

# Comparison of 2 diets with either 25% or 10% of energy as casein on energy expenditure, substrate balance, and appetite profile.

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# Comparison of 2 diets with either 25% or 10% of energy as casein on energy expenditure, substrate balance, and appetite profile<sup>1–3</sup>

Ananda Hochstenbach-Waelen, Margriet AB Veldhorst, Arie G Nieuwenhuizen, Margriet S Westerterp-Plantenga, and Klaas R Westerterp

## ABSTRACT

**Background:** An increase in the protein content of a diet results in an increase in satiety and energy expenditure. It is not clear to what extent a specific type of protein has such effects.

**Objective:** The objective was to compare the effects of 2 diets with either 25% or 10% of energy from casein (25En% and 10En% casein diets), as the only protein source, on energy expenditure, substrate balance, and appetite profile.

**Design:** During a 36-h stay in a respiration chamber, 24 healthy subjects [12 men and 12 women; body mass index (in kg/m<sup>2</sup>): 22.4 ± 2.4; age: 25 ± 7 y] received isoenergetic diets according to subject-specific energy requirements: 25En% diet (25%, 20%, and 55% of energy as protein, fat, and carbohydrate, respectively) and 10En% diet (10%, 35%, and 55% of energy as protein, fat, and carbohydrate, respectively) in a randomized crossover design. Three days before the diets began, the subjects consumed a similar diet at home. Energy expenditure, substrate oxidation, and appetite scores were measured.

**Results:** The 25En% casein diet resulted in a 2.6% higher 24-h total energy expenditure (9.30 ± 0.24 compared with 9.07 ± 0.24 MJ/d; *P* < 0.01) and a higher sleeping metabolic rate (6.74 ± 0.16 compared with 6.48 ± 0.17 MJ/d; *P* < 0.001) than did the 10En% casein diet. With the 25En% casein diet, compared with the 10En% casein diet, the subjects were in positive protein balance (0.57 ± 0.05 compared with -0.08 ± 0.03 MJ/d; *P* < 0.0001) and negative fat balance (-0.83 ± 0.14 compared with 0.11 ± 0.17 MJ/d; *P* < 0.0001), whereas positive carbohydrate balances were not significantly different between diets. Satiety was 33% higher with the 25En% casein diet than with the 10En% casein diet (*P* < 0.05).

**Conclusion:** A 25En% casein diet boosts energy expenditure, protein balance, satiety, and negative fat balance, which is beneficial to body weight management. *Am J Clin Nutr* 2009;89:831–8.

## INTRODUCTION

Obesity is a major health concern worldwide, and treatment for this problem is necessary (1). Body weight management requires a multifactorial approach, because several pathways are involved in the system of body weight regulation. Recent findings suggest that an elevated protein intake affects both short- and long-term mechanisms by increasing satiety, despite similar or lower energy intakes, via increased thermogenesis, increased

storage of fat-free mass, and lower energy efficiency during overfeeding (2–5). The measured thermic effect of the 3 separate macronutrients is highest for protein (20–30%), followed by carbohydrate (5–10%) and fat (0–3%) (6). Previous studies found a higher diet-induced thermogenesis (DIT) (7, 8) and a higher sleeping metabolic rate (SMR) (8) during a relatively high-protein diet. Moreover, proteins are the most satiating, followed by carbohydrates, and then fats (2, 5). Mechanisms that may contribute to protein-induced satiety are increases in energy expenditure (9, 10) and in anorexigenic hormone concentrations (10). Currently, it is not clear to what extent these effects hold for specific types of protein.

This study investigated possible differences between 2 diets, with either 25% or 10% of energy as casein as the only protein source (25En% and 10En% casein diets). Casein is known as a “slow” protein, which means that it clots in the stomach and results in delayed gastric emptying (11). In a previous study, we compared 2 breakfasts, both providing 20% of the daily energy intake and consisting of either 25En% or 10En% casein as the only protein source, with respect to satiety, relevant blood variables, and subsequent energy intake (12). A breakfast with 25En% from casein was more satiating than was a breakfast with 10En% from casein and coincided with prolonged elevated concentrations of plasma amino acids. To confirm this observation over 24 h, and to determine energy expenditure at the same time, we conducted an actual respiration chamber study. We investigated 2 diets in which we exchanged energy from protein (25En% compared with 10En%) with energy from fat (20En% compared with 35En%), whereas the carbohydrate content (55En%) remained the same. The carbohydrate content was the same with both diets because ingestion of this nutrient results in insulin secretion, and insulin is involved in protein metabolism (13).

The aim of this study was to compare the effects of a 25En% casein diet with those of a 10En% casein diet on energy ex-

<sup>1</sup> From the Department of Human Biology, Nutrition and Toxicology Research Institute Maastricht, Maastricht University, Maastricht, Netherlands, and the Top Institute Food and Nutrition, Wageningen, Netherlands (AH-W, MABV, AGN, MSW-P, and KRW).

<sup>2</sup> Supported by Top Institute Food and Nutrition, Wageningen, Netherlands.

<sup>3</sup> Reprints not available. Address correspondence to A Hochstenbach-Waelen, Department of Human Biology, Maastricht University, PO Box 616, 6200 MD Maastricht, Netherlands. E-mail: ananda.waelen@hb.unimaas.nl.

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penditure, substrate balance, appetite profile, and relevant blood variables to determine a possible effect of one single protein, namely casein, and to determine the magnitude of the difference.

