

# Single-Protein Casein and Gelatin Diets Affect Energy Expenditure Similarly but Substrate Balance and Appetite Differently in Adults

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# Single-Protein Casein and Gelatin Diets Affect Energy Expenditure Similarly but Substrate Balance and Appetite Differently in Adults<sup>1–3</sup>

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## Abstract

Increasing the protein content of a diet results in increased satiety and energy expenditure (EE). It is not clear whether the magnitude of these effects differs between proteins differing in concentrations of indispensable amino acids (IAA). We hypothesized that a protein lacking IAA may stimulate appetite suppression and EE and may limit positive protein balance. Therefore, we compared appetite, EE, and substrate balances between gelatin (incomplete protein) and casein (complete protein) in single-protein diets with either 25 or 10% of energy (En%) from protein. During a 36-h stay in a respiration chamber, 23 healthy men ( $n = 11$ ) and women ( $n = 12$ ) (BMI,  $22.2 \pm 2.3$  kg/m<sup>2</sup>; age,  $25 \pm 7$  y) consumed 4 isoenergetic diets: 25 En% (25/20/55 En% protein/fat/carbohydrate) and 10 En% (10/35/55 En% protein/fat/carbohydrate) casein or gelatin diet in a randomized crossover design. For 3 d before the study, participants consumed a diet at home with similar macronutrient distribution as the diet they would receive during the subsequent stay in the chamber. Hunger was suppressed 44% more ( $P < 0.05$ ) and protein balance was more negative when consuming the 10 En% gelatin diet ( $-0.17 \pm 0.03$  MJ/d) compared with the 10 En% casein diet ( $-0.07 \pm 0.03$  MJ/d;  $P < 0.05$ ); carbohydrate and fat balances did not differ between the treatments. EE did not differ when participants consumed the 25 En% or 10 En% diets. Participants were in higher protein balance ( $0.56 \pm 0.05$  vs.  $0.30 \pm 0.04$  MJ/d;  $P < 0.0001$ ), lower carbohydrate balance ( $0.86 \pm 0.14$  vs.  $1.37 \pm 0.17$  MJ/d;  $P < 0.01$ ), and similar negative fat balance when they consumed the 25 En% casein compared with the 25 En% gelatin diet. In conclusion, when we compared the effects of an incomplete protein (gelatin) and a complete protein (casein) at 2 concentrations over 36 h, gelatin resulted in a greater appetite suppression; casein caused a greater positive (smaller negative) protein balance, and effects on EE did not differ. In terms of weight loss for people with obesity, the greater hunger-suppressing effect of gelatin may play a role in reducing energy intake if this effect is maintained when consuming a gelatin diet in the long term. In addition, long-term use of casein may contribute to preservation of fat-free mass. *J. Nutr.* 139: 2285–2292, 2009.

## Introduction

Obesity is a major health concern worldwide and effective treatment 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 elevation of protein intake affects both short- and long-term mechanisms (2–7). First, an increased protein intake increases satiety despite similar or lower energy intake. Second, thermogenesis is increased. Third, energy efficiency is lower

during overfeeding. Fourth, fat free mass is preserved. Dietary protein-induced satiety may be due to amino acid composition (6,8–13), anorexigenic hormone concentrations (6), and energy expenditure (EE)<sup>6</sup> (6,7).

Currently, it is not clear whether such effects differ between proteins differing in concentrations of indispensable amino acids (IAA). Casein and gelatin are 2 contrasting proteins with respect to their amino acid composition. Casein is a complete protein, because it contains all IAA, whereas gelatin is an incomplete

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<sup>3</sup> Supplemental Tables 1–3 are available with the online posting of this paper at [jn.nutrition.org](http://jn.nutrition.org).

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<sup>6</sup> Abbreviations used: AAC, area above the curve; AEE, activity-induced energy expenditure; AUC, area under the curve; BMR, basal metabolic rate; DIT, diet induced thermogenesis; EE, energy expenditure; En%, percentage of energy; GLP-1, glucagon-like peptide-1; IAA, indispensable amino acid; PAL, physical activity level; PYY, peptide-tyrosine-tyrosine; RMR, resting metabolic rate; SMR, sleeping metabolic rate; TEE, total energy expenditure; TFEQ, 3-factor eating questionnaire; VAS, visual analogue scale.

protein, because it lacks the essential amino acid tryptophan and contains low amounts of, e.g. methionine and histidine. Contrasting gelatin and casein is of interest with respect to appetite, EE, and protein balance for the following reasons.

