating behavior

After three months of energy restriction, the groups did not differ in changes in physical activity, measured with the Baecke questionnaire (Table 3). Finally, over the 12 months, physical activity had not changed over time, nor had it become different between groups.

With respect to eating behavior, during the first three months, as well as during the next nine months, TFEQ scores of cognitive restraint (F1) increased, and scores of disinhibition (F2) and hunger (F3) decreased in all groups ($p<0.01$, Table 3). The decrease in BW was related to the decrease in hunger score ($r=0.3$, $p<0.01$) and to the decrease in disinhibition score ($r=0.2$, $p<0.01$). Cognitive restraint score was a function of disinhibition score ($r=-0.4$, $p<0.01$) and of hunger score ($r=-0.3$, $p<0.01$); disinhibition score was a function of hunger score ($r=0.5$, $p<0.01$). Over the 12 months, TFEQ scores of cognitive restraint (F1) had increased, and scores of disinhibition (F2) had decreased ($p<0.01$, Table 3). Finally, hunger scores (F3) were decreased in all diets ($p<0.01$). Changes of cognitive restraint score and hunger score over the 12 months were inter-related ($r=-0.3$, $p<0.01$); both were related to BW loss ($r=0.3$; $r=-0.3$; $p<0.01$).

Table 2
Metabolic profile (mean ± SD) of the subjects (n = 132), 33 in each group: HPNC, HPLC, NPNC, NPLC; age 50 ± 12 yrs. Measurements after phase 1: two weeks run-in; phase 2: three months weight loss; phase 3: nine months weight maintenance.

	Phase	HPNC	HPLC	NPNC	NPLC
SBP (mm Hg)	1	130 ± 11	133 ± 15	134 ± 16	138 ± 23
	2	119 ± 10*	123 ± 14*	125 ± 14*	127 ± 19*
	3	124 ± 1*	126 ± 15*	127 ± 15*	131 ± 19*
DBP (mm Hg)	1	81 ± 8	81 ± 8	87 ± 12	80 ± 8
	2	72 ± 7^{*,a,b,c}	77 ± 11^{*,a}	82 ± 11^{*,b}	75 ± 11^{*,c}
	3	76 ± 8*	77 ± 12*	81 ± 13*	76 ± 13*
Glucose (mmol/L)	1	5.2 ± 0.7	5.5 ± 0.5	5.6 ± 1.6	5.4 ± 0.9
	2	5.1 ± 0.4	5.4 ± 0.7	5.3 ± 1.0	5.0 ± 0.7
	3	5.2 ± 0.6	5.2 ± 0.7	5.5 ± 1.0	5.1 ± 0.7
Insulin (mUnits/mL)	1	14.0 ± 7.4	14.3 ± 8.2	14.2 ± 5.7	13.7 ± 7
	2	8.6 ± 5.0*	7.9 ± 7.0*	9.5 ± 5.6*	8.0 ± 5.2*
	3	9.8 ± 4.6^{*,a}	6.8 ± 8.1^{*,a,b,c}	9.6 ± 4.9^{*,b}	9.0 ± 5.6^{*,c}
HOMA-index	1	3.2 ± 0.4	3.5 ± 0.5	3.5 ± 0.5	3.3 ± 0.5
	2	1.9 ± 0.2*	2.4 ± 0.4*	2.3 ± 0.3*	2.0 ± 0.2*
	3	2.3 ± 0.3*	1.6 ± 0.2*	2.4 ± 0.4	2.0 ± 0.3*
TAG (mmol/L)	1	1.7 ± 0.9	1.6 ± 0.7	1.8 ± 0.8	1.4 ± 0.6
	2	1.2 ± 0.5*	1.1 ± 0.8*	1.3 ± 0.6*	1.1 ± 0.5
	3	1.6 ± 0.7	1.4 ± 0.8	1.4 ± 0.8	1.5 ± 0.6
LDL (mmol/L)	1	2.6 ± 0.8	3.1 ± 0.8	3.0 ± 0.7	2.9 ± 1.0
	2	2.7 ± 0.8	3.0 ± 0.8	2.9 ± 0.7	2.9 ± 1.0
	3	3.0 ± 0.7*	3.4 ± 0.9*	3.4 ± 0.7*	3.3 ± 0.6*
HDL (mmol/L)	1	1.3 ± 0.3	1.4 ± 0.4	1.3 ± 0.3	1.4 ± 0.4
	2	1.3 ± 0.3	1.4 ± 0.4	1.3 ± 0.3	1.4 ± 0.4
	3	1.5 ± 0.2	1.6 ± 0.3	1.5 ± 0.3	1.5 ± 0.4
Tot. chol. (mmol/L)	1	4.7 ± 0.8	5.1 ± 1.0	5.1 ± 0.9	4.9 ± 1.2
	2	4.5 ± 0.4	4.7 ± 1.0	4.7 ± 0.8	4.7 ± 0.6
	3	5.2 ± 0.7*	5.5 ± 1.3*	5.1 ± 0.9*	5.2 ± 0.7*

