Protein leverage affects energy intake of high-protein diets in humans[1–3]

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ABSTRACT

Background: The protein leverage hypothesis requires specific evidence that protein intake is regulated more strongly than energy intake.

Objective: The objective was to determine ad libitum energy intake, body weight changes, and appetite profile in response to protein–to–carbohydrate + fat ratio over 12 consecutive days and in relation to age, sex, BMI, and type of protein.

Design: A 12-d randomized crossover study was performed in 40 men and 39 women [mean ± SD age: 34.0 ± 17.6 y; BMI (in kg/m²): 23.7 ± 3.4] with the use of diets containing 5%, 15%, and 30% of energy from protein from a milk or plant source.

Results: Protein-content effects did not differ by age, sex, BMI, or type of protein. Total energy intake was significantly lower in the high-protein (7.21 ± 3.08 MJ/d) condition than in the low-protein (9.33 ± 3.52 MJ/d) and normal-protein (9.62 ± 3.51 MJ/d) conditions ($P = 0.001$), which was predominantly the result of a lower energy intake from meals ($P = 0.001$). Protein intake varied directly according to the amount of protein in the diet ($P = 0.001$). The AUC of visual analog scale appetite ratings did not differ significantly, yet fluctuations in hunger ($P = 0.019$) and desire to eat ($P = 0.026$) over the day were attenuated in the high-protein condition compared with the normal-protein condition.

Conclusions: We found evidence to support the protein leverage hypothesis in that individuals underate relative to energy balance from diets containing a higher protein–to–carbohydrate + fat ratio. No evidence for protein leverage effects from diets containing a lower ratio of protein to carbohydrate + fat was obtained. It remains to be shown whether a relatively low protein intake would cause overeating or would be the effect of overeating of carbohydrate and fat. The study was registered at clinicaltrials.gov as NCT01320189.   *Am J Clin Nutr* 2013;97:86–93.

INTRODUCTION

Obesity is reaching epidemic proportions in a growing number of countries and is associated with several related health problems such as type 2 diabetes and cardiovascular diseases (1, 2). The development of obesity results from a chronic energy imbalance, with energy intake exceeding energy expenditure. After the focus of weight-loss strategies regarding food intake regulation on changes in carbohydrate and fat consumption, the role of protein has recently received considerable attention (2). According to the protein leverage hypothesis developed by Simpson and Raubenheimer (3), even small changes in the dietary percentage of protein may have substantial effects on energy intake. Early studies in insects (4), a reanalysis of data from studies in chickens (5) and rats (6, 7), laboratory studies in rodents (8, 9), and a more recent study in free-living monkeys (10) suggest that protein intake is maintained at a more constant level than is that of carbohydrate and fat. However, it is still unknown whether dietary protein exerts leverage to drive energy intake in humans or whether overeating predominantly occurs with carbohydrate and fat, thereby automatically decreasing the relative proportion of protein in the diet (11, 12), whereas overeating of protein is prevented by its highly satiating effect (13). Weigle et al (14) showed that a high-protein diet (30% of energy from protein) compared with a normal-protein diet produced a sustained decrease in ad libitum energy intake and related weight loss. Griffioen-Roose et al (15) showed in a 4-d study that subjects increased their protein intake in a compensatory way during ad libitum feeding after a low-protein diet (5% of energy from protein). Also, Gosby et al (16) showed from a short-term, in-house dietary manipulation study that lean subjects consumed on average 10% more energy on a moderate-low protein diet (10% of energy from protein) compared with a normal-protein diet. Proposed mechanisms for the inverse relation between dietary protein intake and energy intake may be increased satiety due to a relatively high protein content (17, 18). Several studies showed that, in energy balance, high-protein diets reduced postprandial hunger and increased postprandial satiety (14, 19, 20).

The protein leverage hypothesis requires specific evidence on cause and effect—ie, that protein intake is regulated more strongly than energy intake or that, depending on energy intake, with absolute protein intake being relatively stable (in g/kg body weight) (1), relative protein intake automatically varies with energy intake. Therefore, we carried out a controlled dietary intervention study to determine ad libitum energy intake, body weight changes, and appetite profile in response to protein–to–

carbohydrate + fat ratio over 12 consecutive days and in relation to age, sex, BMI, and type of protein. Following the analyses by Stock (21) and by Simpson and Raubenheimer (3), isoenergetic diets with a large range in protein concentrations (5%, 15%, and 30% of energy from protein) were provided whereby protein was exchanged for carbohydrate. Proteins from varying sources, namely milk protein (whey protein with α-lactalbumin) and plant protein (soy protein), were used, because they may differ in their effects on energy intake or in possible adaptations in appetite profile (22–24).

