



Randomized trial on protein vs carbohydrate in *ad libitum* fat reduced diet for the treatment of obesity

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OBJECTIVE: To study the effect on weight loss in obese subjects by replacement of carbohydrate by protein in *ad libitum* consumed fat-reduced diets.

DESIGN: Randomized dietary intervention study over six months comparing two *ad libitum* fat reduced diets (30% of total energy) strictly controlled in composition: High-carbohydrate (HC, protein 12% of total energy) or high-protein (HP, protein 25% of total energy).

SETTING AND PARTICIPANTS: Subjects were 65 healthy, overweight and obese subjects (50 women, 15 men, aged 18–55 y) randomly assigned to HC ($n=25$), HP ($n=25$) or a control group (C, $n=15$). All food was provided by self-selection in a shop at the department, and compliance to the diet composition was evaluated by urinary nitrogen excretion.

MAIN OUTCOME MEASURE: Change in body weight, body composition and blood lipids.

RESULTS: More than 90% completed the trial. Weight loss after six months was 5.1 kg in the HC group and 8.9 kg in the HP group (difference 3.7 kg, 95% confidence interval (CI)(1.3–6.2 kg) $P < 0.001$), and fat loss was 4.3 kg and 7.6 kg, respectively (difference 3.3 kg (1.1–5.5 kg) $P < 0.0001$), whereas no changes occurred in the control group. More subjects lost >10 kg in the HP group (35 %) than in the HC group (9 %). The HP diet only decreased fasting plasma triglycerides and free fatty acids significantly.

CONCLUSIONS: Replacement of some dietary carbohydrate by protein in an *ad libitum* fat-reduced diet, improves weight loss and increases the proportion of subjects achieving a clinically relevant weight loss. More freedom to choose between protein-rich and complex carbohydrate-rich foods may allow obese subjects to choose more lean meat and dairy products, and hence improve adherence to low-fat diets in weight reduction programs.

Keywords: low-fat diets; *ad libitum*; high-protein; high-carbohydrate; cardiovascular risk factors; blood lipids; body composition; obesity

Introduction

The prevalence of obesity is increasing rapidly in the Western world, and its comorbidities are of major concern. To prevent obesity, it is recommended that fat should be no more than 30% of the energy intake. The background for this advice is that overconsumption of high-fat foods plays a role in weight gain and obesity in susceptible individuals.^{1–3} This concept has been used clinically to induce and maintain weight loss in obese subjects by administration of low-fat diets consumed *ad libitum*. However, there is some debate about the efficiency of the low-fat *ad libitum* principle, as compared to calorie counting.^{4–6} A reduction in energy intake can be achieved by a reduction in dietary fat content, which can induce a modest weight loss, but the optimal relative proportion of dietary carbohydrate and protein, both in terms

of potential weight loss and of potential adverse effects, has never been addressed in long-term intervention studies.

A number of short-term studies suggest that protein per kJ exerts a more powerful effect on satiety than both carbohydrate and fat.^{1,3,7–15} If this is also true in the long-term, replacing some of the dietary carbohydrate by protein should improve the weight loss obtained by using low-fat diets under *ad libitum* conditions. In contrast, observational studies have found that dietary protein content is positively associated with body fatness.¹⁶ We therefore undertook the present study to compare two *ad libitum*, strictly controlled, low-fat diets, with respect to changes in body weight, body composition and blood lipids in obese subjects over a period of six months.

Subjects and methods

Subjects

Included in the study were 65 overweight and obese subjects ($25 < \text{body mass index (BMI)} < 34 \text{ kg/m}^2$)

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of both genders, aged 18–56 y, (Table 1). All subjects were volunteers recruited through advertisement or a waiting list. They underwent a brief medical screening examination, including a medical history, a routine physical examination and blood tests (haemoglobin, leucocytes, sodium, potassium, glucose, alkaline phosphates and electrocardiogram (ECG)) before enrollment. In addition to normal screening results, the subjects all met the criterion of being weight stable for ≥ 2 months before entry. This was confirmed by weighing at the department.

The subjects of the intervention group were randomly assigned to either high-carbohydrate (HC: 25 subjects) or high-protein (HP: 25 subjects) diet, both low in fat (30% of total energy) or to a control group (C: 15 subjects) (Table 2). To ensure group matching with respect to BMI, gender, age and smoking habits, a third party, who did not know the subjects or their identity, exchanged group membership of six subjects. Alcohol intake, assessed by 7 d dietary records, was equal in the three groups. The subjects in the three groups had similar histories with respect to the course of their obesity; of the subjects in the HC, HP and C groups, 20%, 28% and 20%, respectively, reported that they were overweight at school start (not statistically significant (NS)), and 16%, 28% and 28% reported that they were overweight at age 18 y (NS). Self help, public health services and alternative therapies for weight reduction had all been used to a similar degree in the three groups.