NPNC: normal-protein normal-carbohydrate; NPLC: normal-protein low-carbohydrate; HPNC: high-protein normal-carbohydrate; HPLC: high-protein low-carbohydrate; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA: homeostatic model assessment; TAG: triacylglycerol; LDL: low-density lipoprotein; HDL: high-density lipoprotein.
$^{*}p<0.01$: changes over time compared with phase 1; ANOVA repeated measures.
$^{a,b,c}p<0.01$: treatment × time interaction, same types indicate significant differences post-hoc; factorial ANOVA with Bonferroni's corrections.

Table 3
Behavioral scores (mean ± SD) (Three Factor Eating Questionnaire and Baecke Questionnaire) of the subjects (n = 132), 33 in each group: HPNC, HPLC, NPNC, NPLC; age 50 ± 12 yrs. Measurements after phase 1: two weeks run-in; phase 2: three months weight loss; phase 3: nine months weight maintenance.

	Phase	HPNC	HPLC	NPNC	NPLC
TFEQ					
F1	1	7.8 ± 4.0	8.2 ± 3.9	7.0 ± 4.1	8.3 ± 4.2
	2	12.8 ± 4.9*	12.6 ± 4.5*	11.4 ± 4.7*	10.5 ± 4.5*
	3	12.3 ± 4.2*	14.1 ± 4.6*	11.6 ± 4.4*	11.0 ± 4.3*
F2	1	7.5 ± 2.6	6.3 ± 2.8	6.2 ± 2.4	6.2 ± 2.7
	2	4.7 ± 1.8*	4.8 ± 1.9*	4.9 ± 1.9*	4.7 ± 1.8*
	3	5.7 ± 2.0*	4.4 ± 1.7*	5.1 ± 2.1*	5.3 ± 2.2*
F3	1	6.1 ± 3.7	5.4 ± 3.1	4.3 ± 3.3	4.3 ± 3.6
	2	3.1 ± 1.6*	3.5 ± 1.7*	2.9 ± 1.9*	3.2 ± 1.8*
	3	4.0 ± 2.3*	3.1 ± 1.7*	3.1 ± 2.8*	3.1 ± 1.9
Baecke: total	1–3	7.5 ± 1.5	7.7 ± 1.3	7.6 ± 1.4	7.7 ± 1.5
Baecke: work	1–3	2.4 ± 0.7	2.5 ± 0.5	2.5 ± 0.5	2.5 ± 0.5
Baecke: sport	1–3	2.1 ± 0.8	2.3 ± 0.7	2.3 ± 0.8	2.3 ± 0.9
Baecke: leisure	1–3	3.0 ± 0.8	2.9 ± 0.9	2.8 ± 0.8	2.9 ± 0.8

TFEQ: Three Factor Eating Questionnaire; F1: factor 1 cognitive restraint; F2: factor 2 disinhibition of eating or emotional eating; F3: factor 3 general hunger; Baecke: the Baecke activity index, total and divided over categories; these activities did not change over time.

* $p<0.01$ or $p<0.001$, phases 2 and 3 compared with baseline or phase 1; ANOVA repeated measures; no significant post-hoc differences or interactions.

4. Discussion

The main question underlying the present study was whether it is the relatively higher protein content of the diet or the relatively lower carbohydrate content of the diet that supports the success of the so called 'low-carb' diets, since these diets are not only relatively low in their carbohydrate content, but also relatively high in their protein content. The study showed irrefutably, that, despite the success all-over with all four diets, the answer is that it is the relatively high-protein content per se, that supports the even greater success, and not the relatively lower carbohydrate content. First of all, one has to be sure that the intended intervention was achieved. Here this appeared to be the case, with the relatively high-protein diets showing a consistently higher daily absolute protein intake of 1.1 g/kg during the energy restriction phase and during the weight maintenance phase. To be able to show this convincingly, the N content of the urine was measured several times.

