



Original Article

A breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP

Margriet A.B. Veldhorst^{a,c,*}, Arie G. Nieuwenhuizen^{a,c}, Ananda Hochstenbach-Waelen^{a,c}, Klaas R. Westerterp^{a,c}, Marielle P.K.J. Engelen^b, Robert-Jan M. Brummer^c, Nicolaas E.P. Deutz^{b,c}, Margriet S. Westerterp-Plantenga^{a,c}

^a Department of Human Biology of the Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, P.O. Box 616, 6200 MD, Maastricht, The Netherlands

^b Department of Surgery of the Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, P.O. Box 616, 6200 MD, Maastricht, The Netherlands

^c Top Institute of Food and Nutrition, P.O. Box 557, 6700 AN Wageningen, The Netherlands

ARTICLE INFO

Article history:

Received 23 July 2008

Accepted 24 December 2008

Keywords:

Energy intake

Satiety

Dietary proteins

Timing

Amino acids

SUMMARY

Background & aims: Dietary protein plays a role in body weight regulation, partly due to its effects on satiety. The objective was to compare the effects of casein-, soy-, whey-, whey without glycomacropeptide (GMP)-, alpha-lactalbumin-, gelatin-, or gelatin with tryptophan (TRP)-protein breakfasts at two concentrations on subsequent satiety and energy intake (EI).

Methods: Twenty-four healthy subjects (mean \pm SEM BMI: 24.8 ± 0.5 kg/m²; age: 25 ± 2 years) received a breakfast; a custard with casein, soy, whey, whey-GMP, alpha-lactalbumin, gelatin, or gelatin + TRP as protein source with either 10/55/35 (normal) or 25/55/20 (high) En% protein/carbohydrate/fat in a randomized, single-blind design. At the precedingly determined time point for lunch, 180 min, subjects were offered an *ad lib* lunch. Appetite profile (Visual Analogue Scales, VAS) and EI were determined.

Results: Both at the level of 10 and 25 En% from protein, EI at lunch was $\sim 20\%$ lower after an alpha-lactalbumin or gelatin (+TRP) breakfast (2.5 ± 0.2 MJ) compared with after a casein, soy, or whey-GMP breakfast (3.2 ± 0.3 MJ, $p < 0.05$). Appetite ratings at 180 min differed 15–25 mm ($\sim 40\%$, $p < 0.05$) between types of protein.

Differences in EI were a function of differences in appetite ratings ($R^2 = 0.4$, $p < 0.001$).

Conclusions: Different proteins (alpha-lactalbumin, gelatin, gelatin + TRP) that are $\sim 40\%$ more satiating than other proteins (casein, soy, whey, whey-GMP) induce a related $\sim 20\%$ reduction of subsequent energy intake.

© 2009 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

1. Introduction

Overweight and obesity are the result of a positive energy balance and since body weight regulation involves several pathways, weight management requires a multi-factorial approach.¹ A relatively elevated protein diet implies this multi-factorial approach through increased postprandial and post-absorptive satiety, increased thermogenesis, preservation of fat-free body mass, and lower energy-efficiency compared with control diets.^{1,2} Although protein has been shown to increase satiety, the subsequent effect, i.e. spontaneously reduced food intake, has been shown in very few studies. Weigle et al. however showed that

a high protein diet reduced *ad lib* food intake while sustaining satiety at a comfortable level.² In the present study we focused on short-term satiety effects, i.e. effects on satiety and subsequent food intake induced by a single meal. A protein that is more satiating and decreases energy intake could potentially be used as part of a weight-loss diet to help people to comply with their diet and actually lose weight.

Data on different types of protein affecting food intake are inconclusive. Although Hall and colleagues found whey to be more satiating than casein,³ Bowen et al., did not find differences in energy intake after casein or whey preloads.^{4,5} A study by Lang et al. found no different effects of egg albumin, casein, gelatin, soy, pea, and wheat gluten on energy intake, and in another experiment, there also were no differences in post-lunch energy intake after a casein-, soy-, or gelatin-lunch.^{6,7} Anderson et al. found that whey and soy protein decreased food intake more than egg protein, 1 h after a preload.⁸

* Corresponding author at: Maastricht University, Department of Human Biology, P.O. Box 616, 6200 MD Maastricht. Tel.: +31 43 388 4596; fax: +31 43 367 0976.
E-mail address: m.veldhorst@hb.unimaas.nl (M.A.B. Veldhorst).