## SUBJECTS AND METHODS

### Subjects

Thirty subjects aged 18–55 y and a body mass index (in kg/m<sup>2</sup>) between 20 and 33 were recruited by advertisements on notice boards of Maastricht University and in local newspapers. Recruitment started on 1 September 2006. The actual study started in October 2006. Subjects underwent a medical screening, and 24 subjects (12 men and 12 women) were selected on the following inclusion criteria: good health, nonsmokers, no use of medication (except for contraceptives), no cow milk allergy, moderate to no alcohol consumption, stable weight during the last 3 mo, not following a diet, and no cognitive dietary restraint. The Dutch translation of the Three Factor Eating Questionnaire was used to assess the eating behavior of the subjects (14). The power calculation of this study was based on DIT measured in a respiration chamber under conditions of high and adequate protein feeding as published by Lejeune et al (8). In this study, DIT values were  $0.91 \pm 0.25$  and  $0.69 \pm 0.24$  MJ/d with high- and adequate-protein diets, respectively. With a sensitivity of 0.80 and a significance level of 0.05, power calculation (within subjects, 2-tailed) indicated a sample size of 12 for each group. The power calculation from our previous study (12) on satiety with casein custard for breakfast indicated, with a sensitivity of 0.80 and a significance level of 0.05, a sample size of 24. Subject characteristics are presented in **Table 1**. Subjects signed an informed consent form before participating in the study. The study protocol was approved by the Medical Ethical Committee of the Maastricht University Medical Center.

### Experimental design

This study had a randomized single-blind crossover design. Subjects came to the university 2 times; each time they stayed for 36 h in a respiration chamber for the measurement of energy expenditure and substrate oxidation. For women, it was important to be in the same phase of their menstrual cycle (15), so the 2 stays in

the respiration chamber were separated by a period of 4 wk. In random order, the subjects received 1 of the 2 diets while in the chamber: the 10En% or a 25En% protein diet with casein as the only protein source. The macronutrient distribution for the diets was as follows: 10En%, 35En%, and 55En% as protein, fat, and carbohydrate, respectively, for the 10En% casein diet; 25En%, 20En%, and 55En% as protein, fat, and carbohydrate, respectively, for the 25En% casein diet. The 2 diets were mainly offered as a custard (one custard with 10En% from casein and one custard with 25En% from casein) produced by NIZO Food Research bv (Ede, Netherlands). The protein content of the custards consisted only of casein (Calcium Caseinate S; DMV International, Veghel, Netherlands), whereas the carbohydrate and fat contents consisted, respectively, of tapioca starch (Farinex VA50T and Perfect-amyl 3108; AVEBE, Veendam, Netherlands) and sunflower-seed oil (Reddy; NV Vandemoortele, Roosendaal, Netherlands). Both custards were citrus-vanilla flavored (Citrus, Vanilla; JB de Lange, Belfeld, Netherlands). Extensive product development and use of a taste panel led to custards that did not differ significantly in color, taste, or viscosity. Three days before their stay in the respiration chamber, the subjects were supplied with a diet at home. This diet had the same macronutrient distribution as the diet they received during the subsequent stay in the respiration chamber, but it consisted of normal food products and various protein sources. During the stay in the respiration chamber, blood samples, (24-h) urine samples, and appetite scores on visual analog scales (VASs) were obtained.

### Energy intake

Calculations for both the diet at home and the diet in the respiration chamber were based on average daily energy requirements. The daily energy requirement for the diet at home was estimated as 1.75 times the basal metabolic rate (BMR) (16). BMR was calculated with the Harris-Benedict formula (17). The energy requirement in the respiration chamber was estimated as 1.35 times the BMR. Daily energy intake was divided over 3 meals: 20% at breakfast, 40% at lunch, and 40% at dinner. Breakfast was given at 0900, lunch at 1345, and dinner at 1930.

### Energy expenditure and substrate oxidation

Subjects stayed in the respiration chambers from 2000 in the evening of the third day of their diet at home (day 3) until 0800 in the morning of day 5. The respiration chamber is a 14-m<sup>3</sup> room furnished with a bed, chair, desk, computer, television, DVD player, video cassette recorder, telephone, intercom, sink, and toilet. During the 36-h stay in the respiration chamber, oxygen consumption and carbon dioxide production were measured. The room was ventilated with fresh air at a rate of 70–80 L/min. Flow was measured by using electronically modified dry gas meters (G6; Gasmeterfabriek Schlumberger, Dordrecht, Netherlands). The concentrations of oxygen and carbon dioxide were measured with dual pairs of infrared carbon dioxide analyzers (ABB/Hartman&Braun Uras, Frankfurt am Main, Germany) and paramagnetic oxygen analyzers (Servomex 4100; Servomex, Crowborough, United Kingdom; ABB/Hartman&Braun Magnos, Frankfurt am Main, Germany). 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

**TABLE 1**  
Subject characteristics<sup>1</sup>

	Value (n = 12 M, 12 F)
Age (y)	25 ± 7
Height (m)	1.75 ± 0.09
Body weight (kg)	68.5 ± 10.5
BMI (kg/m <sup>2</sup> )	22.4 ± 2.4
Fat mass (kg)	15.4 ± 6.7
Fat-free mass (kg)	53.0 ± 9.3
TFEQ1 (cognitive restraint) <sup>2</sup>	6 ± 3
TFEQ2 (disinhibition) <sup>3</sup>	4 ± 2
TFEQ3 (hunger) <sup>4</sup>	5 ± 3

<sup>1</sup> All values are means ± SDs; n = 24. TFEQ, Three Factor Eating Questionnaire.

<sup>2</sup> Factor 1 of the Three Factor Eating Questionnaire.

<sup>3</sup> Factor 2 of the Three Factor Eating Questionnaire.

<sup>4</sup> Factor 3 of the Three Factor Eating Questionnaire.

be measured were selected by a computer that also stored and processed the data (18). With the exception of strenuous exercise and sleeping, subjects were allowed to move freely from 0700 to 2300. Total energy expenditure over 24 h (TEE) and 24-h respiratory quotient (RQ) were calculated from 0730 on the first morning until 0730 on the second morning in the respiration chamber. A radar system based on the Doppler principle was used to measure the physical activity of the subjects in the chamber. The following components of energy expenditure were calculated: SMR, DIT, resting metabolic rate (RMR), and activity-induced energy expenditure (AEE). TEE was calculated by using the equation of Carpenter, as published by Brouwer (19):

$$\text{TEE(kJ/d)} = 16 \times \text{O}_2(\text{L/d}) + 5 \times \text{CO}_2(\text{L/d}) - 0.95 \times \text{P} \quad (1)$$