First, in a previous study (11), higher satiety and lower hunger ratings and/or lower energy intake were observed after a single-protein breakfast with either 10 or 25% of energy (En%) from gelatin compared with a 10 En% or 25 En% protein breakfast with casein as the only protein source. This may relate to a mechanism observed in metazoans, where it was discovered that the transfer RNA/general control nonderepressing 2 kinase/phospho-protein eukaryotic initiation factor 2 $\alpha$  system in the brain can detect a deficiency of IAA in the diet from a decline in serum amino acid levels, leading to a behavioral response that rejects consumption of imbalanced diets (14–17) and thus appears as hunger suppression. In addition, the characteristic of casein as a slow protein, indicating that the plasma appearance of dietary amino acids is slower, lower, and prolonged after digestion and absorption of casein compared with, e.g., gelatin (18), leading to diminished satiety is relevant as well. Second, because amino acid catabolism results in a wide variety of carbon chains and cofactors, large differences exist with respect to the efficacy by which amino acids are oxidized (19). Taking into account the amino acid composition of gelatin and casein and the energy expended for ATP synthesis (19) (15.6 kJ · ATP<sup>-1</sup> · g<sup>-1</sup> for gelatin and 8.2 kJ · ATP<sup>-1</sup> · g<sup>-1</sup> for casein), we hypothesize that gelatin consumption may exert a relatively larger increase in EE. Third, consumption of a complete protein may lead to a more positive protein balance compared with an incomplete protein.

We hypothesize that the use of an incomplete protein (gelatin) compared with a complete protein (casein) in single-protein diets may stimulate appetite suppression and EE and may limit a positive protein balance. To test this hypothesis, we aimed to study the effects of gelatin and casein, contrasted in 10 En% and 25 En% single-protein diets, on appetite profile, EE over 24 h, and substrate balances.

## Materials and Methods

**Participants.** The study protocol was approved by the Medical Ethical Committee of the Maastricht University Medical Center. Thirty participants between 18 and 55 y old with a BMI between 20 and 33 kg/m<sup>2</sup> were recruited by advertisements on notice boards of Maastricht University and in local newspapers. Participants underwent a medical screening and 24 participants (12 men, 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 body weight during the last 3 mo, not following a diet, and not cognitively dietary restrained. The Dutch translation of the 3-factor eating questionnaire (TFEQ) was used for assessing eating behavior of the participants (20). Analyses were executed for 22 participants (11 men, 11 women) for the comparison between the 10 En% protein diets and 23 participants (11 men, 12 women) for the comparison between the 25 En% protein diets, because of dropouts (see Table 1 for participant characteristics). Participants signed an informed consent form before participating in the study.

**Body composition.** Body composition was determined according to the 3-compartment model based on body weight, body volume as measured with hydrodensitometry, and total body water as measured with the deuterium dilution technique (21,22) and was calculated using the combined equation of Siri (23). Body composition measurements were assessed during (deuterium dilution) and immediately after (body weight and hydrodensitometry) the first stay in the respiration chamber.

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

	Value
Men/women, n/n	11/12
Age, y	25 ± 7
Height, m	1.74 ± 0.09
Body weight, kg	67.5 ± 9.6
BMI, kg/m <sup>2</sup>	22.2 ± 2.3
Fat mass, kg	15.1 ± 6.6
Fat-free mass, kg	52.4 ± 9.0
TFEQ1, <sup>2</sup> cognitive restraint	6 ± 3
TFEQ2, <sup>3</sup> disinhibition	4 ± 2
TFEQ3, <sup>4</sup> hunger	5 ± 3

<sup>1</sup> Values are means ± SD, n = 23.

<sup>2</sup> Factor 1 of the TFEQ.

<sup>3</sup> Factor 2 of the TFEQ.

<sup>4</sup> Factor 3 of the TFEQ.

**Experimental design.** The study had a randomized, single-blind, crossover design. Participants came to the university 4 times; each time they stayed for 36 h in a respiration chamber for the measurement of EE and substrate oxidation. For women it was important to be in the same phase of the menstrual cycle (24), so each stay in the respiration chamber was separated by a period of ~4 wk. In random order, the participants received 1 of the 4 diets while in the chamber: a 10 En% or a 25 En% protein diet with either casein or gelatin as the only protein source. The macronutrient distribution for the diets was as follows: 10 En% casein diet and 10 En% gelatin diet, 10/35/55 En% protein/fat/carbohydrate; 25 En% casein diet and 25 En% gelatin diet, 25/20/55 En% protein/fat/carbohydrate. Energy from protein (10 En% vs. 25 En%) was exchanged with energy from fat (35 En% vs. 20 En%, respectively), whereas carbohydrate content (55 En%) remained the same, because carbohydrate ingestion results in insulin secretion and insulin is involved in protein metabolism (25). The 4 diets were mainly offered as custard (one custard with 10 En% from casein, 1 custard with 10 En% from gelatin, 1 custard with 25 En% from casein, and 1 custard with 25 En% from gelatin) produced by NIZO Food Research. The protein content of the 2 casein custards consisted only of casein (Calcium Caseinate S; DMV International), whereas the protein content of the gelatin custards consisted only of gelatin (Solugel LMC/3, PB Gelatins) (see Supplemental Table 1 for amino acid compositions of casein and gelatin). Amino acid compositions were determined as follows. The amino acids were separated by ion exchange chromatography and determined by reaction with ninhydrin using photometric detection. The tryptophan was determined by reversed-phase C18 HPLC with fluorescence detection. The carbohydrate and fat content of all 4 custards consisted of tapioca starch (Farinex VA50T and Perfect-amyl 3108, AVEBE) and sunflower seed oil (Reddy; NV Vandemoortele), respectively. All custards were citrus-vanilla flavored (J.B. de Lange). Extensive product development by food technology and use of a taste panel led to custards that did not differ significantly in color, taste, viscosity, and energy density. Three days before their stay in the respiration chamber, the participants 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 (Supplemental Tables 2 and 3). During the stay in the respiration chamber, blood samples, (24-h) urine samples, and appetite scores on visual analogue scales (VAS) were obtained.

