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Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber¹⁻³

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ABSTRACT

Background: The mechanism of protein-induced satiety remains unclear.

Objective: The objective was to investigate 24-h satiety and related hormones and energy and substrate metabolism during a high-protein (HP) diet in a respiration chamber.

Design: Twelve healthy women aged 18–40 y were fed in energy balance an adequate-protein (AP: 10%, 60%, and 30% of energy from protein, carbohydrate, and fat, respectively) or an HP (30%, 40%, and 30% of energy from protein, carbohydrate, and fat, respectively) diet in a randomized crossover design. Substrate oxidation, 24-h energy expenditure (EE), appetite profile, and ghrelin and glucagon-like peptide 1 (GLP-1) concentrations were measured.

Results: Sleeping metabolic rate (6.40 ± 0.47 compared with 6.12 ± 0.40 MJ/d; $P < 0.05$), diet-induced thermogenesis (0.91 ± 0.25 compared with 0.69 ± 0.24 MJ/d; $P < 0.05$), and satiety were significantly higher, and activity-induced EE (1.68 ± 0.32 compared with 1.86 ± 0.41 ; $P < 0.05$), respiratory quotient (0.84 ± 0.02 compared with 0.88 ± 0.03 ; $P < 0.0005$), and hunger were significantly lower during the HP diet. There was a tendency for a greater 24-h EE during the HP diet ($P = 0.05$). Although energy intake was not significantly different between the diet groups, the subjects were in energy balance during the HP diet and in positive energy balance during the AP diet. Satiety was related to 24-h protein intake ($r^2 = 0.49$, $P < 0.05$) only during the HP diet. Ghrelin concentrations were not significantly different between diets. GLP-1 concentrations after dinner were higher during the HP than during the AP diet ($P < 0.05$).

Conclusion: An HP diet, compared with an AP diet, fed at energy balance for 4 d increased 24-h satiety, thermogenesis, sleeping metabolic rate, protein balance, and fat oxidation. Satiety was related to protein intake, and incidentally to ghrelin and GLP-1 concentrations, only during the HP diet. *Am J Clin Nutr* 2006;83:89–94.

KEY WORDS Satiety, high-protein diet, ghrelin, glucagon-like peptide 1, GLP-1, energy balance

INTRODUCTION

With respect to energy expenditure, it is known that protein has the highest and most prolonged thermic effect of the separate macronutrients (20–30%), followed by carbohydrate (5–15%) and fat (0–3%) (1). Studies measuring diet-induced thermogenesis (DIT) over 24 h (2), over several hours (3), or after a single preload (4) all showed that diets higher in protein exert a greater

effect on energy expenditure than do diets lower in protein. This finding suggests that protein has a lower energy efficiency than does carbohydrate or fat.

Protein has been observed to increase satiety to a greater extent than carbohydrate and fat and can therefore reduce energy intake (5, 6). Differences in 24-h satiety have been related to differences in DIT (7). On the basis of these observations for protein-related satiety, thermogenesis, and energy inefficiency, we previously reported that a protein intake of 18% of energy, compared with 15% of energy, resulted in improved weight maintenance, which could partly be explained by increased postabsorptive satiety, decreased energy efficiency, and improved body composition favoring the maintenance of fat-free mass (8, 9). The release of hormones such as ghrelin and glucagon-like peptide 1 (GLP-1) are thought to influence postingestive satiety. Stimulation of endogenous ghrelin and GLP-1 production seems to be nutrient-specific (10–18).

In the present highly controlled study, we assessed simultaneously several mechanisms of satiety when carbohydrate was exchanged for protein isoenergetically over 4 d, thus keeping the energy density constant. We hypothesized that satiety is related to energy inefficiency (ie, elevated thermogenesis), the satiety hormones (ghrelin and GLP-1), or both. To further unravel the favorable effect that a high protein intake appears to have on body composition, ie, increasing fat free mass at the expense of fat mass (9, 8, 19, 20), special attention was paid to 24-h fat oxidation during a high-protein compared with an adequate-protein diet.