SUBJECTS AND METHODS

The Medical Ethical Committee of the Maastricht University approved the study, and all participants gave written informed consent. The study was registered at clinicaltrials.gov as NCT01320189.

Study subjects

On the basis of the study by Weigle et al (14), power analysis showed that with an α of 0.0167 (significance level for each test: $\alpha = 0.05$, taking into account the Bonferroni correction for multiple testing) and a β of 0.10, $\geq$81 subjects were needed. Ninety subjects were recruited by advertisements in local newspapers and on notice boards at the university; 9 of these subjects dropped out due to lack of time. Two subjects were excluded from the data analysis because of noncompliance, as shown by the urinary nitrogen biomarker. Overall, 79 subjects (40 men and 39 women) were included in the final data analysis. To cover a wide range of BMIs, subjects were normal weight, overweight, or obese [BMI range (in kg/m^2): 18.2–33.9]; to include the life span to a large extent, they were between 18 and 70 y old. BMI and age were equally divided between the male and female groups. Subjects underwent a screening that included anthropometric measurements and the completion of questionnaires.

Body weight was measured by using a digital balance, and height was measured by using a wall-mounted stadiometer. BMI was calculated as body weight (kg) divided by height (m) squared. Waist and hip circumferences were determined with subjects in a standing position by using a tape measure. Waist circumference was measured at the smallest circumference between the rib cage and iliac crest, and hip circumference was measured at the level of the spina iliaca anterior superior. Accordingly, waist-to-hip ratio was calculated by dividing waist by hip circumference.

Subjects completed questionnaires related to health, smoking behavior, use of medication, alcohol consumption, physical activity, eating behavior, anxiety, and liking of the study meals. Subjects were nonsmoking, not using more than a moderate amount of alcohol (>10 drinks/wk), weight stable (body weight change of <3 kg during the last 6 mo and no planned weight change during the study period), and not using medications or supplements except for oral contraceptives in women.

The validated Dutch translation of the Baecke Activity Questionnaire was used to measure habitual physical activity (25). This questionnaire consists of 3 indexes of physical activity, namely work, sport, and leisure time (26). Eating behavior was analyzed by using a validated Dutch translation of the Three-Factor Eating Questionnaire (TFEQ), which measures the 3 factors involved in eating behavior, namely "cognitive restraint of eating," "disinhibition of restraint," and "hunger" (13, 27). On the basis of the median of the TFEQ scores in the population from the south of the Netherlands, dietary restraint was defined by TFEQ dietary restraint scores $\geq$9. Dietary restraint scores <9 indicated dietary unrestraint (28). High disinhibition was defined by TFEQ disinhibition scores $\geq$5, and scores <5 represented low disinhibition. Trait anxiety level was scored by means of the State-Trait Anxiety Inventory questionnaire (29).

Small portions of the study meals were served to test the palatability and acceptability of the study meals, and visual analog scales (VASs) were used to assess liking. These 100-mm scales were anchored with "not at all" at one end and "extremely" at the other end. Subjects had to make a single vertical mark at the appropriate point between the 2 anchors on each scale. Only subjects who rated the taste of the meals as sufficient (VAS score for liking: $\geq$50 mm) were included in the study.

Study design

The study used a single-blind crossover design with 3 randomly sequenced experimental conditions that differed in relative protein content of the provided meals. Subjects visited the university to consume breakfast, lunch, and dinner ad libitum in the research restaurant for 12 consecutive days in each condition. The reason for the use of the 12-d time period is the phenomenon that energy balance is regulated over a week and that at least this period of time is needed to show a possible effect on body weight (30). Subsequent test sessions started 8 wk after the start of the preceding one and included a washout period of $\sim$6 wk to prevent possible treatment-induced effects and to take possible effects of menstrual cycle phase on energy intake of women into account (31, 32). Two different protein isolates were used: whey protein with α-lactalbumin (Hiprotal Whey Protein Alpha and Domo; FrieslandCampina) and soy protein (SUPRO Soy Protein Isolate; Solae LLC). Subjects were randomly assigned to either the whey-protein group or the soy-protein group.