**Energy intake.** Calculations for both the diet at home and the diet in the respiration chamber were based on mean daily energy requirements. The daily energy requirement for the diet at home was estimated by multiplying the basal metabolic rate (BMR) with a physical activity level (PAL) of 1.75, which is the mean PAL for the general population in daily life in The Netherlands (26). BMR was calculated with the equation of Harris-Benedict (27). The energy requirement in the respiration chamber was based on results of a study in which physical

activity was determined in confined conditions (a respiration chamber) resulting in a mean PAL of 1.4 [calculated as 24-h EE/sleeping metabolic rate (SMR)] (28). 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.

**Appetite profile.** On d 4, before and after each meal, appetite profiles were scored at the following time points: 0900 (t0), 0930 (t30), 1000 (t60), 1030 (t90), 1100 (t120), 1200 (t180), 1300 (t240), 1345 (t285), 1415 (t315), 1445 (t345), 1515 (t375), 1545 (t405), 1645 (t465), 1745 (t525), 1930 (t630), 2000h (t660), 2030 (t690), 2100 (t720), 2130 (t750), and 2230 (t810). 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?”

**Blood sampling.** On the first morning of their stay in the respiration chamber (d 4), a Venflon catheter was placed in the antecubital vein for blood sampling. Blood samples were drawn 15 min before each meal and 45 and 75 min after each meal (0845h/t-15, 0945h/t45, 1015h/t75, 1330h/t270, 1430h/t330, 1500h/t360, 1915h/t615, 2015h/t675, 2045h/t705) 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 in EDTA tubes. For PYY analysis, blood was collected in EDTA tubes in which dipeptidyl peptidase IV inhibitor (10 mL/L blood) and aprotinin (500,000 KIU/L or 500 KIU/mL blood) was added. The blood for GLP-1 was collected in EDTA tubes with added dipeptidyl peptidase IV inhibitor (10 mL/L blood). After the collection of blood into the tubes, blood samples were immediately centrifuged for 10 min at 4°C, 3000 × g. 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 were measured by RIA (Human Insulin-Specific RIA kit, Linco Research). Plasma concentrations of PYY were measured by RIA [human PYY (3–36) RIA kit, Linco Research]. Plasma concentrations of active ghrelin were measured by RIA [ghrelin (active) RIA kit, Linco Research]. Plasma glucose concentrations were determined by using the hexokinase method (glucose HK 125 kit, ABX Diagnostics). Plasma active GLP-1 concentrations were analyzed by ELISA (EGLP-35K, Linco Research).

**Plasma amino acid concentrations.** In a previous study, 24 participants were given the same 10 En% casein, 10 En% gelatin, 25 En% casein, and 25 En% gelatin breakfasts and plasma amino acid concentrations were measured during 4 h after the breakfasts (11).

**EE and substrate oxidation.** Participants stayed in the respiration chambers from 2000 of d 3 of their diet at home until 0800 of d 5. The respiration chamber was a 14-m<sup>3</sup> room, furnished with a bed, chair, desk with computer, TV, DVD player, video recorder, telephone, intercom, sink, and toilet. Measurement of EE in the respiration chamber was performed as described before (29). With the exception of strenuous exercise and sleeping, participants were allowed to move freely from 0700–2300. Total EE over 24 h (TEE) and 24-h respiratory quotient 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 participants in the chamber. The following components of EE were calculated: SMR, diet-induced thermogenesis (DIT), resting metabolic rate (RMR), and activity-induced EE (AEE). TEE was calculated by the following equation of Carpenter, as published by Brouwer (30): TEE (kJ/d) = +16 × O<sub>2</sub> (L/d) + 5 × CO<sub>2</sub> (L/d) - 0.95 × P, where P is oxidized protein in g/d. SMR was calculated by assessing the lowest mean activity of the participants during 3 consecutive hours between 0000 and 0700 during the second night of their stay in the respiration chamber. SMR was the mean EE during the 3 consecutive hours in which activity was the lowest. RMR was calculated by plotting EE (y-axis) against radar output (x-axis), both being averaged over 30-min intervals of 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 d 4 until the first voiding on d 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> (2 mol/L), whereas total volume was measured afterward. Nitrogen concentrations were measured with a nitrogen analyzer (CHN-O-Rapid; Heraeus). 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 (30):