In the present study we assessed a possible effect on energy intake by type of protein, offered in two concentrations. The amounts of protein chosen represented the highest recommended protein intake per day (25 En%) versus a normal protein intake per day (10 En%).⁹ Casein was selected as one of the protein types as being a 'slow' protein whereas whey is considered as relatively 'fast' protein, inducing satiety quickly.^{3,10–12} Both whey and whey where glycomacropeptide (GMP) was removed were selected since GMP has been suggested to contribute to the satiating effects of whey.^{13,14} Soy was studied because it is a high quality vegetable protein often used in food products. Alpha-lactalbumin contains high levels of tryptophan (TRP) and relatively low levels of large neutral amino acids (LNAA); whether the increased TRP/LNAA ratio in the plasma¹⁵ would also increase brain serotonin production and influence food intake remains to be elucidated. The oxidation of gelatin is calculated to be highly inefficient causing a high thermogenesis, which could affect satiety. In addition, gelatin was also offered with added TRP, in order to discriminate whether a possible difference between gelatin and alpha-lactalbumin was due to the TRP content.

Timing has been shown to play an important role when studying the effect of protein on food intake,⁸ therefore it is of importance to measure energy intake at a sensitive and relevant moment in time. In a preceding experiment, the moment in time that may be sensitive to show a possible difference in food intake was determined by assessing satiety ratings and blood parameters for 4 h after consumption of the same protein-meals as in the current study. Three hours after breakfast significant differences in the orexigenic hormone ghrelin were present, so this was chosen as the moment in time to offer lunch.^{16–18}

The objective of this study was to evaluate the effect of casein, soy, whey, whey-GMP, alpha-lactalbumin, gelatin, or gelatin with added TRP in two concentrations of protein in the breakfast on energy intake at lunch, which was offered 3 h after breakfast.

2. Subjects and methods

2.1. Subjects

Thirty healthy male and female volunteers (body mass index 22–32 kg/m², age 18–45 years) were recruited by advertisements in local newspapers and on notice boards at the university. They underwent a screening procedure including medical history taking, measurement of body weight and height and cognitively restrained eating, using a Dutch translation of the Three Factor Eating Questionnaire (TFEQ).^{19,20} Twenty-four subjects (10 male, 14 female) were selected on the basis of being in good health, non-smokers, non-vegetarian, not cognitively dietary restraint (TFEQ Factor 1 ≤ 9), not using medication apart from oral contraceptives and at most moderate alcohol users (≤10 alcoholic consumptions per week). Their mean age was 25 ± 2 years, and their body weight was 72.8 ± 2.2 kg (BMI: 24.8 ± 0.5 kg/m²). Written informed consent was obtained from all participants and the study protocol was approved by the Medical Ethics Committee of the University Hospital Maastricht.

2.2. Study design

A randomized, single-blind, within-subject experimental study was performed. All subjects came to the university on 14 occasions, separated by at least 3 days. On each test day subjects received a subject-specific standardized breakfast. Three hours after breakfast an *ad lib* lunch was offered; appetite ratings were obtained until 6 h after breakfast.

2.2.1. Preceding experiments

In preceding experiments,^{16–18} the sensitive moment in time to offer lunch was determined using the same breakfasts. In those studies the protocol started at 08.00 h after an overnight fast from 22.00 h. A Venflon catheter was placed in a superficial dorsal vein of the hand for blood sampling. To obtain arterialized venous blood samples the hand was placed in a thermostatically controlled hot box at 60 °C for 20 min before the sampling time. A basal blood sample was taken and appetite ratings were scored. After 5 min a second basal blood sample was obtained and breakfast was offered ($t = 0$ min). After the first and the last bite, taste perception was scored. Appetite ratings were completed just before breakfast and at 20, 40, 60, 80, 100, 120, 180, and 240 min after breakfast. Blood samples for urea and amino acid determination were obtained at –5 min and subsequently just after the appetite ratings; blood samples for determination of glucose, insulin, and ghrelin concentrations were obtained before and 40, 60, 120, and 180 min after breakfast. Venous blood samples for determination of GLP-1 concentration were obtained separately before, and at 30, 60, 90, 120, and 180 min after breakfast by means of a Venflon catheter placed in an antecubital vein.²¹ Subjects were allowed to drink maximally two glasses of water spread over the morning. Details on analyses and results were described previously.^{16–18} In summary, these experiments revealed that differences in concentrations of insulin, GLP-1 or certain amino acids, depending on the type of protein used, coincided with the differences in satiety among different proteins served at breakfast. However, the effects of these hormones and metabolites were different for each protein.

