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ORIGINAL ARTICLE

The effect of a low-fat, high-protein or high-carbohydrate *ad libitum* diet on weight loss maintenance and metabolic risk factors

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Background: High-protein (HP) diets are often advocated for weight reduction and weight loss maintenance.
Objective: The aim was to compare the effect of low-fat, high-carbohydrate (HC) and low-fat, HP *ad libitum* diets on weight maintenance after weight loss induced by a very low-calorie diet, and on metabolic and cardiovascular risk factors in healthy obese subjects.
Design: Forty-eight subjects completed the study that consisted of an energy restriction period of 5–6 weeks followed by a weight maintenance period of 12 weeks. During weight maintenance subjects received maltodextrin (HC group) or protein (HP group) (casein (HPC subgroup) or whey (HPW subgroup)) supplements (2×25 g per day), respectively and consumed a low-fat diet.
Results: Subjects in the HP diet group showed significantly better weight maintenance after weight loss (2.3 kg difference, $P = 0.04$) and fat mass reduction (2.2 kg difference, $P = 0.02$) than subjects in the HC group. Triglyceride (0.6 mM difference, $P = 0.01$) and glucagon (9.6 pg ml^{-1} difference, $P = 0.02$) concentrations increased more in the HC diet group, while glucose (0.3 mM difference, $P = 0.02$) concentration increased more in the HP diet group. Changes in total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, insulin, HOMAir index, HbA1c, leptin and adiponectin concentrations did not differ between the diets. No differences were found between the casein- or whey-supplemented HP groups.
Conclusions: These results show that low-fat, high-casein or whey protein weight maintenance diets are more effective for weight control than low-fat, HC diets and do not adversely affect metabolic and cardiovascular risk factors in weight-reduced moderately obese subjects without metabolic or cardiovascular complications.
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Keywords: low-fat, high-protein diet; body weight maintenance; insulin; glucagon; cardiovascular risk factors; lipid metabolism; blood pressure

Introduction

In overweight and obese people weight loss is known to improve glycemic control, blood lipid profiles and other conditions that may contribute to the development of type 2 diabetes and cardiovascular disease.[1,2] High-protein (HP) diets are considered to be more effective regarding weight loss and weight maintenance than diets with higher carbohydrate-to-protein or fat-to-protein ratios because of their high-satiating and thermogenic effect.[3–9] In addition to their beneficial effect on weight management, dietary proteins also influence glucose and lipid metabolism. Short-term intervention studies have often shown a positive effect of high-protein weight reduction diets on glycemic control, mostly in combination with higher weight loss.[10] In those studies it is often difficult to separate the effects of weight loss and protein intake *per se* on glucose metabolism. Longer term intervention and observational studies, on the other hand, suggest that a high habitual protein intake may be associated with impaired glucose metabolism and increased incidence of type 2 diabetes.[10,11] Exchanging carbohydrates for proteins has been shown to reduce fasting low-density lipoprotein (LDL) cholesterol and triglyceride (TG) concentrations and to increase high-density lipoprotein (HDL)

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concentration, but again some of this effect may be mediated by a higher weight loss on the high protein diets.[12] Higher intake of dietary proteins has also been associated with lower blood pressure in cross-sectional and observational studies. This is especially true for dairy proteins, but well-controlled intervention studies on the blood pressure effects of an exchange of dietary carbohydrate for proteins independent of weight changes are lacking.[12,13]

On the basis of their absorption pattern, dietary proteins can be divided in so-called fast and slow digestible proteins.[14] Fast proteins, such as whey protein, are soluble, whereas slow proteins, such as casein, clot in the stomach resulting in a delay in gastric emptying[15,16] and lower but more sustained plasma amino-acid elevations after ingestion compared with fast proteins,[14,16,17] which may be associated with differences in hormonal and thermogenic responses.[18] Hall *et al*.[17] found that higher postprandial circulating levels of amino acids are associated with increased satiety and suggested that fast proteins are, therefore, more satiating than slow proteins. Slow proteins, on the other hand, could induce a prolonged stimulation of gastric feedback signals contributing to meal termination because of the prolonged enhanced gastric volume compared with fast proteins.[19] The role of slow or fast proteins in weight management and the risk of cardiovascular disease and diabetes remain to be investigated.

Over the past years many studies have looked at the effect of HP diets on weight loss. Weight loss is often associated with a subsequent greater weight regain.[20] Therefore, the present study compared the effects of an *ad libitum* diet high in carbohydrate (HC) with an *ad libitum* diet high in protein (HP) on weight regain in healthy obese subjects after substantial diet-induced weight loss. Additionally, the effects on body composition, blood pressure, and parameters of glucose and lipid metabolism were studied. To our knowledge, only two weight regain prevention studies have been done so far.[6,7] These studies looked at the effect of increased protein intake without controlling what protein had been exchanged for (carbohydrate or fat, or both), which is the main difference with the present study, where the exchange between protein and carbohydrates was investigated while fat intake was kept similar between groups. A secondary aim was to compare, in this setting, a HP diet with intact casein supplements (a slow protein) and a HP diet with intact whey supplements (a fast protein).

Methods

Subjects

Sixty overweight and obese subjects (BMI $\geqslant 27\,kg/m^2$) of both genders, aged 30–60 years were recruited by public advertisement. Subjects underwent a brief medical screening examination, including a medical history, routine physical examination and a fasting blood sample. Subjects had to be weight stable over the past 2 months before enrollment. Subjects were excluded if fasting glucose ($>6\,mmol\,l^{-1}$), TGs ($>2.3\,mmol\,l^{-1}$) or total cholesterol levels ($>6.5\,mmol\,l^{-1}$) were increased, or when diastolic blood pressure exceeded 100 mm Hg. Furthermore, subjects were excluded during the study when they were unable to lose at least 5% of their initial body weight (BW) during the weight loss period.