where P is oxidized protein in g/d. SMR was calculated by assessing the lowest mean activity of the subjects during 3 consecutive hours between 0000 and 0700 during the second night of their stay in the respiration chamber. SMR was the mean energy expenditure during the 3 consecutive hours in which activity was the lowest. RMR was calculated by plotting energy expenditure (*y* axis) against radar output (*x* axis), both being averaged over 30-min intervals in the last 24 h of the stay in the respiration chamber. RMR was calculated by entering the earlier mentioned lowest mean activity into the formula of the linear regression line of the plot. DIT was calculated by subtracting SMR from RMR. AEE was calculated by subtracting RMR from TEE. Substrate oxidation was calculated from 24-h urinary nitrogen, oxygen consumption, and carbon dioxide production. Urine samples (24-h) were collected from the second voiding on day 4 until the first voiding on day 5. To prevent nitrogen loss through evaporation, 24-h urine was collected in containers with 10 mL H<sub>2</sub>SO<sub>4</sub>, whereas total volume was measured afterward. Nitrogen concentrations were measured with a nitrogen analyzer (CHN-O-Rapid; Heraeus, Hanau, Germany). Protein oxidation (g/d) was calculated by multiplying 24-h urinary nitrogen (g/d) by 6.25. Carbohydrate (g/d) and fat oxidation (g/d) were calculated with the following equations of Carpenter, as published by Brouwer (19):

$$\text{Carbohydrate oxidation} = -2.97 \times \text{O}_2(\text{L/d}) + 4.17 \times \text{CO}_2(\text{L/d}) - 0.39 \times \text{P} \quad (2)$$

$$\text{Fat oxidation} = 1.72 \times \text{O}_2(\text{L/d}) - 1.72 \times \text{CO}_2(\text{L/d}) - 0.32 \times \text{P} \quad (3)$$

### Blood sampling

On the first morning of the stay in the respiration chamber (day 4), a Venflon catheter (Becton Dickinson Infusion Therapy, Helsingborg, Sweden) was placed in the antecubital vein of each subject for blood sampling. Blood samples were drawn 15 min before each meal and 45 and 75 min after each meal, ie, at 0845 (–15 min), 0945 (45 min), 1015 (75 min), 1330 (270 min), 1430 (330 min), 1500 (360 min), 1915 (615 min), 2015 (675 min), and 2045 (705 min), for the measurement of plasma glucose, insulin, ghrelin, glucagon-like peptide-1 (GLP-1), and peptide-tyrosine-tyrosine (PYY) concentrations. The blood for insulin,

glucose, and ghrelin analysis was collected into EDTA-containing tubes. For PYY analysis, blood was collected into EDTA-containing tubes in which dipeptidyl peptidase IV inhibitor (10 μL/mL blood) and aprotinin (500 KIU/mL blood) was added. The blood for GLP-1 was collected into EDTA-containing tubes to which dipeptidyl peptidase IV inhibitor (10 μL/mL blood) was added. After the collection of blood into the tubes, blood samples were immediately centrifuged for 10 min at 4°C at 3000 rpm. For ghrelin analysis, phenylmethylsulfonyl fluoride, dissolved in methanol, and hydrochloric acid were added to the plasma. Plasma samples were immediately frozen in liquid nitrogen and stored at –80°C until analyzed further. Plasma concentrations of insulin, PYY, and active ghrelin were measured by radioimmunoassay (Linco Research Inc, St Charles, MO). Plasma glucose concentrations were determined by using the hexokinase method (Glucose HK 125 kit; ABX diagnostics, Montpellier, France). Plasma active GLP-1 concentrations were analyzed by enzyme-linked immunosorbent assay (EGLP-35K; Linco Research Inc, St Charles, MO).

### Appetite profile

On day 4, before and after each meal, appetite profiles were scored at the following time points: 0900 (0 min), 0930 (30 min), 1000 (60 min), 1030 (90 min), 1100 (120 min), 1200 (180 min), 1300 (240 min), 1345 (285 min), 1415 (315 min), 1445 (345 min), 1515 (375 min), 1545 (405 min), 1645 (465 min), 1745 (525 min), 1930 (630 min), 2000 (660 min), 2030 (690 min), 2100 (720 min), 2130 (750 min), and 2230 (810 min). Appetite was scored with a 100-mm anchored VAS. Four questions were asked, anchored by “not at all” to “extremely,” namely “How satiated do you feel?”, “How full do you feel?”, “How hungry are you?”, and “How is your desire to eat?”.

### Body composition

Body composition was determined according to the 3-compartment model with the use of hydrodensitometry and the deuterium dilution (<sup>2</sup>H<sub>2</sub>O) technique (20, 21) and was calculated by using the combined equation of Siri (22).

### Statistical analysis

Data from energy expenditure and substrate balances are presented as means ± SEMs, whereas appetite scores and blood data are presented as mean changes from baseline (Δ) ± SEM, unless otherwise indicated. The area under the curve (AUC) or above the curve (AAC) of the changes over time (at 0900–2230 for appetite scores and at 0845–2045 for blood variables) were calculated by using the trapezoidal method. A 2-factor repeated-measures analysis of variance was carried out for determination of possible differences between the 25En% and 10En% casein diets. To determine relations between variables, regression analyses were performed. The level of statistical significance was set at *P* < 0.05. Statistical analyses were performed by using StatView 5.0 (SAS Institute Inc, Cary, NC).