Approval was obtained from the Municipal Ethical Committee of Copenhagen and Frederiksberg. The study was performed in accordance with the Helsinki II declaration, and each subject signed an informed consent document before the study commenced.

Study design

The study was conducted as a dietary intervention for six months, strictly controlled in terms of macronutrient composition. The outcome measures were changes in body weight and composition, proportion of subjects achieving a certain weight loss (>5 kg and >10 kg), total and intra-abdominal fat mass and changes in the plasma values of total and high density lipoprotein (HDL) cholesterol, triglycerides and free fatty acids.

Diet

All the food for the intervention groups was provided by a shop at the Department and could be consumed *ad libitum*. The C group was instructed not to change anything in their dietary habits whilst shopping in ordinary food shops. The targeted composition of the two diets was: HP: 25% of energy (E%) as protein and 45 E% as carbohydrate; HC: 12 E% as protein and 58 E% as carbohydrate (See Table 2). A variety of different food items made up an all-round assortment offered by the shop and this covered the most common foods. The selection varied seasonally.

Table 1 Physical characteristics of subjects in the two intervention groups and one control group^a

	Age (y)	Gender (M/F)	Smoking ^b	Body weight (kg)	Height (cm)	BMI (kg/m ²)	Body fat (kg)
High-carbohydrate (n = 25)	39.4 ± 2.0	6/19	9 (5.7 ± 1.5)	88.6 ± 1.9	169.5 ± 0.0	30.8 ± 0.4	30.5 ± 1.5
High-protein (n = 25)	39.8 ± 1.9	6/19	9 (8.0 ± 2.0)	87.0 ± 1.9	170.0 ± 0.0	30.0 ± 0.4	28.5 ± 1.4
Controls (n = 15)	37.6 ± 2.2	3/12	5 (9.1 ± 2.4)	88.1 ± 1.8	171.0 ± 0.0	30.3 ± 0.7	29.6 ± 1.8

^aValues are means ± s.e.m. There were no differences between groups by ANOVA.

^bNumber of smokers in group (number of cigarettes smoked per day).

BMI = body mass index.

Table 2 Macronutrient composition and energy content in intervention diets^a

	High-carbohydrate			High-protein		
	Targeted ^b	Actual		Targeted ^b	Actual	
		0–3 months ^c	4–6 months ^d		0–3 months ^c	4–6 months ^d
Energy from protein (%)	12	12.1 ± 0.1	12.2 ± 0.1	25	24.7 ± 0.1	24.1 ± 0.2
Energy from carbohydrate (%)	58	59.4 ± 0.2	59.0 ± 0.2	45	45.9 ± 0.2	46.8 ± 0.2
Energy from fat (%)	30	28.5 ± 0.2	28.8 ± 0.2	30	29.4 ± 0.2	29.1 ± 0.2
Total energy (MJ/d)	-	10.6 ± 0.3**	11.2 ± 0.5**	-	8.6 ± 0.4	9.3 ± 0.4
Fiber content (g) ^e	-	-	22.8 ± 1.6**	-	-	18.6 ± 1.4
Alcohol (g) ^e	-	-	14.5 ± 3.2	-	-	14.4 ± 2.7
Energy density (kJ/g) ^f	-	4.9 ± 0.1	5.0 ± 0.1	-	4.7 ± 0.1	5.0 ± 0.2

^aPlus-minus values are means ± s.e.m.

^bThe targeted macronutrient composition according to the protocol.

^cDietary composition, intake and energy density as registered by the shop computer system during the initial three months of dietary intervention, calculated as mean daily values.

^dDietary composition, intake and energy density as registered by the shop computer system during the last three months of dietary intervention, calculated as mean daily values.

^eData from 7 d dietary records.

^fCalculated without drinks, on the basis of computer registrations.

** $P < 0.001$, * $P < 0.01$ as compared to the corresponding value in the high-protein group.

Protein sources were primarily dairy products and meat (beef, pork, poultry, lamb, fish and offal). Carbohydrate sources were primarily vegetables, fruits, breads, rice and pasta, but chocolate and simple sugars, in the form of sweets, were also available.