Of the 60 subjects included, 48 (31 women and 17 men) completed the study, 6 subjects dropped out during the weight loss period (4 subjects were unable to comply with the energy restriction and 2 subjects lost less than 5% of their initial BW) and 6 during the intervention (2 were not able to comply and 4 due to various personal reasons).

The Medical Ethics Committee of the Maastricht Academic Hospital and University approved the study and all subjects gave their written informed consent before entering the study.

Experimental design

Subjects were matched for BMI, age and gender, and randomly assigned to the high-carbohydrate (HC), HP casein (HPC) or HP whey (HPW) group. The study consisted of a 5-week weight loss period using a very low caloric (liquid) diet (VLCD) and a 12-week weight maintenance period with 1 week in-between to change back from liquid to normal food. Subjects came to the laboratory in a fasting state at the beginning of the energy restriction period (week 0), after changing back completely to normal food (week 6) and 2, 4, 8 and 12 weeks thereafter (that is, weeks 8, 10, 14 and 18 of the study). BW and blood pressure were measured at all visits to the lab. At weeks 0, 6 and 18 waist and hip circumference were measured, fasting blood samples were drawn and body composition was determined by underwater weighing. Twenty-four hour urine samples were collected at weeks 0 and 18 for determination of nitrogen excretion to check compliance to the diets.

Diets

During the 5-week VLCD period subjects consumed a liquid diet providing 500 kcal per day (Modifast, Nutrition et Santé, Belgium). Subjects were allowed to eat an unrestricted amount of vegetables (all vegetables except for HC containing pulse crops). During week 6, the liquid diet was gradually replaced by normal *ad libitum* meals into which supplements were gradually incorporated according to the dietary group the subject was randomized to. During the whole weight maintenance period subjects received dietary counseling from a dietician to maintain a fat intake of approximately 30 % of energy intake (excluding the supplements) in all groups and a carbohydrate intake of at least 55% of energy intake in the HC group, and a protein intake of at least 25% of energy intake in both HP groups. In addition, the HC group consumed maltodextrin supplements (AVEBE, Veendam, The Netherlands) twice a day (50 g per day). The HPC and

HPW groups consumed intact casein (Kerry Ingredients, Tralee, Ireland) or whey supplements (Carberry, Cork, Ireland) twice a day (50 g per day), respectively. Although due to the dietary counseling subjects knew in which diet group (HC or HP) they were enrolled, subjects in the HP group were blinded for the kind of protein they were supplemented with. Maltodextrin supplements were orange flavored, whereas both protein supplements were strawberry flavored. Subjects were asked to consume one supplement in the morning as (part of) breakfast and the other in the afternoon (±1.5 h before dinner). Total energy intake was *ad libitum* during the weight maintenance period.

Measurements

Anthropometric measurements. All subjects were weighed in underwear on the same decimal scale throughout the study (Seca, Model 861, Hamburg, Germany; accuracy of 0.1 kg). Height was measured using a wall-mounted stadiometer (Seca, Model 225, Hamburg, Germany; accuracy of 1.0 mm). Waist circumference was measured at the site of the smallest circumference between the rib cage and the ileac crest, with subjects in standing position. Hip circumference was measured at the site of the largest circumference between the waist and the thighs. Both waist and hip circumferences were measured with an accuracy of 1.0 mm.

Body composition was determined by measuring body mass in air and underwater on a digital balance, accurate to 0.01 kg (Sauter type E1200, Ebingen, Germany). Lung volume was measured simultaneously with the helium dilution technique using a spirometer (Volugraph 2000, Mijnhardt, The Netherlands). Body density was used to calculate body fat according to the two-compartment model as described by Siri.[21]

Blood sample analysis. Fasting blood samples were collected in EDTA-containing tubes for glucose, insulin, glucagon, leptin, adiponectin, free fatty acids, TGs and HbA1c analyses. EDTA blood to which aprotinin (5 kIU ml^{-1} blood; Sigma-Aldrich Nederland, Zwijndrecht, The Netherlands) was added was used for glucagon analysis. Whole blood was stored for HbA1c determination. The remainder of the blood samples was centrifuged at 1000 *g* at 4 °C for 10 min. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at −45 °C. Glucose, total cholesterol, HDL, LDL, and TG (kits from Roche Diagnostics, Basel, Switzerland) and free fatty acids (kit from Wako Chemicals, Neuss, Germany) were analyzed by enzymatic tests on a Roche/Hitatchi Cobas automated analyzer (Roche, Basel, Switzerland). Insulin was analyzed by radioimmunoassay (Human Insulin Specific RIA kit, LINCO, MO, USA), as was glucagon (Glucagon RIA kit, LINCO, MO, USA). Leptin and adiponectin were analyzed using multiplexed sandwich immunoassays based on flowmetric Luminex xMAP technology as described earlier.[22] HbA1c concentration was determined on a Tosoh analyzer by High Pressure Liquid Chromatography (Tosoh Bioscience, San Francisco, USA). HOMA index for insulin resistance (HOMAir) was calculated by the following formula: (glucose)*(insulin)/22.5.[23]

Dietary compliance. Before the start of the VLCD period and at the end of the weight maintenance period, subjects filled out a 3-day food record (2 week days and 1 week end day). The food records were analyzed with a computer program according to the Dutch food table (Komeet 4.0).

Nitrogen content in 24-h urine collections was analyzed (Elemental Analyzer, CHN-O-Rapid, Heraeus, Osterode, Germany). Earlier, it has been reported that on average 24-h urine nitrogen is a constant proportion (±80%) of dietary nitrogen.[24] Therefore, we used the following formula to calculate the amount of proteins ingested during urine collection:

Grams protein ingested per day = (urinary $N + 0.2$* urinary N)*6.25[24] with urinary N in grams.

By using subjects' daily energy intake, as determined by 3-day food records, the contribution of protein intake to total energy intake was estimated.