### RESULTS

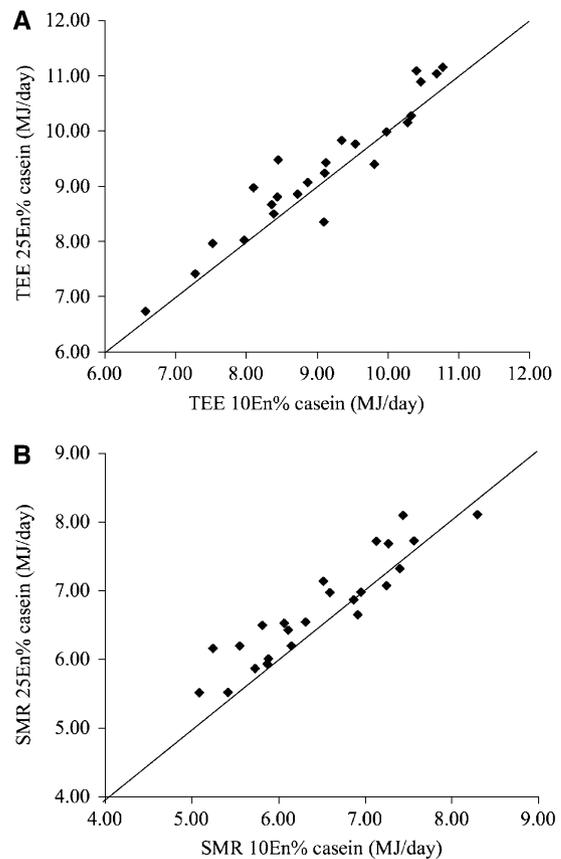
TEE increased 2.6% more with the 25En% casein diet than with the 10En% casein diet: 9.30 ± 0.24 and 9.07 ± 0.24 MJ/d,

respectively ( $P < 0.01$ ). The components of TEE—SMR and RMR—were  $6.74 \pm 0.16$  compared with  $6.48 \pm 0.17$  MJ/d ( $P < 0.001$ ) and  $7.44 \pm 0.17$  compared with  $7.24 \pm 0.18$  MJ/d ( $P < 0.01$ ) with the 25En% and 10En% casein diets, respectively. DIT and AEE were not significantly different between the 2 diets. DIT was  $0.70 \pm 0.06$  and  $0.76 \pm 0.09$  MJ/d (NS) and AEE was  $1.86 \pm 0.11$  and  $1.83 \pm 0.11$  MJ/d (NS) with the 25En% and 10En% casein diets, respectively. Radar counts (activity) during sleep were not different between the 2 diets ( $P = 0.27$ ). TEE and SMR for both diets for each subject are plotted in **Figure 1** in 2 graphs with the line of identity.

Subjects were in a slightly positive energy balance with both diets ( $P < 0.0001$ ; **Figure 2A**). Subjects were in a significantly lower positive energy balance with the 25En% casein diet than with the 10En% casein diet ( $P < 0.01$ ). With respect to macronutrient balances, both the protein and fat balances were significantly different between the 2 diets ( $P < 0.0001$ ), whereas carbohydrate balances were not (**Figure 2B**). The 25En% casein diet resulted in a positive protein balance ( $P < 0.0001$ ), negative fat balance ( $P < 0.0001$ ), and positive carbohydrate balance ( $P < 0.0001$ ); the 10En% casein diet resulted in a negative protein balance ( $P < 0.05$ ), fat balance (NS), and positive carbohydrate balance ( $P < 0.0001$ ). The RQ did not differ between the 2 diets, which was  $0.87 \pm 0.00$  with the 25En% casein diet and  $0.86 \pm 0.00$  with the 10En% casein diet. Protein oxidation was higher ( $P < 0.0001$ ) and fat oxidation was lower ( $P < 0.0001$ ) with the 25En% casein diet than with the 10En% casein diet, whereas carbohydrate oxidation was similar with both diets (**Table 2**).

Hunger was significantly more suppressed with the 25En% casein diet than with the 10En% casein diet for AAC as well as at various time points for hunger ratings over time ( $P < 0.05$ ; **Figure 3**). With respect to hunger ratings, significant differences of 41% AAC on the VAS were present. Ratings for desire to eat and hunger were similar (data not shown). In accordance with the hunger ratings, subjects reached higher satiety levels with the 25En% casein diet than with the 10En% casein diet for AUC as well as at various time points for satiety ratings over time ( $P < 0.05$ ). A significant difference in satiety ratings of 33% AUC on the VAS was present. Ratings for fullness and satiety were similar (data not shown). With the 25En% diet, changes in satiety and changes in hunger were related to changes in SMR ( $r^2 = 0.261$  and  $0.205$ , respectively;  $P < 0.05$ ). With an increased SMR, satiety increased and hunger decreased. Both relations were not present with the 10En% diet.

With both diets, plasma glucose, insulin, GLP-1, and PYY concentrations increased after each meal, whereas plasma ghrelin concentrations decreased (**Figure 4**). Compared with the 10En% casein diet, glucose concentrations were significantly lower after lunch and dinner and insulin concentrations were significantly lower after dinner with the 25En% casein diet. The AUC for plasma glucose was significantly lower with the 25En% casein diet than with the 10En% casein diet:  $5.1 \pm 1.7$  compared with  $7.3 \pm 1.5$  mmol/L · h ( $P < 0.05$ ). After each meal, GLP-1 concentrations were significantly lower with the 25En% casein diet than with the 10En% casein diet, whereas PYY concentrations were significantly lower after breakfast and lunch. Ghrelin concentrations were not significantly different between the 2 diets. No correlations between these blood variables and appetite scores were found.

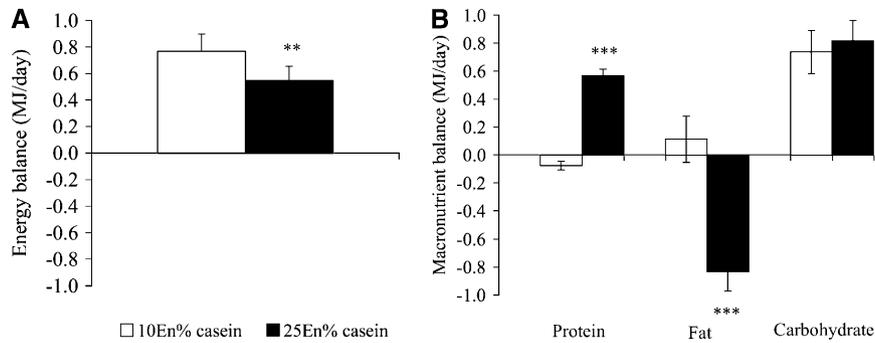


**FIGURE 1.** Total energy expenditure over 24 h (TEE; A) and sleeping metabolic rate (SMR; B) for individual subjects during consumption of a diet with 25% of energy (25En%) from casein compared with a diet with 10% of energy (10En%) from casein ( $n = 24$ ). The lines of identity are shown. Two-factor repeated-measures ANOVA was used to determine differences between the 25En% and 10En% casein diets for TEE (mean  $\pm$  SEM:  $9.30 \pm 0.24$  and  $9.07 \pm 0.24$  MJ/d, respectively;  $P < 0.01$ ) and SMR (mean  $\pm$  SEM:  $6.74 \pm 0.16$  and  $6.48 \pm 0.17$  MJ/d, respectively;  $P < 0.001$ ).