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

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

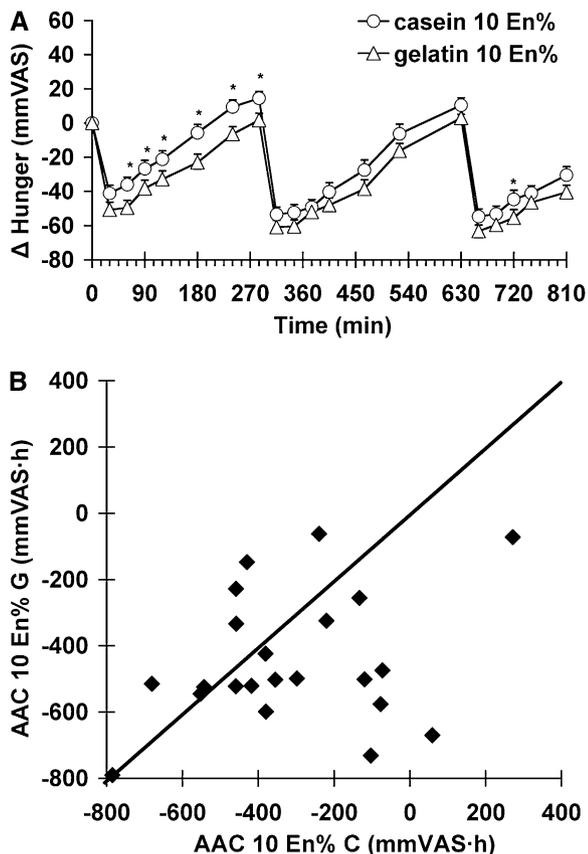
Energy balance in the respiration chamber was calculated as 24-h energy intake minus 24-h EE. Substrate balance in the respiration chamber was calculated as 24-h substrate intake minus 24-h substrate oxidation.

**Statistical analysis.** Data from EE and substrate balances are presented as means ± SEM, whereas appetite scores and blood data are presented as mean changes from baseline (Δ) ± SEM, unless otherwise indicated. The area under/above the curve (AUC/AAC) of the changes over time (0900–2230 for appetite scores, 0845–2045 for blood variables) were calculated by using the trapezoidal method. A 3-factor ANOVA was carried out to compare men with women for the difference between casein and gelatin under 10 En% as well as under 25 En% protein conditions on all variables. Men and women were not affected differently in any of these variables tested and so their data were combined. A repeated-measures ANOVA was conducted for determination of possible differences between the 10 En% casein and 10 En% gelatin diets and between the 25 En% casein and 25 En% gelatin diets on all variables and all time points (for VAS and blood variables). To determine relations between variables, regression analyses were performed. The level of significance was set at *P* < 0.05. Statistical analyses were performed using StatView 5.0 (SAS Institute).

## Results

**Energy balance.** Participants were slightly in a positive energy balance when they consumed each of the 4 diets (*P* < 0.0001). Energy balances did not differ when participants consumed the 10 En% casein (0.82 ± 0.14 MJ/d) and 10 En% gelatin diets (0.87 ± 0.14 MJ/d) or the 25 En% casein (0.56 ± 0.11 MJ/d) and 25 En% gelatin diets (0.70 ± 0.14 MJ/d).

**Appetite and plasma hormone concentrations.** Baseline ratings for hunger and fullness did not differ before each of the diet periods. Hunger was significantly more suppressed when participants consumed the 10 En% gelatin diet compared with the 10 En% casein diet at several time points measured after breakfast and dinner (Fig. 1A). The AAC for the hunger scores was 44% lower when participants consumed the 10 En% gelatin diet (-446 ± 43 mm VAS·h) compared with the 10 En% casein diet (-311 ± 54 mm VAS·h; *P* < 0.05) (Fig. 1B). Fullness score was larger at t810 min when participants consumed the 10 En% gelatin diet (38 ± 6 mm VAS) compared with the 10 En% casein diet (25 ± 7 mm VAS; *P* < 0.05). The AUC for the fullness



**FIGURE 1** Changes from baseline ( $\Delta$ ) of hunger scores when participants consumed the 10 En% casein diet and the 10 En% gelatin diet measured with anchored 100-mm VAS (A). Values are means  $\pm$  SEM,  $n = 22$ . \*Different from the 10 En% gelatin diet at that time,  $P < 0.05$ . AUC of hunger scores for each participant after consumption of a diet with 10 En% from casein compared with a diet with 10 En% from gelatin (B). The line of identity is shown,  $n = 22$ .

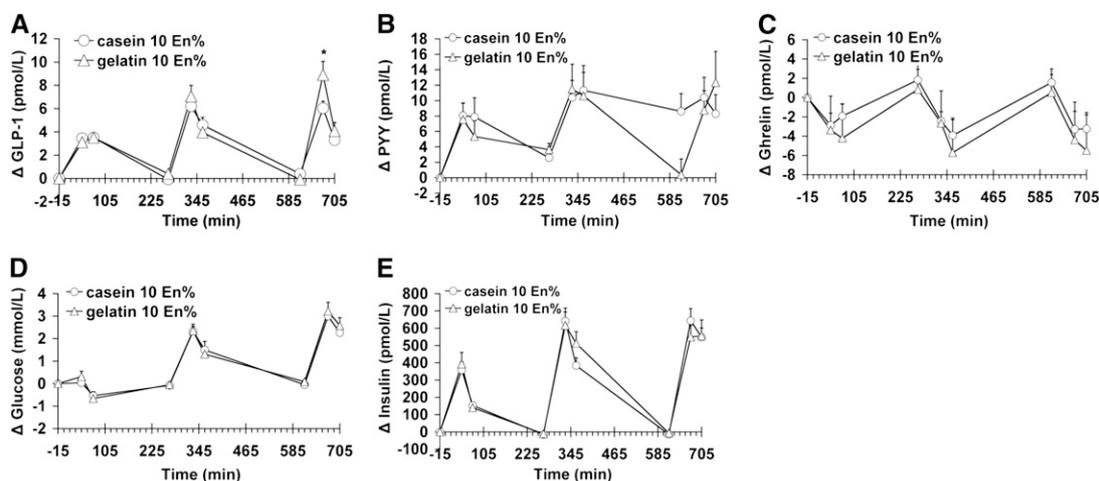
scores over time did not differ between the 2 10 En% protein diets. When participants consumed the 25 En% gelatin diet compared with the 25 En% casein diet, hunger was more suppressed at t810 ( $-49 \pm 4$  vs.  $-34 \pm 5$  mm VAS;  $P < 0.01$ ), but AAC did not differ. Fullness ratings did not differ after they consumed the 25 En% protein diets. Taken together, the gelatin-