The complete analysis showed a diet × time interaction effect of the diets.

Post-hoc, the specific comparisons showed that the two HP diets, namely HPNC vs. HPLC and the two NP diets, namely NPNC vs. NPLC did not show significantly different effects. Significant differences in loss of body-weight were observed when comparing HP diets with NP diets, namely HPLC vs. NPLC and HPNC vs. NPNC over the complete period of time, i.e. over the 3 months and over the 12 months. In parallel significant differences were observed in decreases in FM when comparing these 4 diets. When in focusing on 'low-carb' diets, larger body-weight loss and body-weight maintenance was achieved when 'low-carb' was in the presence of high-protein, but not when it was in the presence of normal-protein. LCHP vs. LCNP showed larger effects, while effects from LCHP did not differ significantly from NCHP, and LCNP did not differ significantly from NCNP. Likewise, NCHP vs. NCNP showed larger effects on body-weight and body-fat. FFM decreased slightly in all diet groups, without differences between diets. Body composition, in terms of FM, changed in parallel to BW loss and BW maintenance, following the different diets. Reductions in BF% were the largest in the two HP diets, while reductions in FFM were not different between the diets. Therefore the improvement of body-composition was rather due to the larger loss of FM, than the preservation of FFM.

The result from the present 2 × 2 analysis that shows that it is likely to be the HP condition and not the LC condition that supports the relatively more favorable body-weight management effect also suggests that in energy-restricted diets of two up to six months with lowered carbohydrate in combination with elevated protein intake compared with control diets [1–4,6,14–17], the relatively higher protein content must have made a major contribution to the success. However, since there are three macronutrients, we cannot exclude an effect from the fat content. In order to test for the different fat-contents of the diets, regression analyses were performed to test the possible relationship between changes in body-weight, body-fat, and metabolic parameters and energy percentage from fat in the diet. Relationships between body-weight changes, or changes in FM, or in FFM and energy percentage of fat in the diet were not statistically significant, nor were relationships between changes in any of the metabolic parameters and energy percentage of fat in the diet.

Previous studies, with protein intake being the same, have indicated that a low fat diet shows a larger weight loss than a high fat diet [18], yet in the present study, the lower fat contents (HPNC and NPNC) vs. the higher fat contents (HPLC and NPLC) did not show a difference in body-weight loss. Another factor may be that those 9 subjects who consumed an HP diet and could not achieve the relatively high protein diet content with the given recipes, were provided with protein sachets; however, their protein-intake was not different from the subjects not receiving any supplements. Nevertheless, this may be considered as a limitation of the study, due to lack of blinding. A further limitation to the study is that although the four groups were successfully stratified with respect to age, BMI, body-weight, physical activity, and attitude toward eating, further stratification was lacking, and possible confounders such as SES, smoking and alcohol intake were not taken into account.

In concurrence with our observations, previous studies already indicated the effect of increasing daily protein intake on BW loss [8,19,20], although the carbohydrate content was not taken into account. When studies did not find differences in BW loss or in BW maintenance thereafter, then mostly the intervention is not sufficiently sensitive, for instance when the differences in protein intake are too small, often uncontrolled for [21], or when the protein contents of the placebo still are sufficiently large. Also a large European study showed that a modest increase in protein content and a modest reduction in the glycemic index led to a relative improvement in maintenance of weight loss [22]. The protein and carbohydrate contents of that diet were similar to our HPNC diets, as well as the glycemic index.

The efficacy of elevated protein energy-restricted diets on decreasing BW and FM may relate to an elevated protein-induced satiety, and preservation of FFM and its related preservation of energy expenditure. Although the prescribed energy-restriction was isoenergetic for the four diets, differences in energy intake in relation to the higher satiating effect of protein cannot be excluded, since the study was free-running, and in practice the energy-intake restriction had to take place following the volunteers' own applications of the prescribed diets.