Physical activity. To check whether subjects kept their physical activity constant during the intervention period, they had to fill in the Baecke questionnaire for physical activity[25] before and after the weight maintenance period.

Statistical analyses

All data are presented as means ± s.e.m. Statistical analysis was performed using SPSS for Mac OS X software (SPSS, Chicago, IL). Changes from baseline (week 0) or from week 6 were calculated. Differences between groups at baseline, week 6 and week 18, and differences in changes in weight loss (week 6–week 0) and weight maintenance (week 18–week 6) between groups were tested by one-way analysis of variance with group (diet: HC vs HP or HPC vs HPW) as factor. Within-group changes over time were analyzed by paired-samples *t*-tests. BW changes and changes in systolic and diastolic blood pressure during the weight maintenance period (changes from week 6 to week 8, 10, 14 and 18) were analyzed using repeated measures analysis of variance with changes over time as within-subject variables and group (HC vs HP; HPC vs HPW) as between-subject variables. All statistical tests were performed two-tailed and a P-value <0.05 was considered statistically significant.

Results

Baseline characteristics of the study population are shown in Table 1. The subgroups were well balanced for age, BMI, waist and hip circumference, blood pressure, and fasting plasma glucose, cholesterol and TG concentrations.

Table 1 Baseline characteristics of subjects in the high-carbohydrate (HC), high-protein casein-enriched (HPC) and high-protein whey-enriched (HPW) groups

	HC	*HPC*	*HPW*	P-*value**
N (M/F)	16 (6/10)	14 (5/9)	18 (6/12)	0.97
Age (years)	46.0 ± 2.2	45.4 ± 2.2	44.9 ± 2.0	0.93
BW (kg)	97.8 ± 4.0	95.1 ± 6.4	96.4 ± 3.0	0.92
BMI (kg/m^2)	32.4 ± 1.2	32.9 ± 1.6	33.4 ± 1.0	0.84
Body fat (%)	39.2 ± 1.9	42.0 ± 1.3	41.2 ± 1.4	0.45
Fat mass (kg)	38.5 ± 2.8	40.23 ± 3.3	39.65 ± 2.0	0.90
Fat-free mass (kg)	59.26 ± 2.9	54.82 ± 3.60	56.23 ± 2.0	0.54
Waist (cm)	108.2 ± 3.1	108.5 ± 4.4	109.1 ± 2.4	0.98
Hip (cm)	110.5 ± 2.4	112.0 ± 2.6	116.2 ± 2.6	0.24
Glucose (mM)	5.4 ± 0.1	5.2 ± 0.1	5.5 ± 0.1	0.18
HbA1c (%)	5.42 ± 0.07	5.38 ± 0.09	5.40 ± 0.09	0.92
Insulin (μU ml^{-1})	20.7 ± 3.0	15.7 ± 1.8	20.0 ± 1.4	0.25
HOMAir Index	5.0 ± 0.8	3.6 ± 0.5	4.9 ± 0.4	0.19
Glucagon (pg ml^{-1})	70.0 ± 7.4	69.1 ± 5.6	75.8 ± 6.6	0.74
Total cholesterol (mM)	4.8 ± 0.2	4.9 ± 0.2	5.2 ± 0.3	0.45
Triglycerides (mM)	1.6 ± 0.3	1.4 ± 0.2	1.7 ± 0.2	0.77
Systolic BP (mm Hg)	135.6 ± 4.4	127.9 ± 6.3	130.4 ± 3.9	0.52
Diastolic BP (mm Hg)	87.9 ± 11.4	83.4 ± 3.0	84.2 ± 2.2	0.46

Values are means ± s.e.m. **P*-values from one-way analysis of variance.

Table 2 Energy and macronutrient intake at the beginning (week 0) and at the end (week 18) of the study in the high-carbohydrate (HC) and high-protein (HP) diet groups.

	Protein intake (% of energy intake)	*Carbohydrate intake (% of energy intake)*	*Fat intake (% of energy intake)*	*Energy intake (kcal day^{-1})*
Week 0				
HC ($N=16$)	16.0 ± 0.8	48.2 ± 1.6	34.9 ± 1.4	2398 ± 141
HP ($N=32$)	16.7 ± 0.6	44.3 ± 1.0	35.5 ± 0.7	2161 ± 93
HPC ($N=14$)	18.0 ± 1.0	44.4 ± 1.7	34.7 ± 1.2	2045 ± 142
HPW ($N=18$)	15.7 ± 0.7	44.1 ± 1.3	36.2 ± 0.9	2252 ± 122
Week 18[a]				
HC ($N=16$)	15.8 ± 0.6	62.7 ± 2.4[#]	21.2 ± 1.5[#]	1868 ± 142[#]
HP ($N=32$)	34.9 ± 1.1[#]	42.2 ± 0.8	24.0 ± 1.1[#]	1828 ± 73[#]
HPC ($N=14$)	34.5 ± 1.3[#]	42.3 ± 1.2	23.5 ± 1.5[#]	1848 ± 108
HPW ($N=18$)	35.2 ± 1.6[#]	42.1 ± 1.1	24.3 ± 1.7[#]	1812 ± 103[#]

Abbreviations: HPC = casein-enriched high-protein group; HPW = whey-enriched high-protein group. [a]Supplements taken into account. [#]Significantly different from week 0 as determined by one-way analysis of variance ($P<0.005$).