The subjects collected their food from the shop twice a week. Food items could be chosen freely within the dietary design and individual 'shoppings' were registered in a computer system designed specifically for the purpose, described in detail previously.¹⁷ At each shop visit, all food items were selected by the subject and bar code scanned by a dietician. This made it possible to monitor achievement of the scheduled macronutrient distribution and, if necessary, to modify the selected provisions. Uneaten food and left-overs, weighed to the nearest 1 g, were taken into account in the calculation of the energy content of the actual selection. We used bar codes, unique for each food item, and uncoded information about energy and macronutrient composition of the food item. The information used was provided by the database, Dankost[®] dietary assessment software (National Food agency of Denmark, Søborg, Denmark) or by the food manufacturers. The calculated energy content of the food was not known by the subjects.

Subjects were instructed thoroughly in how to prepare the foods, but they could also choose ready-prepared dishes. The aim was to fully control the dietary composition of the LP and HP subjects' food, and they were encouraged to collect all their foods, including 'empty calories' and caloric beverages (except for alcohol) from the grocery store. Any deviation from this principle should be recorded analogous to recording of food waste and leftovers.

For validation of the food registration method in the shop the protein intake was monitored by an objective biological markers, 24 h urinary nitrogen excretion (24-h UN) each month and the completeness of the urine sample was controlled.¹⁷ Urine was collected at baseline and at three months and six months of the study. A questionnaire investigation was performed after six months of dietary intervention, to assess the impact of the dietary intervention on the quality of life.¹⁸ The questionnaires were structured with mostly precoded response categories and a few open questions. The subjects were asked to rate numerous life quality variables relating to the dietary alteration/intervention. These included physical, social and psychological well-being, acceptability and palatability, and discomforts from different organ systems such as nausea, constipation, frequent bowel movements, abdominal pain, musculo-skeletal discomforts and tiredness.

The subjects were instructed not to change their physical activity pattern or smoking habits during the study. The subjects were also allowed to leave their alcohol habits unchanged, given that the intake was no

more than 20 g/d. This was controlled by self-reporting of alcohol intake at each visit in the shop.

Anthropometric measurements and body composition

Body weight was measured weekly, with subjects wearing light clothing, on a decimal scale (Seca model 707, Copenhagen, Denmark) in both intervention groups. Subjects in the control group were only weighed at baseline and after three and six months. Sagittal diameter and waist and hip circumferences were measured in all groups at baseline and at the end of the study. Body composition was determined by a dual energy X-ray absorptiometry (DEXA) scanning (Hologic 1000/W, Hologic, Inc., Waltham, MA, software version 5.61). Subjects wore only underwear and a cotton T-shirt during the scan. For quality control, spine phantoms were scanned daily.

Intra-abdominal adipose tissue was estimated from DEXA-scans and anthropometry by the equation given by Treuth *et al.*¹⁸

Intra-abdominal fat area (cm²) = $-208.2 + 4.62(\text{sagittal diameter, cm}) + 0.75(\text{age, y}) + 1.73(\text{waist, cm}) + 0.78(\text{trunk fat, \%})$.

Laboratory analyses

Venous blood samples were drawn from an antecubital vein after an overnight fast. After centrifugation, aliquots were stored at -20°C , prior to analysis. Plasma cholesterol, HDL-cholesterol and triglycerides were determined enzymatically with a Cobas Mira-Analyzer (Boehringer Mannheim GmbH, Mannheim, Germany) and plasma nonesterified fatty acids (NEFA) were determined by an enzymatic colorimetric method using a Wako NEFA C test kit (Wako Chemicals GmbH, Neuss, Germany).

Statistical analysis

Differences between groups in proportion of subjects achieving a certain weight loss, that is, ≥ 5 kg or ≥ 10 kg after three months and six months, respectively, were tested by a chi-squared test and the difference between intervention groups is expressed as odds-ratio (OR). Group differences in changes in body weight and blood lipids after 0, three months and six months of intervention, were analyzed by a mixed model for analysis of variance, with interaction between 'group' and 'time' included as fixed effects and 'subjects' included as random effect. One chi-squared test was performed to test for equal baseline levels of blood lipids in the three groups and another chi-squared test was made to test for the effect of time in the control group. Changes in body weight, composition and blood lipids are given as expected mean \pm s.e.m. (or 95% confidence intervals (CI)), with corresponding *P*-values estimated under the statistical

model. Differences between groups in intra-abdominal fat area were tested by one-way ANOVA. $P < 0.05$ was considered significant.

Life quality variables were tested with nonparametric statistics: Chi-squared tests for differences between groups in yes/no questions and Kruskal-Wallis one-way analysis of variance by ranks for group differences with respect to multiple choice questions (four choices). To account for the multiple comparisons the significance level was set as $P < 0.01$. Statistics Analysis Package, SAS[®] 6.10 (SAS Institute, Cary, NC, USA) and SigmaStat[®] 1.0 (Jandel Scientific GmbH, Erkrath, Germany) were used in the statistical analysis.