SUBJECTS AND METHODS

Subjects

Twelve healthy women with a body mass index (BMI; in kg/m^2) of 20–25 and aged 18–40 y were recruited by advertisements placed on notice boards at Maastricht University. All subjects underwent a medical screening, and all were in good health,

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TABLE 1
Subject characteristics at baseline¹

	Value
Age (y)	21 ± 3
Body weight (kg)	60.6 ± 4.4
Height (m)	1.70 ± 0.07
BMI (kg/m ²)	21.1 ± 1.5
Fat mass (kg)	14.7 ± 2.2
Fat-free mass (kg)	45.8 ± 3.1
Body fat (%)	24.3 ± 2.7
Dietary restraint ²	5 ± 4

¹ All values are $\bar{x} \pm SD$; $n = 12$.

² Factor 1 of the Three-Factor Eating Questionnaire was used.

nonsmokers, not using medication, and at most moderate alcohol users. The baseline characteristics of the subjects are presented in **Table 1**. Written informed consent was obtained from all participants. The Medical Ethics Committee of the Academic Hospital in Maastricht approved the study.

Experimental sessions

The study had a single-blind, randomized, crossover design. The subjects underwent two 36-h sessions in a respiration chamber for the measurement of energy expenditure and substrate oxidation. The 2 sessions were conducted 4 wk apart to maximize the potential that each subject was in the same phase of their menstrual cycle. Three days before each session, subjects were provided with a diet to consume at home. The adequate-protein (AP) diet and the high-protein (HP) diet were randomly assigned over both sessions. The AP diet provided 10%, 60%, and 30% of energy from protein, carbohydrate, and fat, respectively. The HP diet provided 30%, 40%, and 30% of energy from protein, carbohydrate, and fat, respectively. A detailed composition of the diets is presented in **Table 2**.

TABLE 2
Composition of the high-protein (HP) and adequate-protein (AP) diets

	HP diet	AP diet
Breakfast	Bread	Bread
	Butter	Butter
	Chicken filet	Coconut bread
	Milk	Orange juice
	Merengue	
Lunch	Bread	Bread
	Soy milk	Orange juice
	Fruit yogurt	Soy dessert
	Tuna in water	Tuna in oil
	Tomato	Tomato
	Cucumber	Cucumber
	Feta cheese	Cottage cheese
	Salad dressing	Salad dressing
		Corn
	Banana	
Dinner	Rice dish with ham	Rice with curry chicken
	Soup	Soup
	Soy milk	Orange juice
	Muesli bar	Muesli bar
		Fruit cocktail
Energy density (kJ/g)	4.1	4.3

Energy intake

During each experimental session, the subjects were fed with a study diet designed to provide energy balance. The energy content of the diet that the subjects consumed at home was based on basal metabolic rate (BMR), which was calculated with the equation of Harris-Benedict (21). BMR was multiplied by an activity index of 1.7 (22). To determine the appropriate level of energy intake for attaining energy balance in the respiration chamber, the sleeping metabolic rate (SMR) was measured during the first night and multiplied by an activity index of 1.5 (22). Energy intake was divided over the meals: 20% for breakfast (0900), 40% for lunch (1345), and 40% for dinner (1930).

Blood sampling

On the morning of day 4, a polytetrafluoroethylene catheter was placed in the antecubital vein for blood sampling. During each session in the respiration chamber, 9 blood samples (at 0845, 0930, 1015, 1330, 1415, 1500, 1915, 2000, and 2045) were taken for the measurement of plasma ghrelin and GLP-1 concentrations. In addition, a blood sample for the measurement of fasting serum cortisol and growth hormone concentrations was taken at the first blood sampling time point (0845). The blood for the serum measurements was allowed to clot at room temperature for 20 min. Immediately after clotting, the blood samples were put on ice and serum was extracted by centrifugation (1500 × g, 10 min, 4 °C). The blood for plasma measurements was centrifuged immediately. All samples were frozen in liquid nitrogen and stored at −80 °C until analyzed. Plasma concentrations of active ghrelin were measured by radioimmunoassay (Linco Research Inc, St. Charles, MO). Plasma active GLP-1 samples were analyzed by enzyme-linked immunoradiometric assay (EGLP-35K; Linco Research Inc, St Charles, MO). Serum growth hormone concentrations were assayed by using the DELFIA method (Wallac Oy, Turku, Finland). Serum cortisol was determined with a direct radioimmunoassay after denaturation of trancortin by heating at 60 °C as described by Sulon et al (23).