Composition of the study diets and subject compliance

The self-reported energy intake and dietary macronutrient composition before the start of the study (week 0) and at the end of the weight maintenance period of the HC, HPC and HPW groups (week 18) are shown in Table 2. At the end of the intervention period the HC group reported to consume 63 En% carbohydrates and 16 En% protein, whereas both HP groups reported to consume approximately 35 En% proteins and 42 En% carbohydrates. Total energy intake was significantly lower compared with week 0 for all diets ($P<0.005$) except HPC ($P=0.226$). HC and HP groups differed significantly from each other in carbohydrate intake and in protein intake ($P<0.005$) but not in fat intake. Carbohydrate supplements accounted for ± 6.5 En% of total carbohydrate intake in the HC group (thus 56.5 En% through diet, 6.5 En% through supplements), whereas protein supplements accounted for 10 En% of total protein intake (25 En% through diet and 10 En% through supplements). The difference in reported protein intake between the groups is supported by the urinary nitrogen excretion data. Urinary nitrogen excretion at the end of the intervention was significantly higher in the HP groups compared with the HC group (16.0 ± 0.7 g per 24 h vs 9.8 ± 0.9 g per 24 h, $P<0.001$) and was similar for both protein groups (16.5 ± 1.0 g per 24 h vs 15.7 ± 1.0 g per 24 h for HPC and HPW, respectively, NS). When estimating protein intake from 24-h urinary nitrogen excretion and (reported) total daily energy intake as determined from food records, subjects in the HC group consumed ± 16.5 En% proteins, whereas the HPC and HPW group consumed 28.1 and 27.2 En% proteins, respectively. Although all subjects (independent of group) reported to have increased their physical activity ($P=0.004$), no differences in change in physical activity, as determined by the Baecke questionnaire, were found between groups ($P=0.63$).

Weight loss period

The VLCD-induced effects are shown in Table 3. The energy restriction lowered BW, fat mass, fat-free mass, waist and hip circumference (all $P\leqslant0.001$), and blood pressure ($P\leqslant0.001$). A significant decrease was found in all blood lipids ($P\leqslant0.001$) except free fatty acids ($P=0.07$). Furthermore, plasma glucose, insulin, glucagon and leptin concentrations, and LDL/HDL ratio were significantly reduced ($P<0.001$). HbA1c was significantly increased ($P=0.03$), whereas the increase in adiponectin did not reach statistical significance ($P=0.23$).

Ad libitum *weight maintenance period*

During the *ad libitum* weight maintenance period, subjects in the HC group gained, whereas subjects in both protein groups lost approximately an additional 1.0 kg BW (Figure 1b, Table 4). Repeated measures analysis showed a significant effect for the time-by-treatment interaction for BW changes during weight maintenance ($P=0.01$) when HC and HP were compared. No time-by-treatment effect was found when comparing the two protein groups ($P=0.63$) but correlation analysis showed that BW change was positively associated with total energy intake ($P=0.03$). The change in body fat percentage (week 18–week 6) was significantly different between the HC and HP groups ($P=0.02$) (Table 4) but no difference was found between HPC and HPW ($P=0.19$) (Table 5). Waist circumference increased slightly in the HC group, whereas it decreased in the HP group resulting in a significant difference between groups ($P=0.05$) (Table 4). No difference in waist or hip circumference change was found between HPC and HPW groups ($P=0.70$ and

$P=0.48$, respectively) (Table 5). No statistically significant differences were found between HC and HP groups or between HPC and HPW groups regarding changes in blood pressure (systolic blood pressure $P=0.12$, diastolic blood pressure $P=0.09$) (Tables 4 and 5) and no significant time-by-treatment interactions were found (HP vs HC: $P=0.42$ and $P=0.26$, respectively; HPW vs HPC: $P=0.35$ and $P=0.17$, respectively).

Fasting glucose concentrations increased significantly more from week 6 to week 18 in the HP group ($P=0.02$). The difference in changes in fasting insulin between the HC and HP groups did not reach statistical significance ($P=0.10$) (Table 4). Fasting glucagon concentrations increased significantly on the HC diet compared with the HP diet ($P=0.02$) (Table 4). The increase in HDL cholesterol was more pronounced in the HP than in the HC group, although not significantly ($P=0.08$) (Table 4). The change in LDL/HDL ratio was not significantly different between HC and HP ($P=0.15$, Table 4).

When comparing the two HP diet groups (HPC and HPW), only the difference in change in LDL/HDL ratio was significant and in favor of the HPC diet group (Table 5).

Table 3 Variables (mean ± s.e.m.) at baseline (week 0) and after weight loss (week 6) ($N=48$).

Variable	*Week 0*	*Week 6*
BW (kg)	96.5 ± 2.5	87.1 ± 2.3$^{\$}$
Body fat (%)	40.8 ± 0.9	36.5 ± 1.0$^{\$}$
Fat mass (kg)	39.4 ± 1.5	32.0 ± 1.4$^{\$}$
Fat-free mass (kg)	56.8 ± 1.6	55.0 ± 1.5$^{\$}$
Waist (cm)	108.6 ± 1.8	98.4 ± 1.3$^{\$}$
Hip (cm)	113.1 ± 1.4	105.2 ± 1.3$^{\$}$
Systolic BP (mm Hg)	131.4 ± 2.7	124.3 ± 2.0$^{\$}$
Diastolic BP (mm Hg)	85.2 ± 1.5	79.7 ± 1.1$^{\$}$
Glucose (mmol l^{-1})	5.4 ± 0.1	5.0 ± 0.1$^{\$}$
HbA1c (%)	5.4 ± 0.05	5.5 ± 0.06*
Insulin (μU ml^{-1})	19.0 ± 1.3	14.5 ± 1.2$^{\$}$
HOMAir Index	4.6 ± 0.3	3.3 ± 0.3$^{\$}$
Glucagon (pg ml^{-1})	71.9 ± 3.8	54.9 ± 2.7$^{\$}$
Total cholesterol (mmol l^{-1})	5.0 ± 0.1	4.2 ± 0.1$^{\$}$
HDL (mmol l^{-1})	1.1 ± 0.05	1.0 ± 0.03$^{\$}$
LDL (mmol l^{-1})	3.3 ± 0.1	2.6 ± 0.1$^{\$}$
LDL/HDL ratio	3.2 ± 0.2	2.7 ± 0.1$^{\$}$
Triglycerides (mmol l^{-1})	1.6 ± 0.1	1.3 ± 0.1$^{\$}$
FFA (mmol l^{-1})	0.6 ± 0.04	0.5 ± 0.03$^{\$}$
Leptin (ng ml^{-1})	34.9 ± 3.3	15.5 ± 1.4$^{\$}$
Adiponectin (μg ml^{-1})	10.8 ± 1.1	11.7 ± 1.3

Abbreviations: BW = body weight; BP = blood pressure; FFA = free fatty acids; HDL = high-density lipoprotein; HPC = casein-enriched high-protein group; HPW = whey-enriched high-protein; LDL = low-density lipoprotein. *,$^{\$}$Significantly different from week 0 as determined by paired-samples *t*-test analysis ($P<0.05$ and $P\leqslant0.001$, respectively).