Diet composition

The 3 applied conditions differed in relative protein content of the meals: 5% of energy, 15% of energy, and 30% of energy from protein (**Table 1**). Because the intake and storage for carbohydrate are regulated over a shorter period of time than for fat (33) and to prevent bias caused by the possible effects of energy density on energy intake, the fat content between meals and between conditions was maintained at a constant proportion (35% of energy from fat) (33). Another reason to keep the proportion of fat relatively constant is to prevent subjects from overeating on a high-fat diet, which is usually caused by taste and energy density (34, 35). Thus, protein was completely exchanged by carbohydrate. The resulting protein–to–carbohydrate + fat ratios (% of energy) of the diets were 5:95 (low protein), 15:85 (normal protein), and 30:70 (high protein). All meals (breakfast, lunch, and dinner) within each condition had the same macronutrient composition. Moreover, all food items and the energy density, weight, and volume of the meals were the same between conditions (Table 1).

TABLE 1
Composition of the study diets: whey and soy protein combined

	Percentage of energy from protein		
	5%	15%	30%
Breakfast			
Protein (% of energy)	5.05 ± 0.03[1]	15.01 ± 0.03	30.00 ± 0.04
Carbohydrate (% of energy)	59.93 ± 0.09	49.90 ± 0.20	34.94 ± 0.22
Fat (% of energy)	35.01 ± 0.06	35.09 ± 0.18	35.06 ± 0.18
Protein:carbohydrate + fat (% of energy)	$5:95$[2]	$15:85$	$30:70$
Energy density (kJ/g)	7.21 ± 0.33	7.23 ± 0.04	7.18 ± 0.10
Lunch			
Protein (% of energy)	5.15 ± 0.20	14.95 ± 0.03	30.00 ± 0.02
Carbohydrate (% of energy)	59.80 ± 0.21	50.00 ± 0.03	35.04 ± 0.05
Fat (% of energy)	35.06 ± 0.01	35.04 ± 0.00	34.96 ± 0.05
Protein:carbohydrate + fat (% of energy)	$5:95$	$15:85$	$30:70$
Energy density (kJ/g)	8.69 ± 0.34	8.72 ± 0.20	8.69 ± 0.27
Dinner			
Protein (% of energy)	5.20 ± 0.13	14.99 ± 0.01	30.06 ± 0.08
Carbohydrate (% of energy)	59.82 ± 0.03	49.97 ± 0.01	35.65 ± 0.15
Fat (% of energy)	34.98 ± 0.12	35.04 ± 0.01	35.04 ± 0.07
Protein:carbohydrate + fat (% of energy)	$5:95$	$15:85$	$30:70$
Energy density (kJ/g)	5.49 ± 0.07	5.49 ± 0.07	5.46 ± 0.10

[1] Mean ± SD (all such values).
[2] Mean ratio (all such values).

The energy content of the meals was calculated from the nutrition information on the food items or from the standard Dutch NEVO food-composition table. Breakfast was composed of breakfast cereals (Kellogg's Nederland) moistened with orange juice (not milk). Lunch consisted of bread with vegetable salad, and dinner was composed of pasta with tomato sauce. Initially, all meals were low in protein content. Whey and soy protein isolates were used to exchange carbohydrate with protein in the normal- and high-protein conditions to reach the proportions of protein according to the study design.

Food was served as ready-to-eat meals to prevent selective consumption of food items within meals. Three different variants for breakfast, lunch, and dinner were offered in each condition to decrease the possible effects of boredom on energy intake and appetite profile. Meals were provided ad libitum for 30 min, and subjects were instructed to eat until they felt comfortably full. A fixed amount of 300 mL of water per subject was offered with each meal.

After each meal, snack items were provided in individual boxes for ad libitum consumption at home. All snack items were very low in protein content ($\sim$5% of energy from protein) to reduce interference with the protein intake during the test meals. The provided snack items were the same after each meal and in each condition. Subjects were instructed to bring all leftovers back during their visit to the research restaurant for the subsequent meal. Subjects were allowed to drink water, tea, and coffee without added milk or sugar ad libitum between the meals, but no other foods or beverages were allowed to be consumed.