Results

Compliance and acceptability

Two subjects dropped out of each intervention group, due to change of address or non-compliance, and one subject was excluded from the control group, due to elective surgery. A total of 60 subjects completed the trial (92%), 23 in each intervention group and 14 in the control group.

Table 2 shows the average daily macronutrient intake, energy intake and energy density in the six intervention months, separated into two three month periods. The achievement of the targeted differences in protein intakes in the intervention groups was supported by the use of 24 h UN as an objective marker of protein intake. At baseline, dietary protein intake calculated from 24 h UN was similar in the three groups and did not change in the control group over the period. However, in the HP group, protein intake increased from a baseline value of 91.4 g/d (81.0–101.82) to a six months intervention average of 107.8 g/d (102.2–112.1 g/d) ($P < 0.05$), while correspondingly, a decrease from 91.1 g/d (82.5–99.7 g/d) to 70.4 g/d (64.8–76.0 g/d) ($P < 0.05$) was observed in the HC group (Group difference: $P < 0.0002$). Dietary fiber intake changed from 17.8 g/d at baseline to 18.6 g/d in the intervention period in the HP group, whereas dietary fiber intake correspondingly changed in the (HC) group from 16.1 g/d to 22.8 g/d. Hence, the increase in daily dietary fiber content was 7 g lower in the HP group than in the HC group ($P < 0.05$). Alcohol intake at baseline was 17.7 ± 3.3 g/d in the (HC) group, 15.0 ± 2.2 g/d in the HP group and 11.1 ± 3.0 g/d in the C group (NS) and did not change during dietary intervention.

There were no significant group differences in the questionnaire responses in any of the measures of appetite or palatability. None of the subjects in either group responded that they most of the time felt hungry soon after a meal or felt a bit hungry during the whole day. Both in the HP and in the (HC) group, only 4% of the subjects did not agree that low-fat food is as

attractive and tastes as good as 'normal' food. To assess if differences in weight loss may have been influenced by palatability and acceptability, we analyzed the weight loss in sub-groups of both intervention groups and found no evidence to support the contention that differences in acceptability affected weight loss. In addition, no differences were found with respect to discomforts from different organ systems, such as tiredness/sleeping problems, shortness of breath, abdominal symptoms (rumbling/distended stomach, constipation/frequent bowel movements, abdominal pain after meal), general edema or discomforts in muscles or joints. The subjects generally considered the dietary alteration to be easier to comply with than they had expected.¹⁷

Body weight and composition

Pre-treatment body weights were similar in all three groups and no significant change occurred in the control group (Figure 1) Weight loss after three months was greater in the HP group than in the HC group: 7.5 kg vs 5.0 kg (difference 2.5 kg (0.6–4.2 kg) $P < 0.02$). After six months, weight loss was 5.0 kg (3.6–6.4 kg) in the HC group and 8.7 kg (7.3–11.9 kg) in the HP group (difference 3.7 kg (1.3–6.2 kg) $P = 0.0002$). After three months of dietary intervention, more subjects had lost ≥ 5 kg body weight in the HP group (19/24 (79%)) than in the HC group (12/23 (52%)) ($P < 0.05$) (Figure 2). After six months, more subjects had lost ≥ 10 kg body weight in the HP group (8/23 $\approx 35\%$) than in the HC group (2/23 $\approx 9\%$) (OR 5.6 (1.1–30.2) $P < 0.001$). At three months, fat loss was 3.8 kg (2.6–5.0 kg) in the HC group and 5.8 kg (4.6–7.0 kg) in the HP group (difference: 2.0 kg (0.4–3.7) $P < 0.02$). After six months, fat loss was 4.3 kg (3.1–