Discussion

The major findings of the present study are that after an initial substantial weight loss, a HP, low-fat diet prevented weight regain and initiated even further modest weight and body fat loss compared with a HC, low-fat diet. Changes of most risk factors during the weight maintenance period were not different on the two diets, except for fasting glucose, which increased more on the HP diet, and fasting TGs, which increased more on the HC diet. No differences between the HPW and HPC diets were found for any of the variables except for the change in LDL/HDL ratio.

Diet composition and compliance

As Table 2 shows, we were successful in increasing carbohydrate intake in the HC group to 62 En% and protein intake in the HPC and HPW group to 34.5 and 35.2 En%, respectively, as calculated from 3-day food records. Although we tried to guide all subjects towards 30 En% fat intake, the reported fat intake in all groups was slightly above 20 En%. Therefore, protein intake in the HC group still accounted for 16 En% of total energy intake. When comparing reported protein intake with protein intake calculated from urinary nitrogen excretion subjects seemed to have over-reported their protein intake and this mainly in both HP groups. However, the difference in protein intake between the HC and HP groups is likely to have been at least 10 En%.

VLCD-induced weight loss

The amount of weight loss after the VLCD was similar in the HC and HP groups as were the changes in other

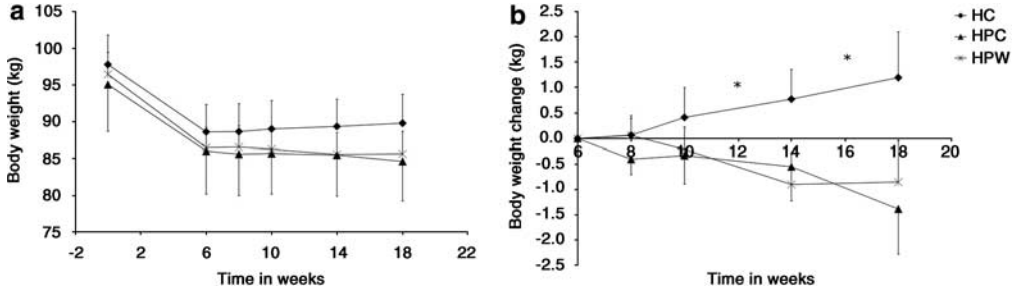

Figure 1 Mean body weight over time (**a**) and body weight change during weight maintenance (compared with immediately after weight loss) (**b**) in the diet groups. *Body weight change significantly different between high-carbohydrate (HC) and high-protein (HP) group ($P<0.05$).

Table 4 Variables (mean ± s.e.m.) for the HC ($N=16$) and HP ($N=32$) groups after weight loss (week 6) and weight maintenance (week 18)