Biomarker of protein intake

Nitrogen excretion, measured from 24-h urine collections at baseline (day 0) and at days 5 and 11, was used as biomarker for protein intake. Urine was collected in 2-L urine bottles with 10 mL of diluted hydrochloric acid (4 mmol/L) added to prevent nitrogen loss through evaporation. Collection started after the first voiding in the morning on the collection days at 0800 and lasted until and with the first voiding on the next day at 0800. The total volume of the 24-h urine was recorded. Urine was gently mixed, and samples were taken and frozen at -20°C until analysis. Nitrogen concentrations were measured with a nitrogen analyzer (CHN-O-Rapid; Hereaus). Total nitrogen output was calculated as 24-h urinary nitrogen plus 10% to account for normal losses via feces and other losses.

Energy and macronutrient intake

Each meal was weighed to the nearest gram before it was provided to the subjects. Leftovers were reweighed, after which energy and macronutrient intakes were calculated per subject. The provided snack items were also recorded, and the leftovers that had to be brought back were weighed to determine the energy and macronutrient intake from the snacks per subject. Mean total energy intake was calculated as the sum of energy intake from meals and the reported mean energy intake from snacks. Individual daily energy requirements were calculated as the resting metabolic rate according to the formula of Harris and Benedict (36) times the physical activity level based on the Baecke Activity Questionnaire (26). Energy balance was determined as the difference between energy requirement and energy intake.

Appetite profile

Appetite profile was measured by means of the VAS. These 100-mm scales were anchored with "not at all" at one end and "extremely" at the other end and combined with questions on feelings of hunger, satiety, fullness, thirst, and desire to eat. Before and after breakfast, lunch, and dinner, subjects had to complete the VAS.

Body weight

Body weight was measured in the fasted state in the morning of days 1, 6, and 12.

Statistical analysis

SPSS version 19 for Macintosh OS X (SPSS Inc) was used to perform statistical analyses. Data are presented as means ± SDs.

Differences in subject characteristics between sexes and protein groups (whey compared with soy) were evaluated by using factorial ANOVA. The AUC over the day was calculated for VASs for hunger, fullness, satiety, and desire to eat by using the trapezoidal method. Amplitude scores were calculated for all appetite measures to assess fluctuations over the day. The scores were calculated by using VASs, by subtracting the minimum score from the maximum score. Factorial ANOVAs with repeated measures were used to test whether nitrogen excretion and VAS ratings changed over time within conditions (low protein, normal protein, high protein) and to test whether nitrogen excretion, VAS ratings, amplitude scores, or the effects of sex and protein group on differences in energy intake, macronutrient intake, and body weight change differed in response to protein–to–carbohydrate + fat ratio. Bonferroni correction for multiple comparisons and post hoc analyses were applied with all ANOVA tests.

Simple linear regression analyses were used to determine the contribution of age and BMI to the prediction of energy intake and body weight changes, in response to protein–to–carbohydrate + fat ratio. Differences were regarded as significant if $P < 0.05$.

RESULTS

Subject characteristics

Subject characteristics are summarized in **Table 2**. No significant differences were observed between the 2 protein groups at baseline with regard to anthropometric measurements, physical activity, eating behavior, and anxiety. Age and BMI ranges were comparable between both protein groups.

Biomarker of protein intake

Baseline nitrogen excretion did not differ significantly between conditions (9.7 ± 3.8 g/d), which indicates that subjects had a normal protein intake at the start of each test period. Nitrogen excretion significantly decreased in the low-protein condition to 5.0 ± 1.6 g/d ($P = 0.001$) and increased in the high-protein condition to 13.7 ± 6.1 g/d ($P = 0.001$) compared with baseline. In the normal-protein condition, nitrogen excretion did not change significantly throughout the test period (8.3 ± 3.4 g/d). Nitrogen excretion differed significantly between the conditions, thereby confirming significant differences in protein intake between conditions ($P = 0.001$). In each condition, no significant differences between the urine collection days 5 and 11 were observed, which indicated stable protein intakes within the conditions over the 12-d test periods.