## DISCUSSION

After consumption of a 25En% protein diet for 3 d, consumption of the 25En% casein diet on day 4 resulted in a 2.6% higher TEE and a higher SMR than did the consumption of the isoenergetic 10En% casein diet on day 4 after consumption of a 10En% protein diet for 3 d. With respect to macronutrient balances, a 25En% casein diet resulted in a positive protein balance and a negative fat balance compared with the negative protein balance and negative fat balance with the 10En% casein diet; carbohydrate balances were the same with both diets. Hunger was suppressed 41% more and satiety increased 33% more with the 25En% casein diet than with the 10En% casein diet. These results were based on isoenergetic diets, which indicated that the observed effects were only due to differences in macronutrient composition between the diets.

Increasing the casein content of the diet, while reducing the fat content of the diet and keeping the carbohydrate content the same, resulted in an increase in TEE of 2.6%. Between the 25En% and the 10En% casein diets, protein was exchanged with fat in an energy amount of 1.52 MJ/d. The difference in TEE was an increase of 0.23 MJ/d with the 25En% casein diet; AEE values were similar between the 2 diets. This implies that the high-



**FIGURE 2.** Mean ( $\pm$ SEM) differences in 24-h energy balances (A) and 24-h macronutrient balances (B) between the diet with 25% of energy (25En%) from casein and the diet with 10% of energy (10En%) from casein ( $n = 24$ ) determined by 2-factor repeated-measures ANOVA. \*\* $P < 0.01$ . \*\*\* $P < 0.0001$ . Mean ( $\pm$  SEM) protein balances with the 25En% casein and 10En% casein diets were  $30.7 \pm 2.6$  and  $-4.1 \pm 1.7$  g/d, respectively. Differences between intake and oxidation or expenditure for each diet (2-factor repeated-measures ANOVA) were as follows: 10En% casein diet [positive energy balance ( $P < 0.0001$ ), negative protein balance ( $P < 0.05$ ), fat balance (NS), positive carbohydrate balance ( $P < 0.0001$ )] and 25En% casein diet [positive energy balance ( $P < 0.0001$ ), positive protein balance ( $P < 0.0001$ ), negative fat balance ( $P < 0.0001$ ), positive carbohydrate balance ( $P < 0.0001$ )].

protein diet resulted in an additional thermogenesis of 15% from the exchanged energy amount, which is lower than the thermic effect of protein known from the literature, ie, 20–30% of protein intake (6). One explanation for the apparently lower thermogenic effect was the higher protein intake together with a lower fat intake with the 25En% casein diet. This resulted in a decreased thermic effect of fat. Second, the theoretical thermic effect of 20–30% was calculated on the basis of complete oxidation of all ingested protein. However, the difference in protein balance between the 25En% and 10En% casein diets resulted in a difference in cumulative protein balance of 0.65 MJ/d. Urea synthesis is an energy-consuming process, which accounts for almost one-third of the thermic effect of protein (23). Because not all protein was oxidized with accompanying urea excretion, less energy was needed, which resulted in a lower net thermic effect.

The higher TEE was mainly due to a higher SMR. We suggest that the increased SMR in the present study may have been due in part to the presence of the thermic effect of the diet, because Reed and Hill (24) found that the thermic response of food may last for >6 h. Because the subjects were already consuming a 25En% protein diet 3 d before they consumed the 25En% casein diet, the higher SMR may in part also have been the result of an adaptation of the body to a high-protein diet with respect to an enhanced protein turnover (25). Because of the similarity in radar counts, it was concluded that sleeping quality was not different between the diets. The higher SMR with the 25En%

casein diet agreed with the observations by Lejeune et al (8), who compared a general high-protein diet (30En%, 30En%, and 40En% as protein, fat, and carbohydrate, respectively) with an adequate-protein diet (10En%, 30En%, and 60En% as protein, fat, and carbohydrate, respectively).