Appetite profile

Appetite profile was measured with the use of anchored 100-mm visual analogue scales (VAS). During each respiration chamber session, these questionnaires were completed before and after every meal. The questions were, “How hungry are you?” and “How satiated are you?” and were anchored by “not at all” and “very.” For the calculation of the 24-h area under the curve (AUC), the VAS ratings were interpolated from the latest measurement at night until the first measurement in the morning (7).

Body composition

Body composition was determined by the 3 compartment model, with the use of hydrodensitometry and the deuterium dilution (²H₂O) technique (24, 25), and was calculated by using the combined equation of Siri (26).

Indirect calorimetry

Oxygen consumption and carbon dioxide production were measured in the respiration chamber (27). The respiration chamber is a 14-m³ room furnished with a bed, chair, computer, television, radiocassette player, telephone, intercom, sink, and toilet. The room was ventilated with fresh air at a rate of 70–80 L/min.

The ventilation rate was measured with a dry gas meter (type 4; Schlumberger, Dordrecht, Netherlands). The concentrations of oxygen and carbon dioxide were measured with the use of an infrared carbon dioxide analyzer (Uras 3G; Hartmann and Braun, Frankfurt, Germany) and 2 paramagnetic oxygen analyzers: Magnos 6G (Hartmann and Braun) and type OAI84A (Servomex, Crowborough, United Kingdom). During each 15-min period, 6 samples of outgoing air for each chamber, 1 sample of fresh air, zero gas, and calibration gas were measured. The gas samples to be measured were selected by a computer that also stored and processed the data (27).

Energy expenditure and substrate oxidation

Twenty-four-hour energy expenditure consists of SMR, DIT, and activity-induced energy expenditure (AEE); 24-h energy expenditure and 24-h respiratory quotient (RQ) were measured from 0800 on day 4 to 0800 on day 5. Activity was monitored with a radar system based on the Doppler principle. SMR was defined as the lowest mean energy expenditure measured over 3 consecutive hours between 0000 and 0700. DIT was calculated by plotting energy expenditure against radar output; both were averaged over 30-min periods. The intercept of the regression line at the lowest radar output represents the energy expenditure in the inactive state (resting metabolic rate; RMR), which consists of SMR and DIT (2). DIT was determined by subtracting SMR from RMR. AEE was determined by subtracting SMR and DIT from 24-h energy expenditure. Carbohydrate, fat, and protein oxidation were calculated from the measurements of oxygen consumption, carbon dioxide production, and urinary nitrogen excretion by using the formula of Brouwer (28). Urine samples (24 h) were collected from the second void on day 4 until the first void on day 5. Samples were collected in containers with 10 mL H₂SO₄ to prevent nitrogen loss through evaporation. Volume and nitrogen concentration were measured, the latter with a nitrogen analyzer (CHN-O-Rapid; Heraeus, Hanau, Germany).

Physical activity

To measure physical activity on day 4, the subjects were asked to wear a tri-axial accelerometer (Tracmor; Philips Research, Eindhoven, Netherlands) (29) during the waking hours. The average counts per day were calculated.

Statistical analysis

Data are presented as means \pm SDs unless otherwise indicated. A repeated-measures analysis of variance was carried out to determine possible differences between conditions. Regression analyses were performed to determine the relations between selected variables. Significance was defined as $P < 0.05$. All statistical tests were performed by using SPSS for WINDOWS (version 11.5; SPSS Inc, Chicago, IL).