In studies where food intake was ad libitum, elevated protein intake led to a decrease in BW [8,23], supported by a higher level of satiety of proteins compared to carbohydrates and fat [7,8]. The high level of satiety of elevated protein intake could influence the compliance to the restriction in energy intake, resulting into successful reduction of BW over time [6,7,19]. In our study with prescribed diets, based on daily menus according to the individual's energy intake level and macronutrient composition of the diet of interest, the overall decrease in BW was related to the decrease in subjective feeling of hunger and decrease in disinhibition of the TFEQ, while hunger, disinhibition, and cognitive restraint were inter-related. This suggests that increase in cognitive restraint with decrease in hunger and decrease in disinhibition reinforced the compliance to the energy intake restriction resulting in successful reduction of BW over time. The increase in cognitive restraint, the decrease in disinhibition, and the decrease in overall hunger score were related to BW loss. A final, consistent BW loss thus partly depends on prevention of suffering from hunger, prevention of disinhibition, both related to, and supporting cognitive restraint, which results altogether in a reduction energy intake.

Previously, also an ad libitum approach of two diets with 30 En% of protein and either 5 or 35 En% of carbohydrate for only four weeks indicated the success of a HPLC diet, since it resulted in a larger reduction of BW of 2 kg between both diets, with decreased BW of −6% vs. −4% due to decreased ad libitum energy intakes ranging from 18 to 83% and from 29 to 94% between these 'ketogenic' and 'non-ketogenic' diets [24]. A suggested mechanism supporting the decrease of BW of such elevated protein, lowered carbohydrate, and relatively high fat diets is the very strong satiety effect created by the ketogenic state of the diet. The formation of ketone bodies such as β-hydroxybutyrate [25] was related to appetite reduction and greater reduction of BW in humans [5,24,26]. Moreover, this type of diet increased energy expenditure, supporting a negative energy balance, via increased gluconeogenesis [27]. Here, measurement of ketone bodies such as β-hydroxybutyrate would have made it possible to compare the degree of ketogenic state of the diets.

The other factors that support the success of a relatively high-protein diet are sustained energy expenditure by the FFM sparing effect, while FM is reduced [7,19,28–34]. This preservation of FFM is crucial, as FFM is the main determinant of resting metabolic rate [30], and it is conditional for a negative energy balance during energy restriction [35]. In addition, elevated protein intake changes substrate oxidation, in that more fat is oxidized than consumed resulting in a negative fat balance, thereby facilitating the reduction of FM [32,36]. In our study, BW and FM decreased relatively more with the relatively high-protein diets, implying a larger FFM sparing effect.

Overall, the metabolic profile was improved in all diet groups. Several metabolic parameters were related to BW loss, indicating a larger effect of weight loss on these parameters than of the diet itself, as was also shown previously [19,37]. These metabolic parameters were: decreases in insulin concentrations, HOMA-index, showing a beneficial effect of reduction of BW on glucose homeostasis, TAG concentrations, systolic and diastolic blood pressures, heart rate, and increases in HDL cholesterol concentrations. Only the HPNC diet vs. all other diets reduced diastolic blood pressure more.

With respect to possible adverse events, some caution is needed when protein intake is elevated in an absolute sense for a longer period in time. There may be adverse effects on blood-pressure, related to the kidney. Especially individuals with sub-clinical renal functioning, such as due to metabolic syndrome or type 2 diabetes mellitus, and elderly are vulnerable. However, the link between protein intake and the initiation or progression of renal disease lacks evidence in healthy individuals [36,38]. Our diets resulted in a decrease in systolic and diastolic blood pressures after weight loss [39].

The novelty of our study is the finding that it is primarily the relatively high-protein intake that underscores the success of the so called 'low-carb' diet that is usually high in protein. Lowered carbohydrate intake per se had no effect on decrease in BW and FM during energy restriction, while daily elevated absolute protein intake of 1.1 vs. 0.7 g/kg BW promoted BW loss while reducing FM during the weight-loss phase. Additionally, we observed that a HPNC diet did not promote BW- or FM-regain, and improves diastolic blood pressure.

5. Conclusion

Body-weight loss and weight-maintenance depends on the high-protein (a relatively high-protein content of 1.1 vs. 0.7 g/kg BW), but not on the 'low-carb' component of the diet, while it is unrelated to the concomitant fat-content of the diet.

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