2.2.2. Breakfast

Breakfast was offered as a custard, with either casein (Calcium Caseinate S, DMV International, Veghel, The Netherlands), soy (Supro[®] 590, The Solae Company, St. Louis, MO, USA), whey (Ultra Whey 90, Volactive Functional Food Products, Orwell, UK), whey-GMP (WPC 80, DMV International, Veghel, The Netherlands), alpha-lactalbumin (BioPURE – Alphasactalbumin[™], Davisco Foods International Inc., Eden Prairie, USA), gelatin (Solugel LMC/3, PB Gelatins GmbH, Nienburg/Weser, Germany), or gelatin + TRP (Solugel LMC/3, PB Gelatins GmbH, Nienburg/Weser, Germany, Tryptophan: Sigma-Aldrich, Steinheim, Germany) with TRP added to the level present in alpha-lactalbumin, as a single protein source, with either protein/carbohydrate/fat: 10/55/35 En% (normal protein) or protein/carbohydrate/fat: 25/55/20 En% (high protein). Protein was exchanged with fat; carbohydrate content was kept constant because its effect on protein metabolism.²² All custards had an energy density of 4 kJ/g. The breakfast contained 20% of daily energy requirement, calculated as basal metabolic rate (BMR), according to the equations of Harris-Benedict, multiplied by an activity index of 1.75 which is the average value reported for the general population in the Netherlands.^{23,24} The mean energy content of the breakfast was 2.39 ± 0.06 MJ.

The 14 custards were produced by NIZO Food Research bv. (Ede, The Netherlands) and had tapioca starch (Farinex VA50T, AVEBE, Veendam, The Netherlands and Perfectamyl 3108 AVEBE, Veendam, The Netherlands) and sunflower oil (Reddy, NV Vandemoortele, Roosendaal, The Netherlands) respectively as carbohydrate and fat source and were citrus-vanilla (Citrus, J.B. de lange, Belfeld, The Netherlands; Vanilla, J.B. de lange, Belfeld, The Netherlands) flavored. Extensive product development and use of a taste panel lead to custards that did not differ in color, taste, or viscosity. The amino acid composition of the 14 different custards is presented in Table 1.

Table 1

Amino acid content of the breakfasts given as a custard with either 10 En% or 25 En% casein, soy, whey, whey-GMP, alpha-lactalbumin, gelatin, or gelatin + TRP protein content (g amino acid/100 g custard).