Energy and macronutrient intake

Age, sex, BMI, and type of protein did not have an effect on differences in energy and macronutrient intake between conditions. Mean protein intake from meals over 12 d varied directly

TABLE 2
Subject characteristics in the whey- and soy-protein groups[1]

	Whey-protein group	Soy-protein group
No. of subjects (M/F)	39 (16/23)	40 (24/16)
Age (y)	34.5 ± 16.8[2]	33.6 ± 18.6
Age range (y)	18–70	18–69
Height (cm)	171.7 ± 10.6	174.0 ± 9.6
Weight (kg)	68.6 ± 13.8	73.6 ± 12.3
BMI (kg/m^2)	23.1 ± 3.5	24.3 ± 3.2
BMI range (kg/m^2)	18.2–33.9	18.1–33.4
Waist circumference (cm)	77.6 ± 11.6	80.9 ± 10.5
Hip circumference (cm)	100.9 ± 6.2	103.2 ± 7.0
WHR	0.77 ± 0.08	0.78 ± 0.08
BMR (MJ/d)	6.5 ± 1.2	6.9 ± 1.0
PAL	1.76 ± 0.15	1.75 ± 0.14
DER (MJ/d)	11.4 ± 2.0	12.1 ± 2.1
Baecke work score	2.4 ± 0.5	2.4 ± 0.6
Baecke sport score	3.3 ± 1.3	3.4 ± 1,1
Baecke leisure-time score	3.4 ± 0.4	3.2 ± 0.6
Baecke total score	9.1 ± 1.4	9.0 ± 1.4
TFEQ dietary restraint score	6.0 ± 4.0	4.8 ± 2.9
TFEQ disinhibition score	3.8 ± 1.9	4.0 ± 1.9
TFEQ hunger score	3.8 ± 3.0	4.0 ± 3.2
STAI score	31.8 ± 8.0	31.3 ± 7.4

[1] There were no significant differences between the protein groups (factorial ANOVA). BMR, basal metabolic rate; DER, daily energy requirement; PAL, physical activity level; STAI, State-Trait Anxiety Inventory; TFEQ, Three-Factor Eating Questionnaire; WHR, waist-to-hip ratio.
[2] Mean ± SD (all such values).

with the amount of protein in the diet ($P = 0.001$; **Table 3**). Mean carbohydrate intake was significantly different between conditions ($P = 0.001$; Table 3), with the highest intake in the low-protein condition and the lowest intake in the high-protein condition.

An increase in protein–to–carbohydrate + fat ratio of meals resulted in significant undereating relative to energy balance ($P = 0.001$), whereas a decrease in protein–to–carbohydrate + fat ratio did not significantly affect energy intake (Table 3; **Figure 1**). As a result of the lower mean energy intake from meals, fat intake was significantly lower in the high-protein condition than in the low-protein ($P = 0.001$) and normal-protein ($P = 0.001$) conditions (Table 3).

The lower mean energy intake from meals in the high-protein condition, compared with the low- and normal-protein conditions, respectively, was also present as a lower mean energy intake during all separate meals: breakfast ($P = 0.001$, $P = 0.004$), lunch ($P = 0.001$, $P = 0.001$), and dinner ($P = 0.013$, $P = 0.001$). Significant differences between the low- and normal-protein conditions were observed in mean energy intake during breakfast ($P = 0.018$) and dinner ($P = 0.047$), with a higher mean energy intake during breakfast and a lower mean energy intake during dinner in the low-protein condition than in the normal-protein condition.

In each condition, individual daily energy intake from meals was similar throughout the days (skewness values of 0.54, 0.66, and 0.35 and kurtosis values of −0.57, −0.26, and −0.60 for the low-, normal-, and high-protein conditions, respectively).

Energy intake from snacks did not significantly differ between conditions (Table 3).

Appetite profile

Before breakfast, VASs for hunger, desire to eat, fullness, and satiety were not significantly different between the conditions.