containing diets, either at a concentration of 10 or 25 En% protein, suppressed hunger more than the casein-containing diets.

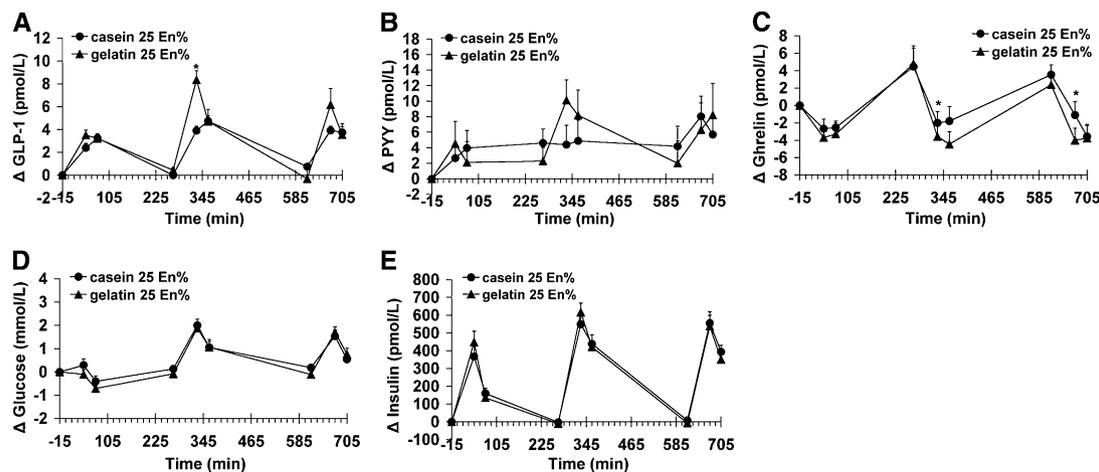
When participants consumed the 10 En% gelatin diet, the plasma GLP-1 concentration was higher after dinner compared with after the 10 En% casein diet ( $P < 0.05$ ; Fig. 2A), whereas plasma PYY (Fig. 2B), ghrelin (Fig. 2C), glucose (Fig. 2D), and insulin (Fig. 2E) concentrations did not differ after the 2 diet periods. When they consumed the 25 En% gelatin diet compared with the 25 En% casein diet, the plasma GLP-1 concentration was higher after lunch ( $P < 0.0001$ ; Fig. 3A), whereas plasma ghrelin concentrations were lower after lunch and dinner ( $P < 0.05$ ; Fig. 3C). Plasma PYY (Fig. 3B), glucose (Fig. 3D), and insulin (Fig. 3E) concentrations did not significantly differ between the 25 En% casein and 25 En% gelatin diet. Taken together, under 10 En% as well as under 25 En% protein conditions, GLP-1 release and/or ghrelin decrease after meals were greater when participants consumed the gelatin diets.

**Amino acids.** In a previous study, 24 participants were given the same 10 En% casein, 10 En% gelatin, 25 En% casein, and 25 En% gelatin breakfasts and plasma amino acid concentrations were measured during 4 h after the breakfasts (11). The AUC for the changes in plasma amino acid concentrations over 4 h after consumption of the 10 En% (Fig. 4A) and 25 En% (Fig. 4B) protein breakfasts were calculated. The plasma concentrations of the essential amino acids histidine, valine, methionine, isoleucine, phenylalanine, tryptophan, and leucine were decreased, expressed as a negative AUC, and lower after consumption of the 10 En% gelatin breakfast compared with the 10 En% casein breakfast ( $P < 0.05$ ). Under 25 En% protein conditions, plasma tryptophan concentration decreased and was lower after consumption of the gelatin compared with the casein breakfast ( $P < 0.05$ ).

**EE.** TEE, RMR, SMR, DIT, and AEE did not differ after participants consumed the 10 En% casein and 10 En% gelatin diets or when they consumed the 25 En% casein and 25 En% gelatin diets (Table 2). Previously, we described the differences between the 10 En% casein and 25 En% casein diets (31) and the differences between the 10 En% gelatin and 25 En% gelatin diets (32).