| | Casein 10% | Soy 10% | Whey 10% | Whey-GMP 10% | Alpha-lactalbumin 10% | Gelatin 10% | Gelatin + TRP 10% | Casein 25% | Soy 25% | Whey 25% | Whey-GMP 25% | Alpha-lactalbumin 25% | Gelatin 25% | Gelatin + TRP 25% |
|----------------------------|------------|---------|----------|--------------|-----------------------|-------------|-------------------|------------|---------|----------|--------------|-----------------------|-------------|-------------------|
| Glutamic acid ^a | 0.477 | 0.328 | 0.381 | 0.378 | 0.316 | 0.229 | 0.229 | 1.127 | 0.816 | 0.957 | 0.922 | 0.790 | 0.576 | 0.576 |
| Aspartic acid ^b | 0.150 | 0.200 | 0.230 | 0.252 | 0.360 | 0.127 | 0.127 | 0.355 | 0.497 | 0.579 | 0.615 | 0.901 | 0.319 | 0.319 |
| Cysteine | 0.009 | 0.022 | 0.055 | 0.071 | 0.115 | 0.001 | 0.001 | 0.021 | 0.054 | 0.139 | 0.172 | 0.288 | 0.002 | 0.002 |
| Serine | 0.120 | 0.089 | 0.099 | 0.088 | 0.095 | 0.074 | 0.074 | 0.283 | 0.220 | 0.249 | 0.216 | 0.239 | 0.186 | 0.186 |
| Histidine | 0.064 | 0.048 | 0.039 | 0.047 | 0.065 | 0.021 | 0.021 | 0.152 | 0.119 | 0.097 | 0.115 | 0.162 | 0.052 | 0.052 |
| Glycine | 0.040 | 0.071 | 0.035 | 0.038 | 0.059 | 0.558 | 0.558 | 0.094 | 0.177 | 0.088 | 0.092 | 0.148 | 1.402 | 1.402 |
| Threonine | 0.090 | 0.066 | 0.150 | 0.106 | 0.114 | 0.042 | 0.042 | 0.214 | 0.164 | 0.378 | 0.259 | 0.285 | 0.106 | 0.106 |
| Arginine | 0.092 | 0.139 | 0.055 | 0.067 | 0.043 | 0.191 | 0.191 | 0.218 | 0.345 | 0.139 | 0.164 | 0.106 | 0.479 | 0.479 |
| Alanine | 0.064 | 0.073 | 0.106 | 0.105 | 0.056 | 0.211 | 0.211 | 0.150 | 0.182 | 0.266 | 0.255 | 0.140 | 0.530 | 0.530 |
| Tyrosine | 0.120 | 0.069 | 0.061 | 0.079 | 0.100 | 0.011 | 0.011 | 0.283 | 0.171 | 0.154 | 0.192 | 0.249 | 0.027 | 0.027 |
| Valine | 0.141 | 0.085 | 0.123 | 0.113 | 0.103 | 0.051 | 0.051 | 0.333 | 0.212 | 0.309 | 0.275 | 0.259 | 0.129 | 0.129 |
| Methionine | 0.064 | 0.022 | 0.048 | 0.051 | 0.028 | 0.019 | 0.019 | 0.152 | 0.056 | 0.121 | 0.125 | 0.069 | 0.048 | 0.048 |
| Isoleucine | 0.112 | 0.089 | 0.141 | 0.126 | 0.136 | 0.035 | 0.035 | 0.265 | 0.222 | 0.355 | 0.307 | 0.339 | 0.087 | 0.087 |
| Phenylalanine | 0.110 | 0.094 | 0.062 | 0.078 | 0.094 | 0.042 | 0.042 | 0.259 | 0.234 | 0.156 | 0.189 | 0.235 | 0.107 | 0.107 |
| Tryptophan | 0.027 | 0.023 | 0.039 | 0.050 | 0.090 | 0.001 | 0.087 | 0.064 | 0.057 | 0.099 | 0.123 | 0.225 | 0.003 | 0.219 |
| Leucine | 0.204 | 0.145 | 0.226 | 0.277 | 0.257 | 0.067 | 0.067 | 0.483 | 0.360 | 0.567 | 0.675 | 0.644 | 0.168 | 0.168 |
| Lysine | 0.172 | 0.110 | 0.201 | 0.230 | 0.246 | 0.087 | 0.087 | 0.405 | 0.274 | 0.504 | 0.560 | 0.614 | 0.219 | 0.219 |
| Proline | 0.230 | 0.087 | 0.128 | 0.097 | 0.057 | 0.316 | 0.316 | 0.544 | 0.216 | 0.321 | 0.238 | 0.142 | 0.792 | 0.792 |

^a Glutamic acid = glutamine + glutamate.

^b Aspartic acid = asparagine.

2.2.3. Study protocol

After an overnight fast from 22.00 h, subjects came to the laboratory in the university building at 08.15 h. The laboratory was a quiet room, free of odors, sounds and other disturbing factors. Subjects sat at separate tables that were at least 2 m apart and were not allowed to talk to each other nor to perform any physical activity. The protocol started at 08.30 h with scoring appetite ratings. Breakfast was offered ($t = 0$ min) and completed within 20 min. With the first and the last bite taste perception was scored. Appetite ratings were completed at 30, 60, 90, 120, and 180 min after breakfast. Immediately after completing the questionnaire at 180 min, subjects were offered an *ad lib* lunch and were instructed to eat just as much till they were satiated. With the first and the last bite of the lunch taste perception was scored. Appetite ratings then were completed at 210, 240, 300, and 360 min after breakfast. Subjects were allowed to drink maximally three glasses of water spread over the entire test period and were allowed to go home 4 h after breakfast; the last two moments of rating were completed at home and returned on the next visit. The subjects were instructed not to perform any heavy physical activity and not to eat or drink for 2 h.

2.3. Measurements

2.3.1. Energy intake at lunch

Lunch consisted of Turkish bread (400 g) with egg salad (400 g) with 13/41/46 En% protein/carbohydrate/fat with an energy density of 11.4 kJ/g. Subjects were instructed to eat till they were comfortably full. Lunch was weighed before and after eating and energy intake was calculated.