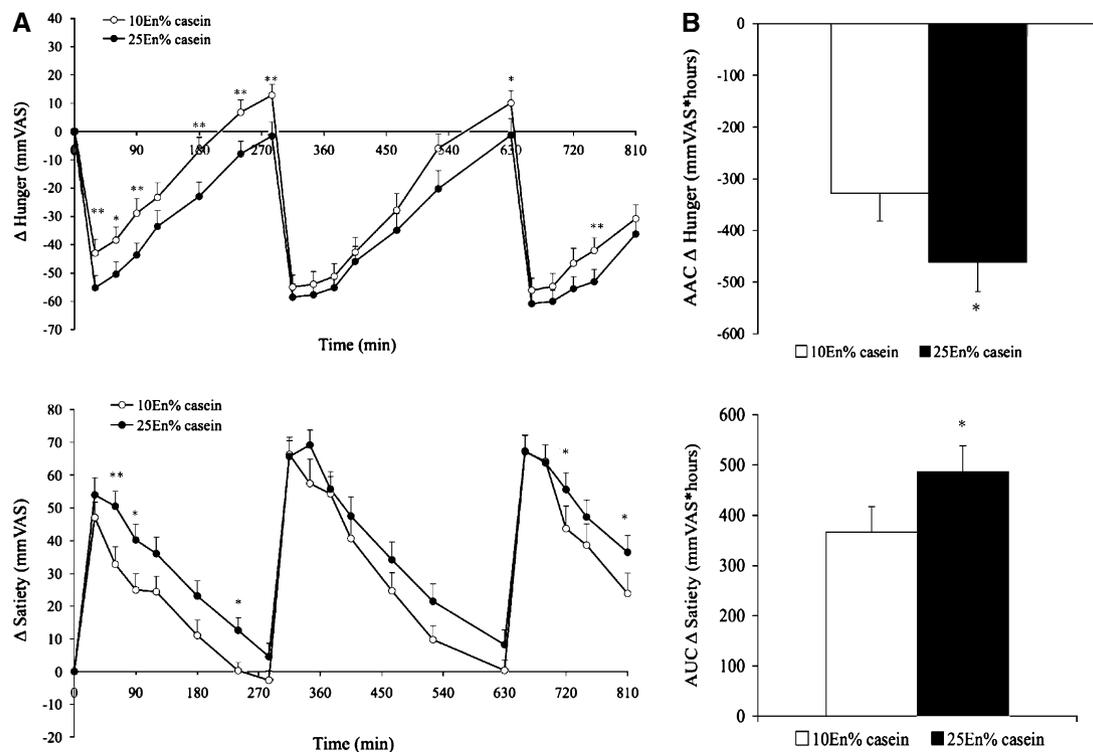
The differences in macronutrient intakes were reflected in the 24-h oxidations of the macronutrients: 24-h carbohydrate oxidation was the same between both diets, whereas 24-h protein oxidation was significantly higher and 24-h fat oxidation was significantly lower with the 25En% casein diet than with the 10En% casein diet. This resulted in a positive protein balance and a negative fat balance. We may conclude from the positive protein balance with the 25En% casein diet that the body is still in positive nitrogen balance after consumption of a high-protein diet for 4 d. Although the body is thought to be in a transitory state of positive nitrogen balance, we expect that a positive protein balance may be sustained for a longer period of time, but to a lesser extent. Soenen et al (26) observed that, in energy balance, a 3-mo dietary intervention with an increased protein intake resulted in a significant increase in fat-free mass of 0.73 kg, independently of change in body weight and with similar physical activity levels during the intervention period. With respect to fat balance, lowering the fat content of the diet while increasing the protein content resulted in a negative fat balance, similar to the finding of Lejeune et al (8). Therefore, a high-protein diet plays a role in the stimulation of fat oxidation per se, even during energy balance. Because of protein turnover,

**TABLE 2**  
Macronutrient intake and energy expenditure/oxidation measured over 24 h in a respiration chamber<sup>1</sup>

	Macronutrient intake		Expenditure/oxidation	
	10En% casein diet	25En% casein diet	10En% casein diet	25En% casein diet
Energy (MJ/d)	9.83 $\pm$ 0.26	9.84 $\pm$ 0.26	9.07 $\pm$ 0.24	9.30 $\pm$ 0.24 <sup>2</sup>
Protein (MJ/d)	1.01 $\pm$ 0.03	2.53 $\pm$ 0.07 <sup>3</sup>	1.09 $\pm$ 0.04	1.96 $\pm$ 0.07 <sup>3</sup>
Protein (g/d)	54.9 $\pm$ 1.5	137.3 $\pm$ 3.6 <sup>3</sup>	59.0 $\pm$ 2.1	106.6 $\pm$ 3.9 <sup>3</sup>
Fat (MJ/d)	3.51 $\pm$ 0.09	2.01 $\pm$ 0.05 <sup>3</sup>	3.40 $\pm$ 0.16	2.84 $\pm$ 0.16 <sup>2</sup>
Carbohydrate (MJ/d)	5.31 $\pm$ 0.14	5.31 $\pm$ 0.14	4.57 $\pm$ 0.21	4.49 $\pm$ 0.15

<sup>1</sup> All values are means  $\pm$  SEMs;  $n = 24$ . 25En%, diet with 25% of energy from casein; 10En%, diet with 10% of energy from casein.

<sup>2,3</sup> Significantly different from the 10En% casein diet (2-factor repeated-measures ANOVA): <sup>2</sup> $P < 0.01$ , <sup>3</sup> $P < 0.0001$ .



**FIGURE 3.** Mean ( $\pm$ SEM) changes from baseline (A) and area above the curve (AAC) and area under the curve (AUC; B) for hunger and satiety scores with the diet with 25% of energy (25En%) from casein and the diet with 10% of energy (10En%) from casein measured with anchored 100-mm visual analog scales (VASs).  $n = 24$ . Two-factor repeated-measures ANOVA: \* $P < 0.05$ , \*\* $P < 0.01$ . For the changes from baseline, significant differences between the 2 diets at the same time point are indicated at various time points.