	HC			*HP*			P-*value**
	Week 6	*Week 18*	*Delta (weeks 18–6)*	*Week 6*	*Week 18*	*Delta (weeks 18–6)*	
BW (kg)	88.58 ± 3.79	89.77 ± 3.92	1.19 ± 0.90	86.28 ± 2.92	85.20 ± 2.86	−1.09 ± 0.59	0.04
Body fat (%)	35.00 ± 2.15	34.85 ± 1.93	−0.14 ± 0.47	37.29 ± 1.08	35.43 ± 1.15$^{\#}$	−1.86 ± 0.46	0.02
Fat mass (kg)	31.19 ± 2.63	31.43 ± 2.53	0.24 ± 0.70	32.47 ± 1.63	30.50 ± 1.62$^{\#}$	−1.96 ± 0.52	0.02
Fat-free mass (kg)	57.38 ± 2.87	58.34 ± 2.93$^{\#}$	0.96 ± 0.38	53.82 ± 1.77	54.70 ± 1.77$^{\#}$	0.88 ± 0.37	0.89
Waist (cm)	97.88 ± 2.81	98.29 ± 2.64	+0.41 ± 0.92	98.68 ± 2.30	96.71 ± 2.28$^{\#}$	−1.97 ± 0.69	0.05
Hip (cm)	105.54 ± 2.28	106.94 ± 2.23	+1.39 ± 0.88	106.01 ± 1.69	105.75 ± 1.63	−0.26 ± 0.77	0.20
Systolic BP (mm Hg)	125.88 ± 3.78	127.5 ± 5.60	+1.63 ± 4.67	123.56 ± 2.37	118.63 ± 2.49$^{\#}$	−4.94 ± 1.77	0.12
Diastolic BP (mm Hg)	81.56 ± 1.90	84.19 ± 4.38	+2.63 ± 3.52	78.75 ± 1.31	76.31 ± 1.32$^{\#\$}$	−2.44 ± 1.16	0.09
Glucose (mmol l^{-1})	5.13 ± 0.12	5.26 ± 0.08	0.13 ± 0.10	4.97 ± 0.08	5.38 ± 0.08$^{\#}$	0.41 ± 0.06	0.02
HbA1c (%)	5.58 ± 0.12	5.60 ± 0.09	0.09 ± 0.07	5.45 ± 0.06	5.53 ± 0.06	0.07 ± 0.07	0.86
Insulin (μU ml^{-1})	15.75 ± 2.39	18.28 ± 3.67	2.53 ± 2.11	13.88 ± 1.33	12.88 ± 0.84	−1.00 ± 1.07	0.10
HOMAir	3.68 ± 0.64	4.31 ± 0.87	0.62 ± 0.47	3.10 ± 0.31	3.12 ± 0.23	0.02 ± 0.24	0.21
Glucagon (pg ml^{-1})	51.02 ± 4.15	63.40 ± 6.18$^{\#}$	12.38 ± 3.57	56.82 ± 3.50	59.58 ± 5.36	2.76 ± 2.23	0.02
Total cholesterol (mmol l^{-1})	4.15 ± 0.27	4.88 ± 0.27$^{\#}$	0.73 ± 0.18	4.16 ± 0.13	4.74 ± 0.14$^{\#}$	0.58 ± 0.10	0.43
HDL (mmol l^{-1})	1.00 ± 0.06	1.10 ± 0.06$^{\#}$	0.10 ± 0.04	1.01 ± 0.04	1.21 ± 0.06$^{\#}$	0.20 ± 0.03	0.08
LDL (mmol l^{-1})	2.52 ± 0.20	2.95 ± 0.18$^{\#}$	0.43 ± 0.13	2.62 ± 0.11	2.98 ± 0.13$^{\#}$	0.36 ± 0.08	0.67
Triglycerides (mmol l^{-1})	1.43 ± 0.18	1.98 ± 0.42	0.56 ± 0.29	1.19 ± 0.10	1.16 ± 0.12$^{\$}$	−0.06 ± 0.06	0.01
FFA (mmol l^{-1})	0.48 ± 0.03	0.39 ± 0.03$^{\#}$	−0.07 ± 0.03	0.49 ± 0.04	0.47 ± 0.03	−0.03 ± 0.04	0.55
LDL/HDL ratio	2.56 ± 0.19	2.73 ± 0.16	0.17 ± 0.14	2.73 ± 0.16	2.65 ± 0.17	−0.08 ± 0.10	0.15
Leptin (ng ml^{-1})	16.13 ± 2.69	23.49 ± 3.43$^{\#}$	7.36 ± 1.48	15.17 ± 1.67	20.31 ± 2.39$^{\#}$	5.13 ± 1.22	0.28
Adiponectin (μg ml^{-1})	10.11 ± 1.76	8.11 ± 0.84	−2.00 ± 1.79	12.50 ± 1.78	11.21 ± 1.20$^{\#}$	−1.29 ± 1.22	0.74

Abbreviations: BW = body weight; BP = blood pressure; FFA = free fatty acids; HC = high-carbohydrate; HDL = high-density lipoprotein; HP = high-protein group; HPC = casein-enriched high-protein group; HPW = whey-enriched high-protein group; LDL = low-density lipoprotein. $^{\$}$Between-group differences: significantly different from HC group as determined by one-way ANOVA analysis ($P<0.05$). $^{\#}$Within-group differences: significantly different from week 6 as determined by paired-samples *t*-test analysis ($P<0.05$). **P*-values for differences in delta values (week 18 to week 6) between the HPC and HPW group as determined by one-way analysis of variance.

Table 5 Variables (mean ± s.e.m.) for the HPC ($N=14$) and HPW ($N=18$) groups after weight loss (week 6) and weight maintenance (week 18)

	HPC			*HPW*			P-*value**
	Week 6	*Week 18*	*Delta (week 18–6)*	*Week 6*	*Week 18*	*Delta (week 18–6)*	
BW (kg)	86.02 ± 5.84	84.64 ± 5.38	−1.39 ± 0.89	86.49 ± 2.72	85.63 ± 3.05	−0.85 ± 0.80	0.66
Body fat (%)	37.13 ± 1.38	35.96 ± 1.56	−1.18 ± 0.58	37.42 ± 1.63	35.02 ± 1.67$^{\#}$	−2.40 ± 0.67	0.19
Fat mass (kg)	32.41 ± 2.97	30.86 ± 2.84$^{\#}$	−1.55 ± 0.69	32.51 ± 1.83	30.22 ± 1.91$^{\#}$	−2.29 ± 0.75	0.49
Fat-free mass (kg)	53.61 ± 3.25	53.77 ± 3.10	0.16 ± 0.53	53.98 ± 1.98	55.41 ± 2.09$^{\#}$	1.43 ± 0.49	0.09
Waist (cm)	99.23 ± 4.35	96.95 ± 3.98$^{\#}$	−2.28 ± 0.83	98.25 ± 2.41	96.52 ± 2.73	−1.73 ± 1.06	0.70
Hip (cm)	104.26 ± 2.77	104.64 ± 2.60	0.37 ± 0.97	107.37 ± 2.12	106.62 ± 2.11	−0.75 ± 1.16	0.48
Systolic BP (mm Hg)	122.57 ± 5.02	119.64 ± 4.96	−2.93 ± 3.27	124.33 ± 1.77	117.83 ± 2.34$^{\#}$	−6.50 ± 1.87	0.33
Diastolic BP (mm Hg)	78.43 ± 2.53	76.64 ± 2.13	−1.79 ± 2.03	79.00 ± 1.32	76.06 ± 1.72$^{\#}$	−2.94 ± 1.37	0.63
Glucose (mmol l^{-1})	5.01 ± 0.16	5.35 ± 0.12	0.34 ± 0.12	4.93 ± 0.08	5.40 ± 0.11	0.47 ± 0.06	0.32
HbA1c (%)	5.38 ± 0.09	5.58 ± 0.11	0.09 ± 0.07	5.39 ± 0.08	5.49 ± 0.07	0.07 ± 0.07	0.49
Insulin (μU ml^{-1})	12.56 ± 1.23	11.26 ± 0.88	−1.29 ± 0.73	14.91 ± 2.16	14.13 ± 1.26	−0.78 ± 1.84	0.82
HOMAir	2.83 ± 0.31	2.69 ± 0.23	−0.14 ± 0.19	3.31 ± 0.49	3.45 ± 0.35	0.15 ± 0.40	0.56
Glucagon (pg ml^{-1})	55.52 ± 3.26	58.60 ± 3.61	3.08 ± 2.73	57.84 ± 5.76	60.35 ± 5.36	2.52 ± 3.42	0.90
Total cholesterol (mmol l^{-1})	4.10 ± 0.13	4.60 ± 0.16$^{\#}$	0.50 ± 0.13	4.20 ± 0.21	4.85 ± 0.22$^{\#}$	0.64 ± 0.14	0.48
HDL (mmol l^{-1})	0.99 ± 0.06	1.22 ± 0.09$^{\#}$	0.23 ± 0.05	1.02 ± 0.06	1.20 ± 0.08$^{\#}$	0.18 ± 0.05	0.48
LDL (mmol l^{-1})	2.56 ± 0.14	2.81 ± 0.15	0.22 ± 0.11	2.62 ± 0.17	3.09 ± 0.20$^{\#}$	0.48 ± 0.12	0.13
Triglycerides (mmol l^{-1})	1.13 ± 0.11	1.08 ± 0.13	−0.05 ± 0.08	1.23 ± 0.17	1.22 ± 0.20	−0.08 ± 0.10	0.98
FFA (mmol l^{-1})	0.50 ± 0.05	0.45 ± 0.06	−0.06 ± 0.08	0.48 ± 0.05	0.48 ± 0.04	0.01 ± 0.05	0.50
LDL/HDL ratio	2.79 ± 0.25	2.50 ± 0.23$^{\#}$	−0.29 ± 0.15	2.68 ± 0.21	2.77 ± 0.25	0.09 ± 0.12	0.05
Leptin (ng ml^{-1})	14.65 ± 2.39	19.02 ± 3.43	4.38 ± 2.18	15.59 ± 2.36	21.31 ± 3.37$^{\#}$	5.72 ± 1.39	0.59
Adiponectin (μg ml^{-1})	11.36 ± 2.17	10.96 ± 1.54	−0.40 ± 1.48	13.39 ± 2.71	11.40 ± 1.80	−1.99 ± 1.86	0.53