2.3.2. Appetite profile

To determine the appetite profile, hunger, fullness, satiety, and desire to eat were rated on 100 mm Visual Analogue Scales (VAS), anchored with 'not at all' and 'extremely'. Subjects were instructed to rate the appetite dimensions by marking the scale at the point that was most appropriate to their feeling at that time.

2.3.3. Taste perception

Taste perception profiles of the custards and lunch were assessed after the first and the last bite using 100 mm Visual

Analogue Scales (VAS), anchored with 'not at all' and 'extremely' on the aspects: pleasantness, sweetness, sourness, saltiness, bitterness, savoriness, crispiness, and creaminess.

2.4. Statistical analysis

Data are presented as mean changes from baseline \pm standard error to the mean (SEM), unless otherwise indicated.²⁵ The area under the curve (AUC) of changes from baseline till 180 min after breakfast (AUC180) was calculated using the trapezoidal method. To determine possible differences between the different types of protein at a concentration of 10 and 25% of energy from protein, a repeated measures ANOVA between factors with protein concentration as factor was carried out. When there was no effect of protein concentration a repeated measures ANOVA with Fisher's PLSD correction for multiple comparisons within one protein type was carried out. Regression analysis was performed to determine the relationships between the difference in energy intake between two different breakfasts and the difference in AUC of hunger or satiety after these two different breakfasts. Glucose, insulin, GLP-1, ghrelin, and amino acid concentrations between different protein types within one concentration were compared using the Mann-Whitney *U*-test.^{16–18} A *p*-value <0.05 was regarded as statistically significant. Statistical procedures were performed using StatView 5.0 (SAS Institute Inc., USA, 1998).

3. Results

3.1. Energy intake

Energy intake at lunch did not differ depending on protein concentration with respect to comparisons between different protein types. After a breakfast with 10% of energy from protein, energy intake at lunch was 0.54 MJ (17%) lower after a breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP than after a breakfast with casein, soy, or whey-GMP ($p < 0.05$, Fig. 1). After a breakfast with 25% of energy from protein, energy intake at lunch was 0.78 MJ (24%) lower after a breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP than after a breakfast with casein, soy, or whey-GMP ($p < 0.05$, Fig. 1). Energy intake at lunch was also

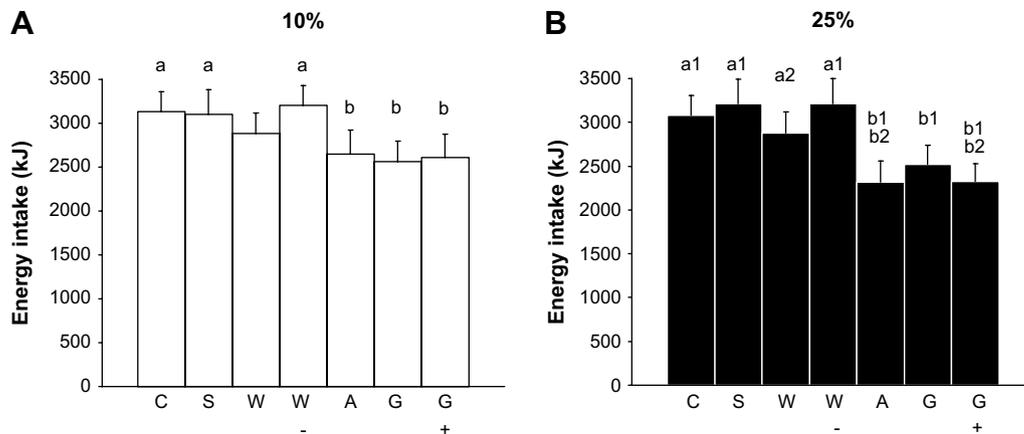


Fig. 1. *Ad lib* energy intake (kJ) at lunch after consumption of a breakfast with 20% of daily energy requirements with either 10 En% (A) or 25 En% (B) from protein with either casein, soy, whey, whey-GMP, alpha-lactalbumin, gelatin, or gelatin + TRP as protein type in 24 subjects (men and women). Values are means \pm SEM, ANOVA repeated measures with Fisher's PLSD correction. C casein, S soy, W whey, W-whey-GMP, A alpha-lactalbumin, G gelatin, G+ gelatin + TRP. a significantly different from b ($p < 0.05$), a1 significantly different from b1 ($p < 0.05$), a2 significantly different from b2 ($p < 0.05$).