RESULTS

There was no difference in total energy intake between conditions (HP: 9.10 ± 0.70 MJ/d; AP: 9.15 ± 0.77 MJ/d). The results on 24-h energy expenditure are shown in **Table 3**. There was a trend ($P = 0.05$) for a greater total energy expenditure during the HP condition. SMR and DIT were significantly greater during the HP condition, whereas AEE was significantly smaller in the HP condition. No differences between conditions

TABLE 3

Total energy expenditure, components of energy expenditure, respiratory quotient (RQ), and nonprotein RQ (NPRQ) during the high-protein (HP) and adequate-protein (AP) diets¹

	HP diet (n = 12)	AP (n = 12)	P ²
SMR (MJ/d)	6.40 \pm 0.47 ³	6.12 \pm 0.40	< 0.05
DIT (MJ/d)	0.91 \pm 0.25	0.69 \pm 0.24	< 0.05
(% of EI)	10.1 \pm 2.7	7.6 \pm 2.5	< 0.05
TEE (MJ/d)	8.99 \pm 0.71	8.67 \pm 0.73	=0.05
AEE (MJ/d)	1.68 \pm 0.32	1.86 \pm 0.41	< 0.05
24-h RQ	0.84 \pm 0.02	0.88 \pm 0.03	< 0.0005
24-h NPRQ	0.86 \pm 0.02	0.90 \pm 0.03	< 0.0005

¹ SMR, sleeping metabolic rate; DIT, diet-induced thermogenesis; EI, energy intake; TEE, total energy expenditure; AEE, activity-induced energy expenditure.

² Repeated-measures ANOVA.

³ $\bar{x} \pm$ SD (all such values).

were found in physical activity measured with the accelerometers, expressed as counts per day. During the HP condition, energy balance was not significantly different from zero; during the AP condition, the subjects were slightly in positive energy balance (HP: 0.11 ± 0.30 MJ/d; AP: 0.47 ± 0.63 MJ/d; $P < 0.05$). RQ was significantly lower in the HP condition, which indicated higher fat oxidation (Table 3). The separate macronutrient balances are shown in **Figure 1**, and there was a significant difference in protein and fat balances between diet conditions. During the HP condition, subjects were in a positive protein and a negative fat balance; the protein and fat balances were significantly different between the 2 diets.

The 24 h-AUC for hunger was significantly smaller in the HP condition (HP: 822 ± 304 mm/24 h) than in the AP condition (1101 ± 256 mm/24 h; $P < 0.005$), whereas the 24-h AUC for satiety was significantly greater in the HP condition (973 ± 178 mm/24 h) than in the AP condition (765 ± 304 mm/24 h; $P < 0.01$). The hunger and satiety ratings during day 4 are shown in **Figure 2**. No significant time-by-treatment interaction was seen. However, compared with the AP condition, the hunger scores were significantly lower and the satiety scores were significantly higher during the HP condition before and after dinner. In the HP

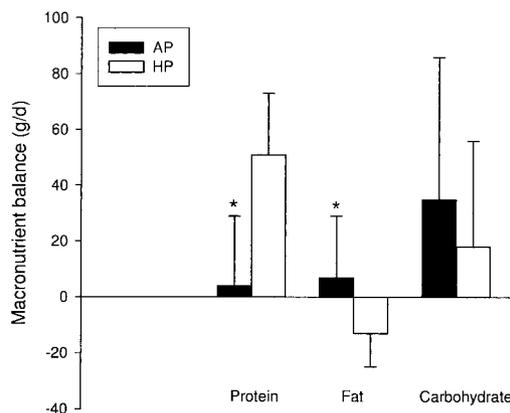


FIGURE 1. Mean (\pm SD) 24-h macronutrient balances on day 4 of the high-protein (HP; n = 12) and adequate-protein (AP; n = 12) diets. *Significantly different from the HP diet, $P < 0.005$ (repeated-measures ANOVA).