Abbreviations: BW = body weight; BP = blood pressure; FFA = free fatty acids; HC = high-carbohydrate; HDL = high-density lipoprotein; HPC = casein-enriched high-protein group; HPW = whey-enriched high-protein group; LDL = low-density lipoprotein. No significant differences found between HPC and HPW group as determined by one-way analysis of variance. $^{\#}$Within-group differences: Significantly different from week 6 as determined by paired-samples *t*-test analysis ($P<0.05$). **P*-values for differences in delta values (week 18 to week 6) between the HPC and HPW group as determined by one-way analysis of variance.

anthropometric measures and blood parameters (despite the fact that subjects were already on different diets for 1 week when week 6 measurements were done) (data not shown). This suggests no important acute effects of the diets on variables measured. The effect of weight loss on fasting glucose, insulin and blood lipids has been reviewed exten-

sively before.[26,27] Our results are in line with these findings. To our knowledge, the effect of weight loss on fasting glucagon levels has not been reported before. We found plasma glucagon levels to be reduced after weight loss. The mechanism underlying this change is unclear and requires further study.

Although we expected adiponectin to increase with BW loss, differences did not reach significance. Recently, Bobbert *et al*.[28] showed that although total adiponectin concentrations did not change significantly after weight loss, which is in accordance with our results, the high and medium molecular weight adiponectin oligomers had significantly increased after weight loss. Unfortunately, in the present study we only measured total adiponectin concentrations. HbA1c increased significantly although the rise is physiologically not relevant. Only with elevated glucose levels, VLCD can induce a decrease in HbA1c.

Weight maintenance: HC vs HP diet

The difference in BW change during weight maintenance between the HC and HP group was mainly caused by the further reduction in fat mass in the HP group, while changes in fat-free mass were similar. The absence of an increase in BW in the HP group is remarkable. Studies looking at weight maintenance over 3 to 6 months after weight loss reported weight regain both in the control (15 En% protein) (2.0 and 3.0 kg during 3 and 6 months weight maintenance, respectively) and in the protein-supplemented group (18 En% protein) (1.0 and 0.8 kg during 3 and 6 months weight maintenance, respectively), although weight regain in the protein-supplemented group was lower.[6,7] In these studies[6,7] subjects in the protein group received protein supplements (Ca-caseinate ± 30 g per day) but no placebo was used in the control group and subjects were asked to adhere to their habitual diet in all groups. No information about carbohydrate or fat content of their diets is available. In particular, changes in *ad libitum* fat intake have a considerable impact on BW changes as described earlier.[29] The combination of a low fat intake and a high protein intake in our HP group may have been responsible for the prevention of weight regain in this group. In fact, BW decreased even further.

Another striking finding was that, although subjects in the HP group reduced their BW during the weight maintenance period this was accompanied by a significant increase in FFM. This is in agreement with studies investigating HP energy-restricted diets, which have shown a fat-free mass preserving effect of such diets.[7]

Previous research showed that HP diets are more satiating than HC diets.[4,6,7,9] We did not find significant differences in self-reported total EI between groups. However, 3-day food records are not able to pick up relatively small differences in total EI because of their limited accuracy. Although differences in total EI were not significantly different, correlation analysis showed that EI was positively associated with BW change. Also, an increase in energy expenditure related to elevated protein synthesis in the HP group[30,31] and the high metabolic cost of metabolizing protein[32] may have contributed to the difference in BW change between the HP and HC diet. The difference in BW changes between the diets cannot be attributed to a more pronounced increase in physical activity in the HP group as determined by the Baecke questionnaire, as subjects reported to have increased their physical activity ($P=0.004$) independent of diet ($P=0.88$). It should be noted that due to the drop-out rate, which was slightly higher than expected, the power of the present study was lower than the preconceived power of 0.8, which was calculated from earlier published results.[7] When calculating the actual power of the study, we found that it was 0.6 when using observed changes in BW and 0.8 when using changes in FM.