TABLE 3
Mean energy and macronutrient intake and change in body weight over 12 d between conditions: whey-protein and soy-protein groups combined ($n = 79$)[1]

	Percentage of energy from protein		
	5%	15%	30%
Energy intake (MJ/d)			
Total	9.33 ± 3.52[2],a	9.62 ± 3.51[a]	7.21 ± 3.08[b]
Snacks	2.15 ± 2.09[a]	2.44 ± 2.35[a]	1.16 ± 1.13[a]
Meals	7.18 ± 2.38[a]	7.13 ± 2.39[a]	6.04 ± 2.12[b]
Breakfast	2.56 ± 0.91[a]	2.38 ± 0.91[b]	2.19 ± 0.93[c]
Lunch	2.10 ± 0.90[a]	2.02 ± 0.88[a]	1.56 ± 0.68[b]
Dinner	2.52 ± 0.99[a]	2.73 ± 1.03[b]	2.29 ± 0.94[c]
Protein intake, meals			
(MJ/d)	0.36 ± 0.12[a]	1.07 ± 0.36[b]	1.81 ± 0.64[c]
(g · kg BW^{-1} · d^{-1})	0.3 ± 0.1[a]	0.9 ± 0.3[b]	1.6 ± 0.5[c]
Carbohydrate intake, meals			
(MJ/d)	4.31 ± 1.43[a]	3.57 ± 1.20[b]	2.12 ± 0.74[c]
(g · kg BW^{-1} · d^{-1})	3.7 ± 1.2[a]	3.1 ± 1.1[b]	1.8 ± 0.6[c]
Fat intake, meals			
(MJ/d)	2.51 ± 0.83[a]	2.50 ± 0.84[a]	2.12 ± 0.74[b]
(g · kg BW^{-1} · d^{-1})	1.0 ± 0.3[a]	1.0 ± 0.3[a]	0.8 ± 0.3[b]
Protein:carbohydrate + fat			
Meals (% of energy)	5:95[3]	15:85	30:70
Total (% of energy)	5:95	12:88	23:77
ΔBW$_{day12 - day1}$ (kg)	−0.9 ± 1.1	−0.8 ± 1.0	−1.0 ± 1.0
Energy balance (MJ/d)	−2.48 ± 2.92[a]	−2.20 ± 2.84[a]	−4.61 ± 4.64[b]

[1] Values with different superscript letters are significantly different, $P < 0.05$ (repeated-measures ANOVA with Bonferroni correction for pairwise post hoc comparisons). BW, body weight.

[2] Mean ± SD (all such values).

[3] Mean ratio (all such values).

Consumption of the meals induced significant decreases in mean VASs for hunger and desire to eat and significant increases in mean VASs for fullness and satiety ($P = 0.001$) in all conditions over the 12-d test periods. Despite differences in energy intake, no significant differences in hunger, desire to eat, fullness, and satiety over the day were observed between conditions, expressed as mean AUC over the 12-d test periods. Significant lower amplitude values showed less fluctuation in hunger ($P = 0.019$) and desire to eat ($P = 0.026$) in the high-protein condition ($57 ± 19$ and $58 ± 21$, respectively) over the day compared with the normal protein condition ($60 ± 19$ and $62 ± 21$, respectively).

Mean VASs for liking showed that subjects rated the meals as sufficiently palatable except for the lunch in the high-protein condition. VAS liking scores for lunch in the high-protein condition were significantly lower than those in the low- and normal-protein conditions ($P = 0.001$). VAS liking scores for dinner were significantly higher in the whey-protein group compared with the soy-protein group (condition × protein interaction, $P = 0.023$), but energy intake during dinner was not significantly different between both protein groups.

Body weight and energy balance

During the test periods, mean body weight gradually decreased from day 1 to day 12 in all 3 conditions ($P = 0.001$), which implies that subjects were in negative energy balance in all conditions (Table 3). The decrease in body weight did not differ between conditions. Subjects consistently regained their original body weight before the subsequent test session started. Mean energy deficit was significantly greater in the high-protein condition than in the low- and normal-protein conditions (Table 3).

DISCUSSION

The protein leverage hypothesis requires specific evidence that protein intake is regulated more strongly than energy intake. Results from our study provide evidence that supports the protein leverage hypothesis with respect to effects from diets containing a higher protein–to–carbohydrate + fat ratio. Individuals underate relative to energy balance, predominantly from meals in the high-protein condition. No evidence for protein leverage effects from diets containing a lower ratio of protein to carbohydrate + fat was obtained. Nitrogen excretion measurements confirmed that protein intake varied directly with the amount of protein in the diet. Differences in energy intake between conditions were not influenced by age, sex, BMI, or type of protein.