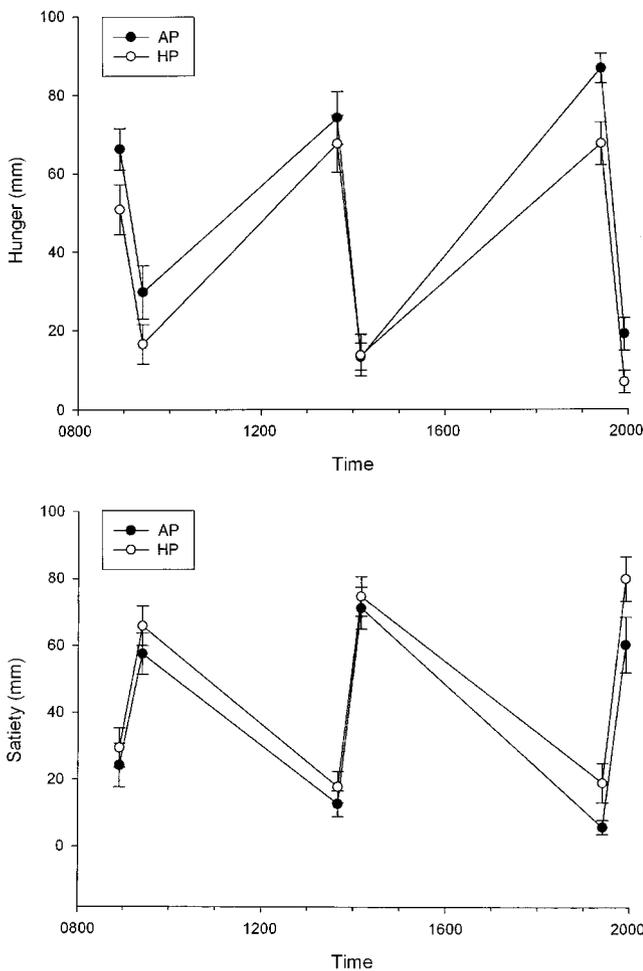


FIGURE 2. Mean (\pm SEM) hunger and satiety ratings measured with the use of an anchored 100-mm visual analogue scale on day 4 of the high-protein (HP; $n = 12$) and adequate-protein (AP; $n = 12$) diets. No significant time-by-treatment interactions were observed.

condition, 24-h satiety (AUC) appeared to be a function of 24-h protein intake (regression analysis: $r^2 = 0.49$, $P < 0.05$; **Figure 3**). This relation was not found in the AP condition (NS).

Ghrelin and GLP-1 concentrations are shown in **Figure 4**. No significant differences in ghrelin concentrations were found between dietary conditions. However, in the AP condition the ghrelin concentrations decreased significantly after lunch and after dinner, whereas in the HP condition this was only seen after dinner. A significant increase in GLP-1 was seen 15 min after lunch and after dinner in both conditions, and the concentrations tended to stay higher for 1 h afterward. The GLP-1 concentration 15 min after dinner was significantly higher during the HP condition than during the AP condition. After breakfast there was a trend ($P < 0.1$) for GLP-1 concentrations to be higher in the HP condition than in the AP condition. Fasting cortisol (HP: $287.0 \pm 86.6 \mu\text{g/L}$; AP: $283.9 \pm 124.0 \mu\text{g/L}$) and growth hormone (HP: $13.6 \pm 15.2 \text{ mU/L}$; AP: $13.7 \pm 12.1 \text{ mU/L}$) concentrations were not significantly different between conditions. During breakfast, the decrease in hunger was related to a decrease in ghrelin in the HP condition ($r^2 = 0.52$, $P < 0.05$) and in the AP condition ($r^2 = 0.45$, $P < 0.05$). In addition, the increase in satiety was related to the decrease in ghrelin after the AP breakfast ($r^2 = 0.53$, $P < 0.05$). Only in the HP condition was

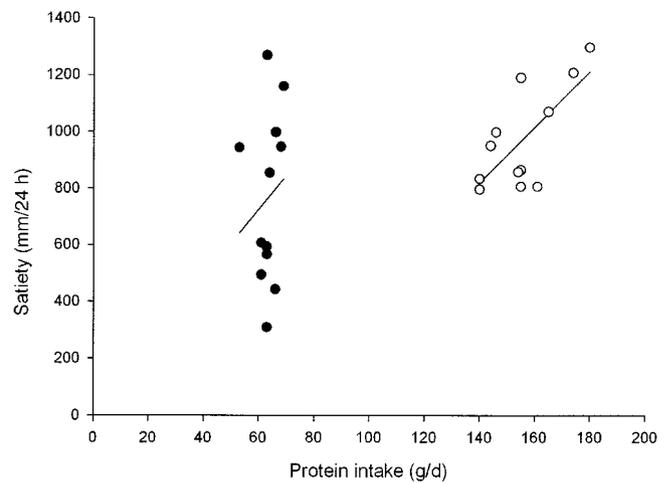


FIGURE 3. Correlation between 24-h satiety (area under the curve) and 24-h protein intake during the high-protein (○; regression analysis: $r^2 = 0.49$, $P < 0.05$) and adequate-protein (●; regression analysis: NS) diets.