0.55 MJ (19%) lower after a breakfast with alpha-lactalbumin or gelatin + TRP than after a breakfast with whey ($p < 0.01$, Fig. 1).

3.2. Taste perception breakfast

Pleasantness of taste of the custards with the first bite was sufficient with a mean value of 55 ± 5 mm without differences between custards.

3.3. Satiety and hunger

Baseline ratings for satiety or hunger were not different between treatments. The changes in appetite ratings did not differ depending on protein concentration with respect to comparisons between different protein types. Within one protein concentration there were various significant differences in the change in satiety or hunger between the seven different breakfasts at several time points, both at the level of 10 and 25% of energy from protein (Fig. 2). Changes in fullness or desire to eat were similar to the changes in satiety or hunger respectively and are therefore not presented separately. The differences in appetite ratings between types of protein at 180 min after breakfast were 30–50% (Fig. 2).

The AUC of changes in appetite ratings over the first 3 h after breakfast, i.e. the AUC180 of satiety or hunger suppression was larger in general after the breakfast with alpha-lactalbumin, gelatin, and/or gelatin + TRP than after casein, soy, whey, and/or whey-GMP, both at 10 and 25% of energy from protein (Fig. 2).

3.4. Correlations

Comparison of the different protein breakfast types at a concentration of 10% of energy from protein revealed that the difference in energy intake at lunch between a breakfast with gelatin + TRP and a breakfast with soy was a function of the difference in the AUC180 of satiety between those two breakfasts ($r = -0.470$, $p < 0.05$), the difference in energy intake at lunch between a breakfast with gelatin and a breakfast with whey-GMP was a function of the differences in the AUC180 of satiety or the AUC180 of hunger between those two breakfasts ($r = -0.641$, $p < 0.001$; and $r = 0.481$, $p < 0.05$ respectively), and the difference in energy intake at lunch between a breakfast with gelatin + TRP and whey-GMP was a function of the differences in the AUC180 of

satiety or the AUC180 of hunger between those two breakfasts ($r = -0.446$, $p < 0.05$; $r = 0.414$, $p < 0.05$ respectively).

Comparison of the different protein types at a concentration of 25% of energy from protein revealed that the difference in energy intake at lunch between a breakfast with gelatin + TRP and a breakfast with soy a function was of the difference in the AUC180 of satiety or the AUC180 of hunger between those two breakfasts ($r = -0.571$, $p < 0.01$; $r = 0.458$, $p < 0.05$ respectively).

3.5. Blood parameters

The comparison of glucose, insulin, GLP-1, ghrelin, and amino acid concentrations, obtained during the preceding experiments,^{16–18} revealed that there were several significant differences in metabolite responses between the different protein breakfasts. These differences are presented in Tables 2A,B.

In general, responses of essential amino acids were more increased after a breakfast with casein, whey, whey-GMP or alpha-lactalbumin than after a breakfast with gelatin or gelatin + TRP. For the non-essential amino acids some amino acid responses were more increased after a breakfast with gelatin or gelatin + TRP compared with casein, soy, whey, whey-GMP or alpha-lactalbumin whereas for other amino acids it was the other way around.

4. Discussion

Ad lib energy intake at lunch was $\sim 20\%$ lower after a breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP than after a breakfast with casein, soy, or whey-GMP, both at the level of 10 and 25% of energy from protein. Moreover, *ad lib* energy intake at lunch also was lower after a breakfast with 25% of energy from alpha-lactalbumin or gelatin + TRP in comparison with a breakfast with 25% of energy from whey. The iso-energetic custards consumed for breakfast were of the same color and viscosity and did not differ in taste. To explain the differences in energy intake at lunch we explored differences in appetite ratings, glucose, insulin, GLP-1, ghrelin, and amino acid concentrations.