During weight maintenance, no significant differences in HOMA index for insulin resistance, insulin or adiponectin, or HbA1c between the HC and HP diet groups were found. However, subjects in the HP group increased their fasting glucose concentration during the weight maintenance period more than subjects in the HC group. This may be related to the gluconeogenesis-stimulating and/or glucagon-stimulating effect of high protein intake, which both will lead to increased hepatic glucose output. Linn *et al*.[33] (2000) showed that hepatic glucose output was higher in subjects habitually consuming a high-protein diet ($>0.8\,g\,kg^{-1}$ per day) than in subjects consuming a lower protein diet ($<0.8\,g\,kg^{-1}$ per day) under fasting conditions. The increased hepatic glucose output under high-protein conditions resulted from increased gluconeogenesis. This was associated with a higher fasting glucagon concentration in the high-protein consumers.[33] In our study, the plasma glucagon concentrations at 18 weeks were not significantly different between the HP and HC group and the increase from week 6 was even significantly higher in the HC group than in the HP group, which makes a role for glucagon less likely. Another possible explanation is that hepatic insulin sensitivity, and thus insulin-induced repression of endogenous glucose output by the liver, was reduced on the HP diet compared with the HC diet. However, HOMAir index did not differ. Although impaired fasting glucose (fasting glucose $\geqslant 6.1$ and $<7.0\,mmol\,l^{-1}$) is a risk factor for the development of impaired glucose tolerance and type 2 diabetes in a high-risk population,[34,35] there is no evidence that elevations of fasting plasma glucose within the normal range, as in the majority of our subject population (two subjects, both from the HP group, had fasting glucose concentrations of 6.1 mM), are associated with increased risk. Furthermore, no adverse effect of the high-protein diet on HbA1c was found in our subjects. The effect of HP diets on glycemic regulation remains unclear.[36] By using obese insulin-sensitive subjects, we expected that if a prolonged HP diet influences glycemic regulation and thus insulin sensitivity, this would affect fasting insulin, glucose, HOMAir and HbA1c in our study population. As we found a slight increase in fasting glucose concentrations, which remained within the normal range, in

the HP group without changes in insulin, HOMAir and HbA1c suggest a subtle, if any, effect of the HP diet on insulin sensitivity in the present study.

When looking at changes in lipid profiles the HC diet induced a significantly higher increase in TGs than the HP diet. This is in agreement with the short-term study reported by Dumesnil *et al*.[37] who studied the effect of an *ad libitum* HP and high-carbohdyrate diet, although in both studies changes in TG concentrations could partly be explained by differences in BW changes between the groups.[37] In a meta-analysis of 60 studies a clear elevation effect of increased carbohydrate intake on TG was found.[38] In moderately hypercholesterolemic subjects, a low-fat, HP diet reduced total cholesterol, LDL cholesterol, VLDL cholesterol, LDL/HDL ratio and TGs and increased HDL cholesterol compared with a low-fat, HC diet.[39] We found no significant differences in changes in total, LDL and HDL cholesterol levels between diet groups, which may be related to our more strict exclusion criteria for elevated TGs and cholesterol at screening and smaller subject number.

There is growing evidence for a beneficial effect of dietary protein on blood pressure.[12] In the DASH[40] and Omni-Heart[41] trials high-protein intake was associated with lower blood pressure. In our study no significant beneficial effect of the high-protein diet on blood pressure could be shown. This again may have been related to the lower (systolic) blood pressure levels at the start of the weight maintenance period (week 6) compared with the DASH and OmniHeart trials, and the smaller number of subjects. However, despite the known blood pressure-lowering effect of the VLCD[42] no rise in blood pressure was noted during weight maintenance in either group and in the HP group systolic blood pressure even tended to decrease.

HPC vs HP whey diet

Boirie *et al*.[14] introduced the concept of fast and slow proteins, where fast proteins are soluble and slow proteins form clots in the stomach resulting in a slower release of amino acids. Calbet and Holst[43] (2004), on the other hand, showed that ingestion of casein, a slow protein, did not induce a difference in gastric emptying as compared with whey protein (fast protein) but did elicit a slower and lower increase of plasma amino-acid levels.[43,44] Lacroix *et al*.[16] (2006) also found that amino-acid kinetics were different for fast and slow proteins. Furthermore, plasma amino-acid levels are found to be associated with satiety.[17,45] In agreement with this, whey has been shown to reduce food intake more than casein in the first 60–90 min after ingestion,[46] but the more prolonged effects have not been compared. In the present study we could not find a significant difference in effect of slow or fast digestible proteins, given as supplement between meals in an *ad libitum* situation, on BW maintenance or any of the other variables studied. Only the LDL/HDL ratio was reduced in the HPC group compared with the HPW group ($P = 0.05$, Table 5) without significant effects on LDL or HDL cholesterol separately. This finding therefore needs confirmation.

In conclusion, the present study showed that a 3-month low-fat, HP diet resulted in a better maintenance of weight loss induced by a very low calorie diet than a low-fat, HC diet. The HP diet was associated with higher fasting glucose and lower fasting TG concentrations than the HC diet. Fasting glucose concentrations, although increased, remained within the normal range. Other risk factors (total cholesterol, LDL and HDL cholesterol, HOMAir index, HbA1c and blood pressure) were not affected by diet composition. No differences between casein- or whey-enriched HP diets were found. These results show that low-fat, HP weight maintenance diets are more effective for weight control than low-fat, HC diets and do not adversely affect metabolic and cardiovascular risk factors in weight-reduced moderately obese subjects without metabolic or cardiovascular complications. The milk protein casein and whey do not differ in outcome parameters.

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