the increase in satiety after dinner related to the increase in GLP-1 ($r^2 = 0.41$, $P < 0.05$).

DISCUSSION

The present study showed that a high-protein diet compared with an adequate-protein diet when eaten over 4 days increased 24-h satiety and decreased hunger, without differences in energy intake. These results support the hypothesis that protein increases satiety to a higher extent than does carbohydrate and fat. This finding is also reflected in the relation between 24-h satiety and protein intake, which was only seen with the HP diet. The protein intake with the AP diet was $1.0 \pm 0.1 \text{ g/kg}$, which should be enough to maintain nitrogen balance based on the current Recommended Dietary Allowance for protein of 0.8 g/kg (30). This is reflected in the protein balance, which was not significantly different from zero during the AP condition. The protein intake during the HP diet was $2.6 \pm 0.3 \text{ g/kg}$, which resulted in a positive protein balance that is likely to be short term (31). Thus, if the protein intake is sufficient to maintain protein balance (as in the AP condition), it is not related to satiety. However, if the protein intake is higher than the protein requirement, satiety is positively related to protein intake.

With respect to the composition of the HP diet, 40% of energy as carbohydrate is considered to be within the range of a normal carbohydrate diet, whereas 30% of energy from protein is above the normal range (30). Therefore, this diet could also be considered to be high in protein. Although the fat content was kept constant at 30% of energy, the fat balance was different between the diets. The negative fat balance seen in the HP condition favors fat loss in the long term. These results are in line with the findings of our previous study, in which subjects with an increased protein intake during weight maintenance had a lower fat mass than did subjects with a lower protein intake (8, 9).

With respect to energy expenditure, a higher DIT and SMR were found during the HP condition. It is suggested that the differences in DIT between the 2 diet conditions may have been due to the body's small storage capacity for protein; hence, it needs to be metabolized immediately. Furthermore, with an increased protein synthesis, the high ATP cost of peptide bond