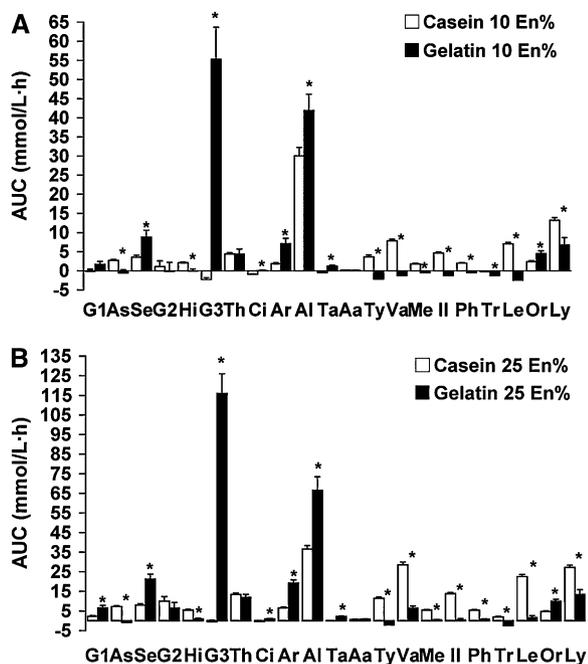
**FIGURE 2** Changes from baseline ( $\Delta$ ) in plasma GLP-1 (A), PYY (B), ghrelin (C), glucose (D), and insulin (E) concentrations when participants consumed the diet with 10 En% from casein and the diet with 10 En% from gelatin. Values are means  $\pm$  SEM,  $n = 22$ . \*Different from the 10 En% casein diet at that time,  $P < 0.05$ .



**FIGURE 3** Changes from baseline ( $\Delta$ ) in plasma GLP-1 (A), PYY (B), ghrelin (C), glucose (D), and insulin (E) concentrations when participants consumed the diet with 25 En% from casein and the diet with 25 En% from gelatin. Values are means  $\pm$  SEM,  $n = 23$ . \*Different from the 25 En% gelatin diet at that time,  $P < 0.05$ .

**Macronutrient balances.** Consumption of both the 10 En% casein and 10 En% gelatin diets resulted in a negative protein balance ( $P < 0.05$ ), a neutral fat balance, and a positive carbohydrate balance ( $P < 0.0001$ ), whereas consumption of both the 25 En% casein and 25 En% gelatin diets resulted in a positive protein balance ( $P < 0.0001$ ), a negative fat balance ( $P < 0.0001$ ), and a positive carbohydrate balance ( $P < 0.0001$ ). Macronutrient balances for both 10 En% protein diets for each participant and macronutrient balances for both 25 En%

protein diets for each participant are plotted in graphs with the line of identity (Fig. 5A–F). Negative protein balance was less when participants consumed the 10 En% casein ( $-0.07 \pm 0.03$  MJ/d) compared with the 10 En% gelatin diet ( $-0.17 \pm 0.03$  MJ/d;  $P < 0.05$ ), whereas neutral fat ( $0.11 \pm 0.17$  vs.  $0.17 \pm 0.15$  MJ/d) and positive carbohydrate balances ( $0.79 \pm 0.15$  vs.  $0.87 \pm 0.17$  MJ/d) did not significantly differ between the diet periods. When participants consumed the 25 En% casein compared with the 25 En% gelatin diet, the positive protein balance ( $0.56 \pm 0.05$  vs.  $0.30 \pm 0.04$  MJ/d;  $P < 0.0001$ ) was larger, negative fat balances ( $-0.85 \pm 0.15$  vs.  $-0.96 \pm 0.16$  MJ/d) did not differ, and the positive carbohydrate balance ( $0.86 \pm 0.14$  vs.  $1.37 \pm 0.17$  MJ/d;  $P < 0.01$ ) was lower. When they consumed the 10 En% casein diet ( $1.08 \pm 0.04$  MJ/d), protein oxidation was lower compared with the 10 En% gelatin diet ( $1.18 \pm 0.05$  MJ/d;  $P < 0.05$ ), whereas fat ( $3.40 \pm 0.17$  vs.  $3.34 \pm 0.16$  MJ/d) and carbohydrate oxidation rates ( $4.52 \pm 0.22$  vs.  $4.44 \pm 0.19$  MJ/d) did not differ between the diet periods. Protein oxidation was lower when participants consumed the 25 En% casein diet compared with the 25 En% gelatin diet ( $1.96 \pm 0.08$  vs.  $2.22 \pm 0.06$  MJ/d, respectively;  $P < 0.0001$ ), fat oxidation did not differ ( $2.85 \pm 0.17$  vs.  $2.96 \pm 0.19$  MJ/d, respectively), and carbohydrate oxidation was higher