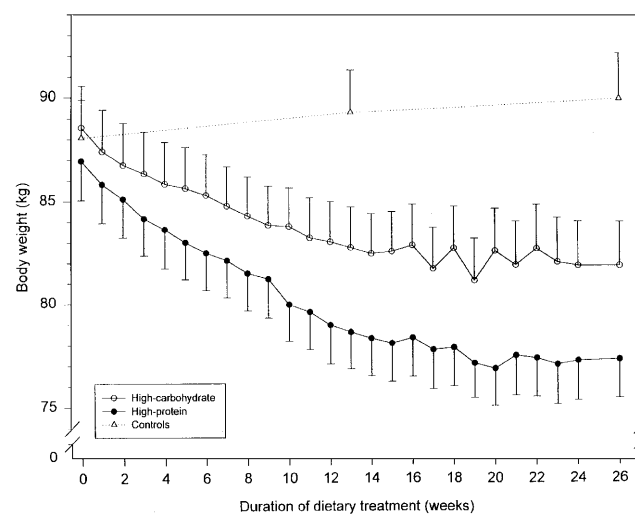


Figure 1 Changes in body weight in overweight and obese subjects randomized to *ad libitum* fat-reduced diets: high-carbohydrate (protein 12% of total energy; $n = 25$), high-protein (protein 25% of total energy; $n = 25$) or to a control group (no intervention; $n = 15$). Values are means \pm s.e.m. There were no differences in baseline values of body weight. Group \times time interaction: $P < 0.0001$.

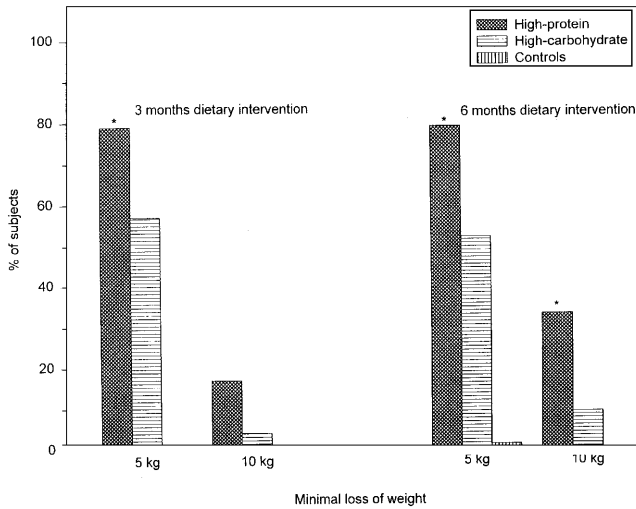


Figure 2 Proportion of subjects having lost >5 kg or 10 kg body weight after three months and six months of dietary intervention. Comparisons between groups were made by a chi-squared test. * $P < 0.05$ for the comparison of difference with the high-carbohydrate group.

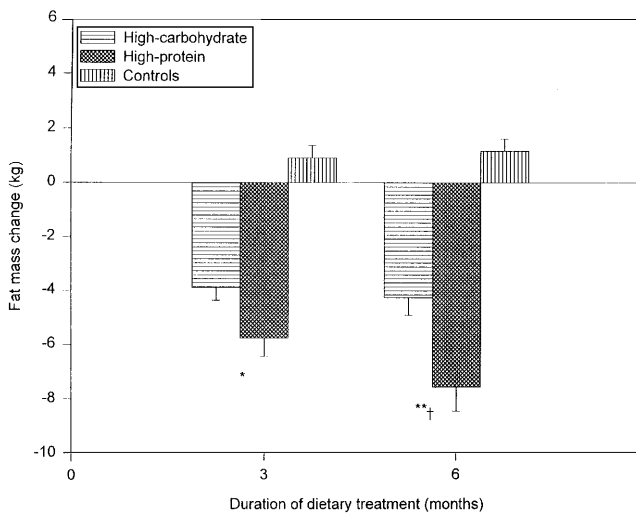


Figure 3 Changes from baseline in body fat mass in overweight and obese subjects randomized to two *ad libitum* fat-reduced diets, either a high-carbohydrate (protein 12% of total energy; $n = 25$), or high-protein (protein 25% of total energy; $n = 25$) or a control group (no intervention; $n = 15$). * $P < 0.02$ for the comparison between the two intervention groups. ** $P < 0.0001$ for the comparison between the two intervention groups. Values are means \pm s.e.m.

5.5 kg) in the HC group and 7.6 kg (6.2–9.0 kg) in the HP group (difference: 3.3 kg (1.1–5.7) $P < 0.0001$) (Figure 3). Intra-abdominal adipose tissue decreased by 33.0 cm² in the HP group and by 16.8 cm² in the HC group ($P < 0.0001$), whereas it increased in the control group by 15.2 cm², differing from both intervention groups ($P < 0.0001$) (Figure 4).