Although the mean energy balance was different between conditions, body weight loss did not differ significantly. This is likely to be due to a fat-free mass–sparing effect in the high-protein condition. Body weight loss with an energy-deficient, normal-protein diet consists of loss of fat mass of 60–75% and of loss of fat-free mass of 25–40%. The corresponding energy equivalent of the body weight loss is ∼30 MJ/kg (37). Body weight loss with an energy-deficient, high-protein diet consists of nearly only loss of fat mass and no loss of fat-free mass. The corresponding energy equivalent of the body weight loss then is ∼52 MJ/kg (21, 37, 38). Although the duration of the test periods was sufficient to measure differences in body weight change, it was not long enough to reliably measure differences in body composition (34, 39).

Appetite scores were similar between the conditions, despite the intervention-induced lower energy intake in the high-protein condition, which indicates that subjects were similarly satiated and satisfied by the diets, including less eating with a high-protein diet. These results confirm the observations by Weigle et al (14). Fluctuation over the day in hunger and desire to eat was lower in the high-protein condition than in the normal-protein condition. Less pronounced fluctuation in appetite may indicate more stable levels of appetite hormones and insulin over the day, which may improve control over food intake (40).

Gosby et al (16) also tested the protein leverage hypothesis and showed that compared with a normal-protein diet (15% of energy from protein), human subjects had a higher energy intake with a moderately low-protein diet (10% of energy from protein), whereas energy intake with a high-protein diet (25% of energy from protein) was not different. Similar between the studies was the aim to test protein leverage in a randomized well-controlled experimental study by using a variety of foods and some food choice wherein protein (not being deficient in amino acids) and carbohydrate were exchanged and energy density and palatability were kept similar. A difference in the design between the present study and that of Gosby et al was the relative protein content of the diets: 5% of energy, 15% of energy, and 30% of energy from protein compared with 10% of energy, 15% of energy, and 25% of energy from protein, respectively. Further differences in the design between the studies were the number of subjects ($n = 79$
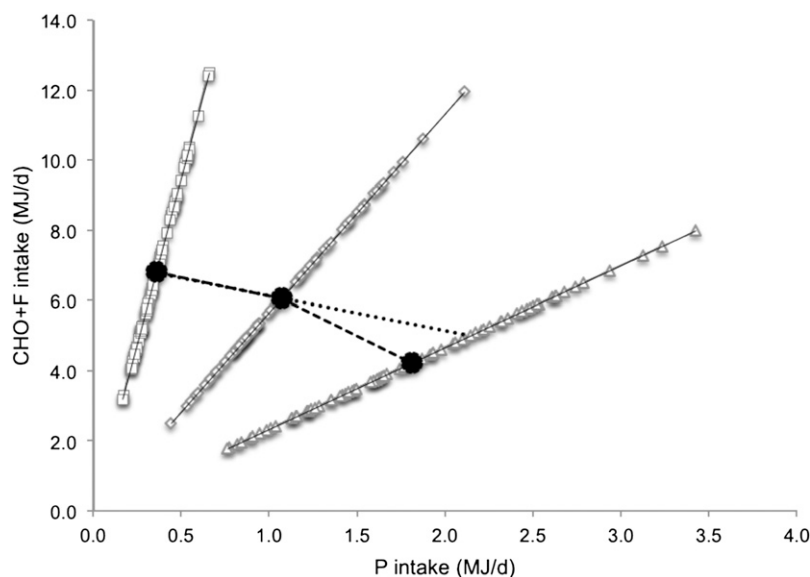
FIGURE 1. Relation between mean protein intake (MJ/d) and mean carbohydrate + fat intake (MJ/d) in response to diets differing in protein–to–carbohydrate + fat ratio for 12 d. Between-subject effects are expressed as individual means for the 5% of energy from protein condition (white squares), the 15% of energy from protein condition (white diamonds), and the 30% of energy from protein condition (white triangles); within-subject effects are expressed as group means (black circles). The dashed line combines group means, and the dotted line represents intakes of protein and carbohydrate + fat that may occur if intakes were regulated to energy intake in the 15% of energy from protein condition. The differences between the dashed line and the dotted line show the degree to which carbohydrate + fat intake is balanced against protein intake. CHO, carbohydrate; F, fat; P, protein.