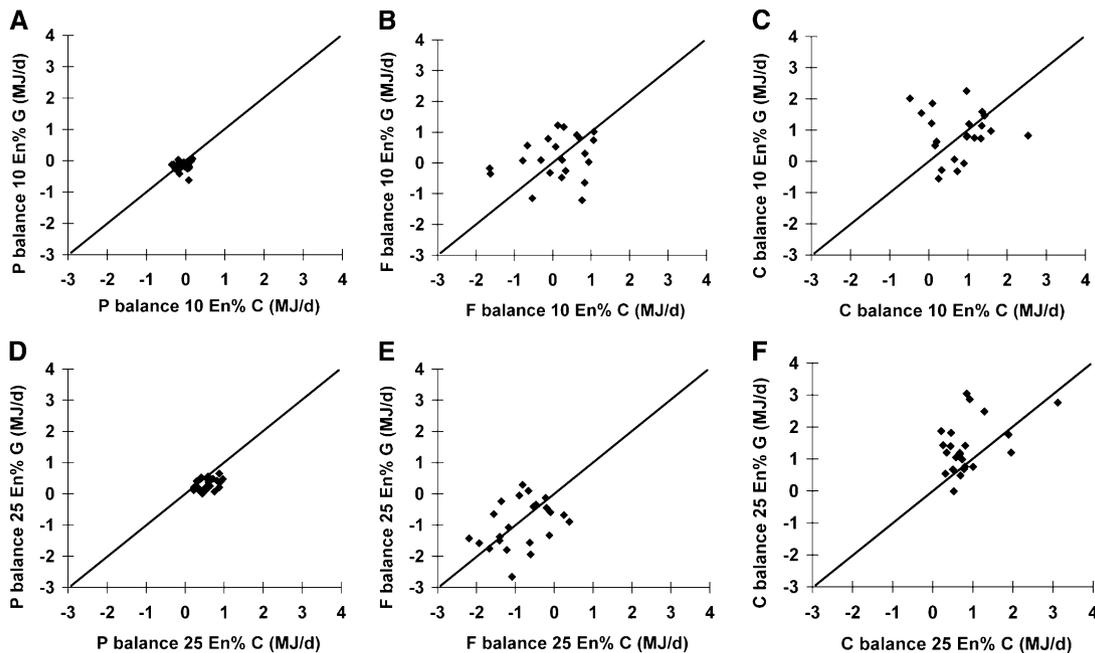
**FIGURE 4** AUC for the changes from baseline in plasma amino acid concentrations during 4 h after consumption of a breakfast with 10 En% from casein or gelatin (A) and after consumption of a breakfast with 25 En% from casein or gelatin (B). Values are means  $\pm$  SEM,  $n = 24$ . \*Different from the casein diet,  $P < 0.05$ . G1, Glutamate; As, asparagine; Se, serine; G2, glutamine; Hi, histidine; G3, glycine; Th, threonine; Ci, citrulline; Ar, arginine; Al, alanine; Ta, taurine; Aa,  $\alpha$ -aminobutyric acid; Ty, tyrosine; Va, valine; Me, methionine; I, isoleucine; Ph, phenylalanine; Tr, tryptophan; Le, leucine; Or, ornithine; and Ly, lysine.

**TABLE 2** TEE, RMR, SMR, DIT, and AEE measured over 24 h in a respiration chamber after consumption of casein or gelatin diets that contained 10 En% or 25 En% protein<sup>1,2</sup>

	10 En%		25 En%	
	Casein diet	Gelatin diet	Casein diet	Gelatin diet
	<i>MJ/d</i>			
TEE	9.01 $\pm$ 0.25	8.97 $\pm$ 0.24	9.26 $\pm$ 0.25	9.12 $\pm$ 0.22
RMR	7.20 $\pm$ 0.18	7.17 $\pm$ 0.20	7.39 $\pm$ 0.17	7.30 $\pm$ 0.18
SMR	6.45 $\pm$ 0.18	6.49 $\pm$ 0.18	6.68 $\pm$ 0.15	6.62 $\pm$ 0.16
DIT	0.75 $\pm$ 0.09	0.68 $\pm$ 0.08	0.71 $\pm$ 0.07	0.68 $\pm$ 0.06
AEE	1.81 $\pm$ 0.11	1.79 $\pm$ 0.07	1.87 $\pm$ 0.11	1.82 $\pm$ 0.07

<sup>1</sup> Values are means  $\pm$  SEM,  $n = 22$  (10 En% protein diets) or 23 (25 En% protein diets).

<sup>2</sup> No significant differences between consumption of the 10 En% protein diets and between consumption of the 25 En% protein diets on all variables.



**FIGURE 5** Protein balance (A), fat balance (B), and carbohydrate balance (C) for each participant after consumption of a diet with 10 En% from casein compared with a diet with 10 En% from gelatin, and protein balance (D), fat balance (E), and carbohydrate balance (F) for each participant after consumption of a 25 En% casein diet compared with a 25 En% gelatin diet. The lines of identity are shown. 10 En% protein diets,  $n = 22$ ; 25 En% protein diets,  $n = 23$ .

( $4.43 \pm 0.14$  vs.  $3.93 \pm 0.13$  MJ/d, respectively;  $P < 0.01$ ). Respiratory quotient significantly differed neither after consumption of the 10 En% casein ( $0.86 \pm 0.0048$ ) and 10 En% gelatin ( $0.86 \pm 0.0046$ ) diets nor after consumption of the 25 En% casein ( $0.87 \pm 0.0037$ ) and 25 En% gelatin ( $0.86 \pm 0.0043$ ) diets. Taken together, when participants consumed both 10 En% and 25 En% diets, negative and positive protein balances, respectively, were higher when they consumed the casein diets. Additionally, the positive carbohydrate balance was less when participants consumed the 25 En% casein compared with the 25 En% gelatin diet.