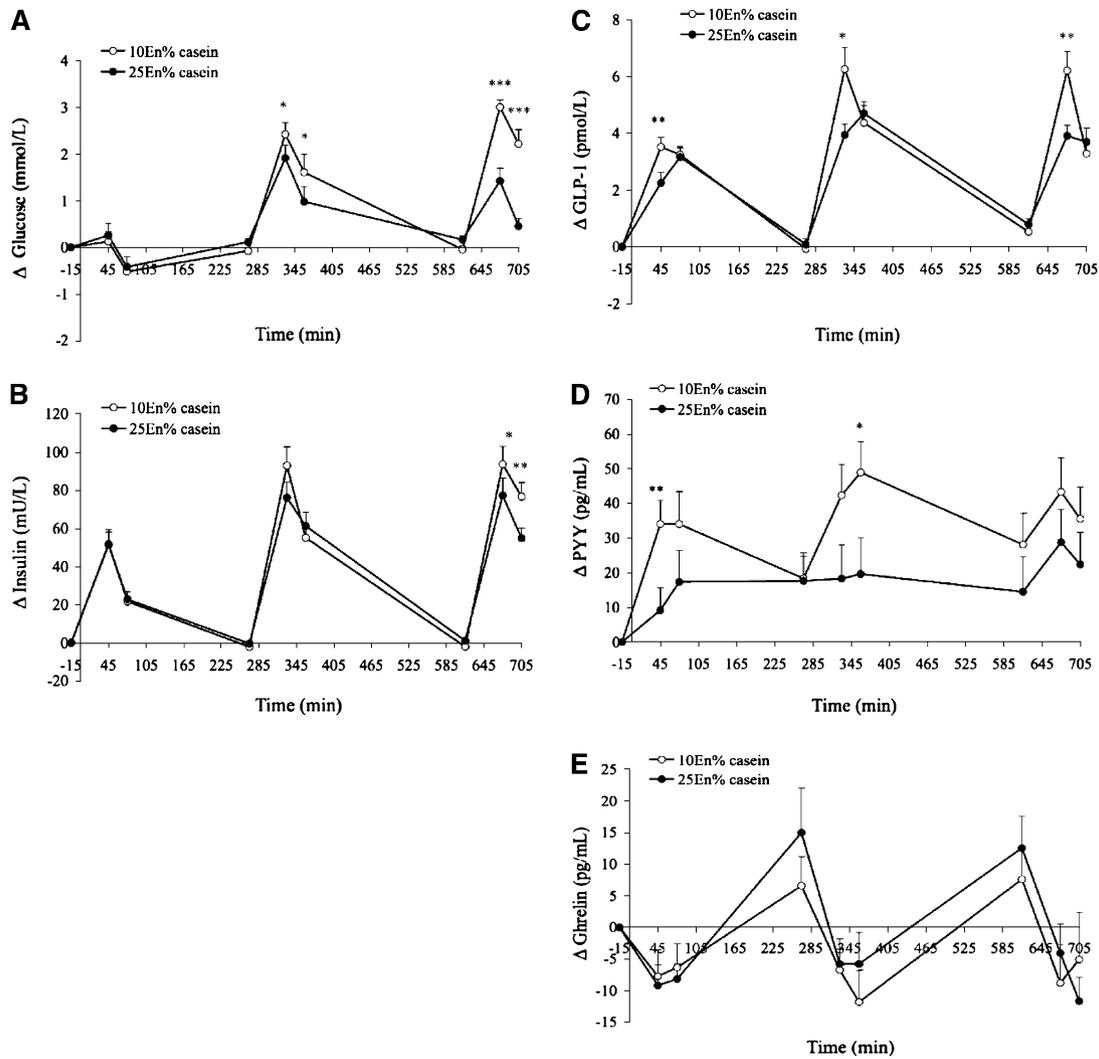
the result of an increased protein intake with the 25En% casein diet was a higher protein synthesis than protein oxidation, which resulted in a positive protein balance—the leverage hypothesis (27). This process may occur at the expense of fat and result in higher fat oxidation. The observed negative fat balance with a high-protein diet may also favor fat loss in the long term, in agreement with the findings of weight-maintenance studies (28, 29). With respect to carbohydrate balances, carbohydrate intake and oxidation were the same between both diets, which resulted in carbohydrate balances that did not differ significantly between the 2 diets. Because of a slight positive energy balance, excess energy may be partly stored as glycogen.

The 25En% casein diet on day 4 resulted in 41% lower hunger and 33% higher satiety ratings than did the isoenergetic 10En% casein diet on day 4. In a previous study (12), we observed higher satiety ratings over 4 h after the consumption of identical vanilla custards at breakfast, which coincided with prolonged elevated concentrations of amino acids, indicating a slower gastric emptying. Thus, prolonged elevated concentrations of amino acids may have contributed to the higher satiety ratings, in agreement with Mellinkoff et al's amino static theory (30). Moreover, SMR contributed to the higher satiety and lower hunger ratings with the 25En% casein diet, in that a significant positive relation was found between satiety and SMR, and a significant inverse relation was found between hunger and SMR. The 25En% protein condition also resulted in a higher protein oxidation and higher energy expenditure. In a previous study, Westerterp-Plantenga et al (9) found that differences in DIT correlated with differences in satiety over 24 h. On the basis of 3 other studies that observed satiety scores under limited

oxygen availability conditions (31–33), Westerterp-Plantenga et al suggested that oxygen availability becomes limiting with an increased metabolic rate at rest, which seems to be perceived by the subjects as a reduction in the possibility to eat and therefore is rated as an increase in satiety. This suggestion might help explain the correlation found between SMR and appetite scores in the present study.

Although the carbohydrate content of both diets was the same (55En%), plasma glucose concentrations were lower with the 25En% casein diet, probably because of slower gastric emptying. The same was reflected in the insulin responses, ie, lower insulin concentrations were observed after dinner with the 25En% casein diet. These results agree with our previous research (12), in which subjects also had lower glucose and insulin responses after consuming the same custards for breakfast. A similar pattern was found for GLP-1 and PYY. Significantly lower plasma GLP-1 and PYY concentrations were observed over time with the 25En% casein meals than with the 10En% casein meals. Again, this may be the result of delayed gastric emptying. However, the less pronounced hormone responses were not reflected in the appetite scores. These results indicate that physiologic responses are not always in line with perceived satiety-related feelings, which indicates that the regulation of appetite is a complex process in which different mechanisms [eg, metabolic rate, (an)orexigenic hormones, and protein metabolism] may play a role and in which not only one factor can be held responsible for the perceived satiety.

This was the first time that 24-h measurements of energy expenditure and macronutrient oxidation were performed to study differences between 2 diets with different concentrations of casein



**FIGURE 4.** Mean ( $\pm$ SEM) changes from baseline in plasma glucose (A), insulin (B), glucagon-like peptide 1 (GLP-1; C), peptide YY (PYY; D), and ghrelin (E) concentrations for the diet with 25% of energy (25En%) from casein and the diet with 10% of energy (10En%) from casein.  $n = 24$ . Significant differences between the 2 diets at the same time point are indicated at various time points (2-factor repeated-measures ANOVA): \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.0001$ .

(25En% compared with 10En%) and with casein as the only protein source. In addition, this study had a high power (24 subjects participated in this research) and the subjects had a broad range of body mass indexes. We conclude that after consumption of a 25En% protein diet for 3 d, consumption of a 25En% casein diet on day 4 boosts energy expenditure, protein balance, satiety, and negative fat balance compared with the consumption of an isenergetic 10En% casein diet on day 4 after consumption of a 10En% protein diet for 3 d. Thus, a 25En% casein diet appears to be beneficial in weight management. Changes in SMR were related to changes in satiety and hunger with the 25En% casein diet.