Blood lipids

No group differences in baseline values of blood lipids were found, and no significant changes were

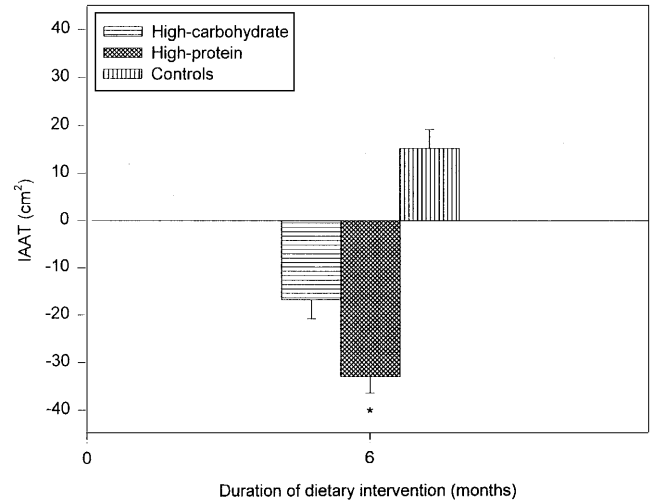


Figure 4 Changes in intra-abdominal adipose tissue (IAAT) estimated from dual energy X-ray absorptiometry (DEXA) scans and anthropometry by the equation given by Treuth *et al*¹⁹: IAAT (cm²) = $-208.2 + 4.62(\text{sagittal diameter, cm}) + 0.75(\text{age, y}) + 1.73(\text{waist, cm}) + 0.78(\text{trunk fat, \%})$. * $P < 0.0001$ for the comparison of changes between high-protein group and the two others. Values are means \pm s.e.m.

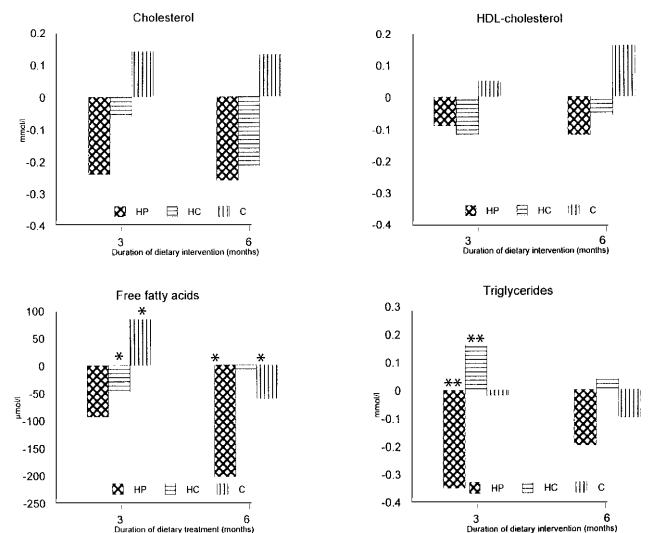


Figure 5 Changes in blood lipids from base-line values in overweight and obese subjects randomized to *ad libitum* fat-reduced diets: high-carbohydrate (HC: protein 12% of total energy; $n = 25$), high-protein (HP: protein 25% of total energy; $n = 25$) or to a control group (C: no intervention; $n = 15$). * $P < 0.05$ and ** $P < 0.01$ for the comparison of change from baseline and for difference between intervention groups and control group. P HDL = high density lipoproteins.

seen in the control group during the six months of intervention (Figure 5). Total cholesterol and HDL-cholesterol decreased in both the HC and HP groups, with no group differences (Figure 5). Plasma free fatty acids decreased by approx 30% after six months in the HP group, while they were unchanged in the HC group ($P < 0.05$). In contrast to the increase in plasma triglycerides after three months in the HC group, a decrease by 0.37 mmol/l (0.15–0.59

mmol/l) was found in the HP group ($P=0.001$). After six months, no significant group differences remained (Figure 5).

Discussion

The present study shows that two diets with a dietary fat content reduced to slightly below 30 E%, cause clinically relevant weight losses during *ad libitum* consumption, compared to a control diet with a fat content of about 40 E%. This study further shows that the HP diet induces a larger weight loss than the HC diet. After six months intervention, the HP diet induced a 3.7 kg (1.3–6.2 kg) larger weight loss, which was mainly due to a reduction in body fat mass. Moreover, in the HP group, 35% of the subjects lost >10 kg, whereas only 9% in the HC group achieved this goal (OR 5.6 (1.1–30.2)). Both fat-reduced diets decreased the intra-abdominal fat stores, but the decrease in the HP group was two-fold greater than in the HC group.