compared with *n* = 22), characteristics of the subjects (mean ± SD age: 34.0 ± 17.6 compared with 24.3 ± 1.3 y; BMI: 23.7 ± 3.4 compared with 21.8 ± 0.4; age range: 18–70 compared with 18–51 y; BMI range: 18–34 compared with 18–26), duration of the experiments (3 times for 12 d compared with 3 times for 4 d), measurement of possible changes in body weight (measurement of significant changes is possible over 12 d, whereas this is not applicable over 4 d), and measurements of further subject characteristics, such as waist-to-hip ratio, dietary restraint, and physical activity scores, and calculations of physical activity level, basal metabolic rate, and daily energy requirement in the present study.

A similarity in the outcome was the higher total energy intake in the lower compared with the higher protein condition. This indicates that a protein-leveraging mechanism might work between relatively low and relatively high protein diets. However, there is a discrepancy in the results between the studies with respect to energy intake from the 15% of energy from protein diets. The present study observed an energy intake similar to the 5% of energy from protein diet, whereas Gosby et al observed an energy intake similar to the 25% of energy from protein diet. The main discrepancy in protein-induced energy intake differences results from the fact that the present study observed these differences from meals, thus as a satiation effect, whereas Gosby et al observed these differences from snacks, thus a satiety and food-choice effect. We speculate that the satiation effect may be caused by the larger difference in the protein content of the diets. Nitrogen contents of the urinary samples indicated large and constant differences in protein intake between the conditions, which was not the case in Gosby et al. The lack of an effect from snack intake in the present study may be due to the availability of only reported snack intake, which has created large SDs and implies the possibility of uncontrolled snack intake during the hours that subjects were away from the study site, a consequence of the number of subjects and the duration of the experiment.

The strength of the present study was the complete control over energy and macronutrient intake from 3 meals per day over a period of 12 d in a large number of subjects, including both sexes and with a large range in age and BMI, and the nitrogen contents of the urinary samples, which indicated large and constant differences in protein intake between the conditions.

Meal times were scheduled in consultation with the subjects, taking their preferred meal times into account. The slight difference in palatability of the lunches observed in the present study was not related to differences in total energy intake.

To unravel the possible mechanism behind the protein leverage hypothesis, it is necessary to show a direct effect on energy intake from a relatively low and a relatively high protein diet. To our knowledge, until now, studies have observed only one side, either from a low-protein diet (15, 16) or from a high-protein diet (14). Observational studies, either from food-intake surveys (1, 41) or from ecological field studies (4, 10), showed a relative constant absolute protein intake, which may fluctuate relatively depending on energy intake. The driving force behind the observations may have been prevention of overeating with a high-protein diet (17, 18, 20, 24, 42–45) and overeating easily with a high-carbohydrate, high-fat diet (33–35, 46, 47). More research is necessary to unravel cause and effect taking all macronutrients into account.

The prevention of overeating with a high-protein diet also is in line with the hypothesis of Stock (21), which suggests that energy efficiency is influenced by dietary protein content. Thermogenesis may be increased with unbalanced diets in an effort to homeostatically expend excess energy (21). Overeating easily may have been prevented in the present study because an exchange of only protein and carbohydrate took place, and possible overeating on a relatively high-fat diet was impossible (47).

Another explanation may be maintenance of protein balance, which is essential for normal growth and development in organisms.

This requires homeostasis in total nitrogen intake and in the intake of essential amino acids. A proposed mechanism to prevent nitrogen imbalance may be related to an observed signaling pathway for detection of amino acid depletion. Reduced concentrations of essential amino acids in the anterior piriform cortex in the brain may lead to deacetylation of the cognate transfer RNA (48). Subsequent activation of general amino acid nonderepressing kinase 2 may phosphorylate eukaryotic initiation factor 2α, a factor involved in the control of the initiation of translation in protein synthesis, leading to behavioral responses (48–56).

To conclude, we found evidence to support the protein leverage hypothesis in that individuals underate relative to energy balance from diets containing a higher protein–to–carbohydrate + fat ratio. No evidence for protein leverage effects from diets containing a lower ratio of protein to carbohydrate + fat was obtained. It remains to be shown whether a relatively low protein intake would cause overeating or would be the effect of overeating of carbohydrate and fat.

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