## Discussion

In this study, we contrasted an incomplete protein (gelatin) and a complete protein (casein) in 10 En% and 25 En% single-protein diets to measure effects on appetite, 24-h EE, and 24-h macronutrient balances. We hypothesized that the use of gelatin compared with casein may stimulate appetite suppression and EE and may limit a positive protein balance. We observed significant differences in appetite profile and macronutrient balance yet no differences in EE. These results were based on isoenergetic diets, indicating that the observed effects were due to differences in amino acid composition of the proteins used in the diets.

The greater hunger suppression found after consumption of the 10 En% gelatin diet compared with the 10 En% casein diet is in accordance with the results of our previous research (11), showing higher satiety and lower hunger ratings after consumption of a 10 En% gelatin breakfast compared with a 10 En% casein breakfast. The mechanism mentioned in the introduction may have contributed to the reduced appetite. Detection in the brain of a deficiency of essential amino acids in the diet from a decline in serum amino acid levels may lead to a behavioral response rejecting consumption of imbalanced diets (14–17) and

thus suppressing hunger. Therefore, the decreased hunger feelings after consumption of a gelatin compared with a casein diet may be understood as an anorexigenic effect on intake of food that does not contain sufficient IAA (17). Under 10 En% conditions, the plasma concentrations of the essential amino acids histidine, valine, methionine, isoleucine, phenylalanine, tryptophan, and leucine decreased and were lower after consumption of the gelatin compared with the casein breakfast, which may indicate a deficiency of these IAA. Under 25 En% conditions, the deficiency of IAA was not so large: only the plasma concentration of tryptophan decreased and was lower after consumption of the gelatin compared with the casein breakfast. Because hunger suppression did not differ after participants consumed both 25 En% protein diets, a role of Trp in hunger suppression seems to be unlikely. Moreover, we previously observed that addition of Trp to gelatin did not affect appetite differentially (8). Deficiency of the other IAA may have been involved in the greater hunger suppression observed after consumption of the 10 En% gelatin diet. In addition, the contribution of ghrelin and/or GLP-1 was probably marginal, because the observed differences after consumption of the casein and gelatin diets in the anorexigenic hormone GLP-1 and/or the orexigenic hormone ghrelin were minor and these hormones and appetite were not correlated.

Contrary to the hypothesis that gelatin oxidation induces a higher EE compared with casein, the higher protein oxidation after consumption of the gelatin diets compared with the casein diets did not result in differences in EE. The reason for this may be that in addition to energy costs for protein oxidation, other factors are also involved in the thermic effect of proteins, such as protein synthesis (with high ATP costs of peptide bond synthesis), high costs for urea production, and gluconeogenesis (4,33). Although not measured directly, protein synthesis was probably higher after consumption of the casein diets compared with the gelatin diets, as we observed a lower protein oxidation with

similar protein intake. The higher protein synthesis may be a factor involved in the similar effect on EE of both protein types.

Compared with the gelatin diet, consumption of the casein diet resulted in a smaller negative and larger positive protein balance under 10 En% and 25 En% protein conditions, respectively. This different effect on protein balance is probably the result of a difference in completeness between both protein sources, because the absence or low amounts of certain essential amino acids in gelatin may have been a limiting factor for postprandial protein synthesis, resulting in a higher oxidation of the free amino acids. For both casein and gelatin, the negative protein balance under 10 En% conditions and the positive protein balance under 25 En% conditions are due to the presence of the total amount of amino acids in the diet, which turned out to be too low, with respect to total body protein turnover, under 10 En% conditions and to be excessive under 25 En% conditions. Under 25 En% protein conditions, the difference in protein balance when participants consumed the 2 diets was more pronounced than under 10 En% protein conditions. This difference in protein balance probably resulted in a more pronounced difference in carbohydrate balance, because a higher protein oxidation after consumption of the 25 En% gelatin diet may have resulted in a lower carbohydrate oxidation, and thus in a larger positive carbohydrate balance, compared with the 25 En% casein diet.

The study results confirm our hypothesis that the use of an incomplete protein (gelatin) compared with a complete protein (casein) in single-protein diets stimulates appetite suppression and limits a positive protein balance. However, in contradiction to the hypothesis, EE was affected similarly. In terms of weight loss for people with obesity, the greater hunger-suppressing effect of gelatin may play a role in reducing energy intake if this effect is maintained when consuming a gelatin diet in the long term. In addition, long-term use of casein may contribute to preservation of fat free mass. In conclusion, when we compared the effects of an incomplete protein (gelatin) and a complete protein (casein) at 2 concentrations over 36 h, gelatin resulted in greater hunger suppression, casein caused a greater positive (smaller negative) protein balance, and effects on EE did not differ.

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A.H.-W., M.S.W.-P., and K.R.W. designed research; A.H.-W. and M.A.B.V. conducted research; A.H.-W. analyzed data; A.H.-W. wrote the paper; A.H.-W. had primary responsibility for final content; and M.S.W.-P. and K.R.W. supervised the project. All authors read and approved the final manuscript.

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