The weight loss on the two *ad libitum* fat-reduced diets was markedly higher than those previously reported in normal weight subjects^{2,19–21} and slightly above the weight losses reported in overweight and obese subjects.²² There are two likely reasons for this. Firstly, the small weight losses observed in some of the low-fat intervention trials can partly be attributed to low adherence to the low-fat diet composition.²³ Most trials using the *ad libitum* low-fat principle reported so far have instructed the subjects how to make the dietary changes, but have not ensured that the subjects actually consumed a diet with the prescribed composition. Adherence to the diet, as assessed by recovered label in expired air in subjects consuming meals enriched with ¹³C-glucose under free-living conditions, has been shown to be positively related to weight loss.²³ In contrast, our shop system, where all foods during six months were free of charge, allowed a more strict control of macronutrient composition, while allowing the subjects freedom to select appropriate food items in the shop. Thus the compliance to the two diets as assessed by UN excretion was high and 92% of the subjects completed the 6 months of treatment.

We find it very likely that the provision of free food during the intervention trial played a role and that the high compliance was also economically motivated. However, in a recent study, obese subjects participated in four different behavioural weight control programs that differed only with respect to the way the food was provided to the subjects: no food provision, meal plans, provision of food (paid for by the subjects) or food provided free.²⁴ Weight losses were similar in the three latter groups, but significantly different from that of the group that received the behavioural program alone. Therefore, it

is unlikely that the provision of free food enhanced weight loss.

Dietary composition was not monitored as closely in the C-group as in the intervention groups, since food was not provided to the control group from the study shop. However, as we chose experimental conditions that were very natural, we do not consider this will invalidate the status of the C-group as a reference group.

The adherence to the dietary compositions of the two intervention groups was high, as assessed by the excretion of 24 h UN, which was used as a marker of protein intake.²⁵ The agreement between the dietary protein intake, as estimated by the shop computer and the UN excretion was very high ($r=0.84$, $P<0.0001$), and the achievement of a two-fold difference in UN excretion between the HP and HC groups supports that statement that the targeted macronutrient compositions of the intervention diets were actually reached.

We find it very likely that some unintended voluntary energy restriction occurred in both intervention groups, due to the subjects being highly motivated to lose weight. This may have enhanced the weight loss in the two intervention groups, but is unlikely to have influenced the weight loss difference between the HP and HC groups.

The mechanisms responsible for the larger weight loss caused by the HP diet than by the HC diet might be due to both a reduced energy intake and a greater thermogenic effect of protein.

We found the reported energy intake during the intervention was lower in the HP group than in the HC group by 2 MJ (0.94–3.05 MJ, $P<0.001$), which is more than sufficient to explain the larger weight loss in the HP group. Rolls *et al*¹² found that high protein and high starch foods produced greater satiety than high fat, high sucrose or mixed content foods. The lower energy intake in the HP group is in accordance with most meal test studies, showing a higher satiating effect of protein than carbohydrate, when compared joule for joule.^{7,9,12,15,26} A high protein intake also seems to be able suppress the following day's energy intake more than an isoenergetic amount of carbohydrate. Stubbs *et al*²⁷ studied the relationship between carbohydrate and protein balances and the next day's spontaneous energy intake during a seven-day stay in a respiration chamber, and found that every megajoule of increased protein stores on day 1 produced a reduction in energy intake on the subsequent day amounting to 2.1 MJ. For a similar increase in carbohydrate stores, the reduction in energy intake was only 0.4 MJ. Thus the more pronounced effect of protein than of carbohydrate in inhibition of energy intake found in short-term studies is confirmed by the present study and shown to be maintained for at least six months.

Palatability of the diet has been shown to be an important determinant of energy intake,²⁸ and the lower energy intake in the HP group than in the HC group could therefore have been due to a lower

palatability of the HP diet. However, we find this explanation unlikely since no differences were found in palatability between the intervention groups after six months. Moreover we found that in the questionnaire on appetite the subjects' responses to questions were independent of their achieved weight loss. Moreover, no differences with respect to physical well-being were found. The subjects generally considered the dietary alteration to be easier to comply with than they had expected (Holm L, SKov AR, Astrup A. unpublished results). The results therefore suggest that the lower energy intake in the HP diet was due to a higher satiating effect of protein than of carbohydrate.

In addition to the effect on energy intake, the HP diet may increase energy expenditure more than the HC diet, as the post-prandial thermogenesis of protein amounts to 30% of its energy content, whereas that of carbohydrate is only 4–8%.^{29,30} On a daily basis, the difference in protein and carbohydrate intakes between the HP group and the HC group can be estimated to produce a difference of about 300 kJ/d, which is only about 15% of the observed difference in energy balance. The greater weight loss caused by the HP diet than the HC diet can therefore mainly be attributed to a reduction in energy intake.