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## REFERENCES

- Wilborn C, Beckham J, Campbell B, et al. Obesity: prevalence, theories, medical consequences, management, and research directions. *J Int Soc Sports Nutr* 2005;2:4–31.
- Westerterp-Plantenga MS. Protein intake and energy balance. *Regul Pept* 2008;149:67–9.
- Westerterp-Plantenga MS, Lejeune MP. Protein intake and body-weight regulation. *Appetite* 2005;45:187–90.
- Halton TL, Hu FB. The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *J Am Coll Nutr* 2004;23:373–85.
- Paddon-Jones D, Westman E, Mattes RD, Wolfe RR, Astrup A, Westerterp-Plantenga M. Protein, weight management, and satiety. *Am J Clin Nutr* 2008;87:1558S–61S.
- Tappy L. Thermic effect of food and sympathetic nervous system activity in humans. *Reprod Nutr Dev* 1996;36:391–7.
- Westerterp KR, Wilson SA, Rolland V. Diet induced thermogenesis measured over 24h in a respiration chamber: effect of diet composition. *Int J Obes Relat Metab Disord* 1999;23:287–92.
- Lejeune MP, Westerterp KR, Adam TC, Luscombe-Marsh ND, Westerterp-Plantenga MS. 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 chamber. *Am J Clin Nutr* 2006;83:89–94.
- Westerterp-Plantenga MS, Rolland V, Wilson SA, Westerterp KR. Satiety related to 24 h diet-induced thermogenesis during high

- protein/carbohydrate vs high fat diets measured in a respiration chamber. *Eur J Clin Nutr* 1999;53:495–502.
10. Veldhorst M, Smeets A, Soenen S, et al. Protein-induced satiety: effects and mechanisms of different proteins. *Physiol Behav* 2008;94:300–7.
  11. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci U S A* 1997;94:14930–5.
  12. Veldhorst MA, Nieuwenhuizen AG, Hochstenbach-Waelen A, et al. Comparison of the effects of a high- and normal-casein breakfast on satiety, 'satiety' hormones, plasma amino acids and subsequent energy intake. *Br J Nutr* 2009;101:295–303.
  13. Tesseraud S, Metayer S, Duchene S, Bigot K, Grizard J, Dupont J. Regulation of protein metabolism by insulin: value of different approaches and animal models. *Domest Anim Endocrinol* 2007;33:123–42.
  14. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res* 1985; 29:71–83.
  15. Webb P. 24-hour energy expenditure and the menstrual cycle. *Am J Clin Nutr* 1986;44:614–9.
  16. Westerterp KR, Speakman JR. Physical activity energy expenditure has not declined since the 1980s and matches energy expenditures of wild mammals. *Int J Obes (Lond)* 2008;32:1256–63.
  17. Harris JA, Benedict FG. A biometric study of human basal metabolism. *Proc Natl Acad Sci U S A* 1918;4:370–3.
  18. Schoffelen PF, Westerterp KR, Saris WH, Ten Hoor F. A dual-respiration chamber system with automated calibration. *J Appl Physiol* 1997;83:2064–72.
  19. Brouwer E. On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals, from gaseous exchange (oxygen intake and carbonic acid output) and urine-N. *Acta Physiol Pharmacol Neerl* 1957;6:795–802.
  20. Schoeller DA, van Santen E, Peterson DW, Dietz W, Jaspán J, Klein PD. Total body water measurement in humans with  $^{18}\text{O}$  and  $^2\text{H}$  labeled water. *Am J Clin Nutr* 1980;33:2686–93.
  21. van Marken Lichtenbelt WD, Westerterp KR, Wouters L. Deuterium dilution as a method for determining total body water: effect of test protocol and sampling time. *Br J Nutr* 1994;72:491–7.
  22. Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. *Nutrition* 1993;9:480–91, discussion 480, 492.
  23. McBride BW, Kelly JM. Energy cost of absorption and metabolism in the ruminant gastrointestinal tract and liver: a review. *J Anim Sci* 1990; 68:2997–3010.
  24. Reed GW, Hill JO. Measuring the thermic effect of food. *Am J Clin Nutr* 1996;63:164–9.
  25. Zangarelli A, Chanseume E, Morio B, et al. Synergistic effects of caloric restriction with maintained protein intake on skeletal muscle performance in 21-month-old rats: a mitochondria-mediated pathway. *FASEB J* 2006;20:2439–50.
  26. Soenen S, Plasqui G, Smeets AJ, Westerterp-Plantenga M. Protein induced increase in stimulated fat oxidation in energy balance. *Clin Nutr Suppl* 2008;3:28.
  27. Wolfe RR. Radioactive and stable isotope tracers in biomedicine: principles and practice of kinetic analysis. New York, NY: Wiley-Liss Inc, 1992.
  28. Westerterp-Plantenga MS, Lejeune MP, Nijs I, van Ooijen M, Kovacs EM. High protein intake sustains weight maintenance after body weight loss in humans. *Int J Obes Relat Metab Disord* 2004;28: 57–64.
  29. Lejeune MP, Kovacs EM, Westerterp-Plantenga MS. Additional protein intake limits weight regain after weight loss in humans. *Br J Nutr* 2005; 93:281–9.
  30. Mellinkoff SM, Frankland M, Boyle D, Greipel M. Relationship between serum amino acid concentration and fluctuations in appetite. *J Appl Physiol* 1956;8:535–8.
  31. Westerterp-Plantenga MS, Westerterp KR, Rubbens M, Verwegen CR, Richelet JP, Gardette B. Appetite at "high altitude" [Operation Everest III (Comex-'97)]: a simulated ascent of Mount Everest. *J Appl Physiol* 1999;87:391–9.
  32. Westerterp-Plantenga MS, Verwegen CR, Ijedema MJ, Wijckmans NE, Saris WH. Acute effects of exercise or sauna on appetite in obese and nonobese men. *Physiol Behav* 1997;62:1345–54.
  33. Schols AM. Nutrition and outcome in chronic respiratory disease. *Nutrition* 1997;13:161–3.