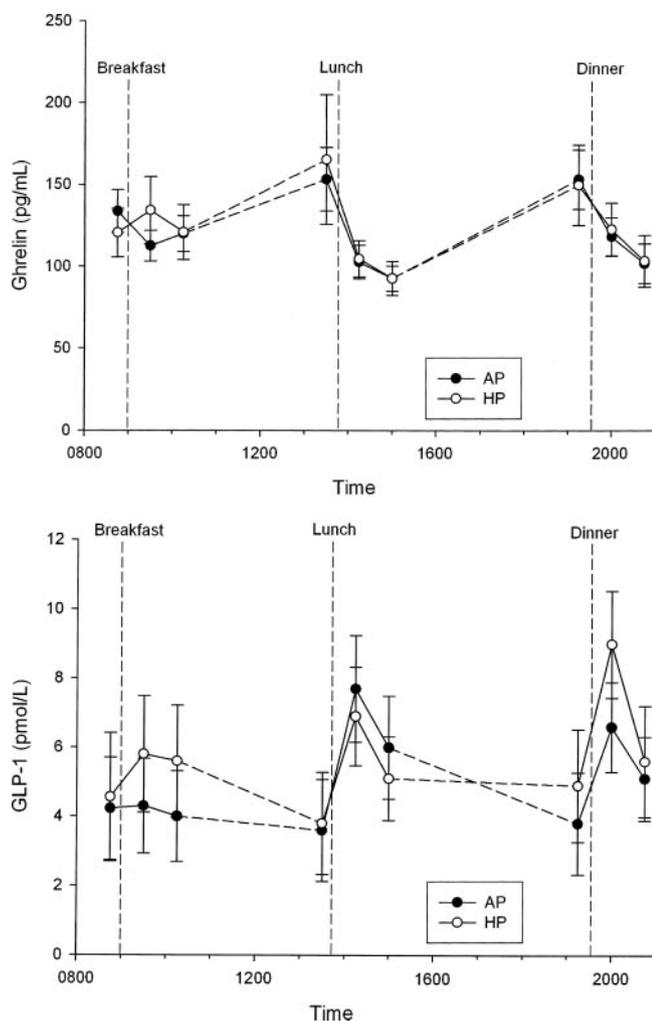


FIGURE 4. Mean (\pm SEM) ghrelin and glucagon-like peptide 1 (GLP-1) concentrations on day 4 of the high-protein (HP; $n = 12$) and adequate-protein (AP; $n = 12$) diets. No time-by-treatment interactions were observed.

synthesis, as well as the high cost of gluconeogenesis and urea production, may be the reason for the higher thermic effect of protein (32, 33). In a respiration chamber experiment, Dauncey and Bingham (34) also showed that nutrient composition can have a marked influence on 24-h energy expenditure. In that study a 12% increase in energy expenditure after a high-protein intake was seen.

The AEE was lower in the HP condition than in the AP condition. This may have been due to the fact that the AP diet was higher in carbohydrate than was the HP diet; the fat content was the same in both diets. Because carbohydrate has a lower DIT than does protein (1), more energy remains for physical activity. However, the physical activity counts measured with the accelerometers showed no differences between the 2 diet conditions. This means that the activity of the subjects was not different during the day between both diets, but more energy was spent on the same activities with the AP diet.

It is thought that postingestive satiety is mediated, among other factors, by the release of hormones such as ghrelin, GLP-1, cholecystokinin, peptide YY, oxyntomodulin, and pancreatic polypeptide. Because of the frequent blood sampling, it was not possible to take all the afferent satiety factors into account.

Therefore, the present study focused on ghrelin and GLP-1 only. A decrease in ghrelin concentrations after a meal was seen during the AP diet, which was relatively high in carbohydrate. This agreed with observations on carbohydrate-rich meals to have a suppressive effect on plasma ghrelin concentrations (15–17). Erdmann et al (17) proposed that this might be due to elevated glucose and insulin concentrations, which can lead to suppression of plasma ghrelin. When 20% of energy from carbohydrate was exchanged for protein, the suppression of ghrelin after a meal was still observed, although it was significant only after dinner. This finding contrasts with the findings of other studies, which showed that ghrelin concentrations increased after an oral protein load (15, 17). However, in the present study the carbohydrate content of the HP diet was probably high enough to elicit an elevated glucose and insulin response, which resulted in suppressed ghrelin concentrations. Endogenous GLP-1 production was reported to be stimulated by meal ingestion, especially by carbohydrate and fat (11, 12, 18). In contrast, if given in a mixed meal, protein can increase GLP-1 concentrations to a greater extent than can carbohydrate and fat (14). In the present study, GLP-1 concentrations increased with both diets after lunch and dinner. After dinner, this increase was greater with the HP diet than with the AP diet, which agrees with the results of Raben et al (14). In the present study we focused on peripheral GLP-1 signals in relation to satiety. However, it is not known how central GLP-1 receptors are involved in food intake regulation in humans (35, 36). With respect to additional regulatory circuits, it has been shown that the availability of nutrients can be sensed at central sites or directly in peripheral tissues (37).

We conclude that an HP diet, compared with an AP diet, when consumed in energy balance over 4 d, increased 24-h satiety, thermogenesis, SMR, protein balance, and fat oxidation. Only in the HP condition was satiety related to protein intake and incidentally to ghrelin and GLP-1 concentrations.

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MPGML designed the experiment, collected the data, analyzed the data, and wrote the manuscript. KRW provided significant advice and helped prepare the manuscript. TCMA provided significant advice with respect to the analysis of the GLP-1 data. NDLM assisted with the data collection and reviewed the manuscript for correct spelling and grammar. MSW-P designed the experiment, helped analyze the data and write the manuscript, and supervised the project. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

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