The mechanisms responsible for the high satiating effect of protein are not known. The energy density of foods is an important determinant of spontaneous energy intake and seems to be responsible for the higher energy intakes observed on high-fat than on low-fat diets.²⁷ However, differences in energy density are unlikely to be involved because the HP diet and the HC diet had similar energy densities of 4.7–5.0 kJ/g. This is in agreement with the finding that a high-protein meal suppressed hunger to a greater extent than two isoenergetic high-fat and high-carbohydrate meals with the same energy density.¹⁵ The change in daily dietary fiber content was expectedly lower in the HP group than in HC group, by 7 g. This difference cannot explain the larger weight loss in the HP group, as the higher fiber intake would rather have contributed to a larger weight loss in the HC group. Possible differences in fat quality may have played a role, but there are no published human data to support that differences in fat types influence the satiating effect of the diet. Consequently, the inhibition of energy intake caused by the HP diet may be due to mechanisms other than the energy density, for example, release of gut peptides, liver metabolism and a direct central effect of certain amino acids.³¹

Obesity is an important risk factor of cardiovascular disease (CVD) and abdominal obesity in particular is strongly associated with an adverse lipid profile, ischaemic heart disease, stroke and premature death.³² Overwhelming epidemiological data have demonstrated a close association between obesity and coronary heart disease (CHD) mortality,^{33,34} which is attributed partly to its effects on plasma lipid metabolism. The dyslipidaemic profile associated with fatness and especially with excessive

intra-abdominal fat deposition is characterised by increased total cholesterol, low density lipoprotein (LDL)-cholesterol, triglyceride and free fatty acid levels, and decreased HDL levels.³⁵ Hence, the larger reduction in the intra-abdominal fat depots in the HP group, may be expected to reduce the risk of these comorbidities. Although a beneficial effect of weight loss on plasma lipids was found in both intervention groups, more favourable improvements were seen in the HP group (Figure 5). There was a slight transient increase in plasma triglycerides after three months in the HC group, whereas a reduction was seen in the HP group. The increase in plasma triglycerides has been reported to occur on isoenergetic low-fat, high carbohydrate diets,^{22,36} but not under *ad libitum* conditions where weight loss is allowed to occur.²² Moreover, plasma NEFA were reduced only in the HP group. The greater improvement in that cardiovascular risk profile after six months on the HP diet may be due to a combination of the greater fat loss, reduction in intra-abdominal fat and to the diet composition *per se*. Intervention studies comparing isoenergetic low-fat diets with either high or low ratios of protein to carbohydrate have demonstrated that, without changes in body weight, the exchange of protein for carbohydrate reduced LDL-cholesterol and triglycerides, and increased HDL-cholesterol in hypercholesterolaemic subjects.^{37,38} The more favourable effects of the HP diet may be only partially attributable to the larger reduction in body fat.

We did not measure blood pressure in the present study, but it is unlikely that dietary protein increases blood pressure.³⁹ Furthermore, weight loss has consistently been associated with clinically relevant reduction in both systolic and diastolic blood pressures.⁴⁰ A recent intervention study on moderately hypertensive patients demonstrated that a fat-reduced diet, rich in fruits and vegetables and low-fat dairy products, providing 18% of energy from protein, reduced systolic and diastolic blood pressure by 5.5 mm Hg and 3.0 mm Hg more than a control diet.⁴¹ This intervention resulted in a weight loss of <0.5 kg, so there is no reason to believe that an increase in dietary protein can offset the beneficial effect of the weight loss on blood pressure in obese subjects.

A protein-rich diet may have other health implications and its effects on osteoporosis, kidney function and colonic cancers are still a matter for debate. We failed to detect any detrimental effect of the HP diet on bone mineral density and kidney size and glomerular function (data not shown), but more studies are needed to elucidate the contribution of high-protein diets to the development of these disorders. The use of fat-reduced, high-protein diets in the treatment of obesity seems justified because the health benefits of a weight loss of the magnitude observed in the present study is associated with a marked improvement in risk factors for non-insulin dependent diabetes and CVD,⁴⁰

and possibly with a reduction in mortality.⁴² The uncertainty about possible adverse effects means that the beneficial effects observed in this treatment program for obesity cannot yet be extrapolated to the recommendation of a high-protein diet to the general population.

Conclusion

The study shows that replacement of some dietary carbohydrate by protein in *ad libitum* fat-reduced diets, for treatment of obesity, improves mean weight loss and increases the proportion of subjects achieving a clinically relevant weight loss. Slight improvements in blood lipids were also observed. More freedom to choose between protein-rich and complex carbohydrate-rich foods may allow obese subjects to eat more lean meat and dairy products and hence improve adherence to low-fat diets during weight reduction programs.

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