One of the explanations for the observed differences in energy intake at lunch were differences in appetite ratings after consumption of the different protein breakfasts. The differences in energy intake between two treatments indeed were a function of the difference in appetite ratings between those two treatments;

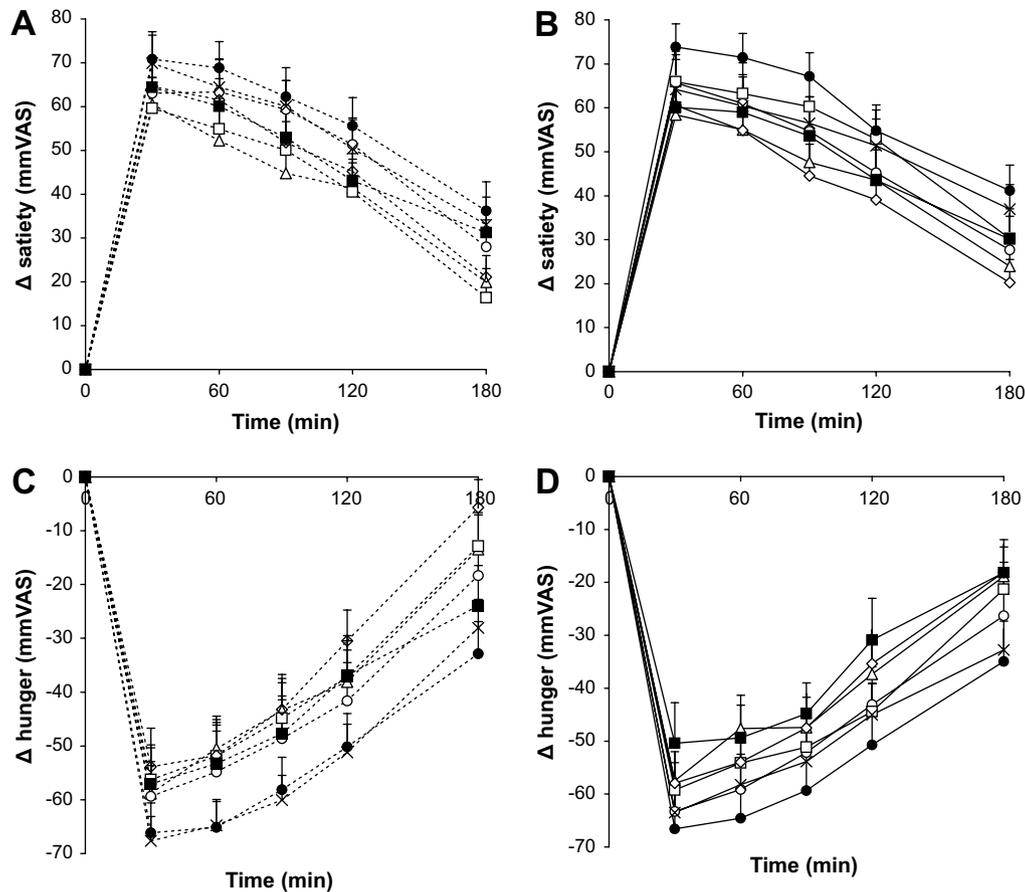


Fig. 2. Changes in satiety and hunger (mmVAS) after consumption of a breakfast with 20% of daily energy requirements with either 10 En% (A + C) or 25 En% (B + D) from protein with either casein, soy, whey, whey-GMP, alpha-lactalbumin, gelatin, or gelatin + TRP as protein type in 24 subjects (men and women). Values are means \pm SEM, ANOVA repeated measures with Fisher's PLSD correction. Δ casein 10%, \circ soy 10%, \square whey 10%, \diamond whey-GMP 10%, \times alpha-lactalbumin 10%, \blacksquare gelatin 10%, \bullet gelatin + TRP 10%, Δ casein 25%, \circ soy 25%, \square whey 25%, \diamond whey-GMP 25%, \times alpha-lactalbumin 25%, \blacksquare gelatin 25%, \bullet gelatin + TRP 25%. Significant differences * p < 0.05. A: 90 min: alpha-lactalbumin/gelatin + TRP > casein/whey*. 180 min: alpha-lactalbumin/gelatin/gelatin + TRP > casein/whey/whey-GMP*. Area Under the Curve: alpha-lactalbumin > casein/whey*, gelatin + TRP > casein/whey*. B: 90 min: gelatin + TRP > casein/soy/whey-GMP*. 180 min: alpha-lactalbumin/gelatin + TRP > casein/soy/whey-GMP*. Area Under the Curve: whey > whey-GMP*, gelatin + TRP > casein/soy/whey-GMP/gelatin*. C: 30 min: alpha-lactalbumin/gelatin + TRP > whey/whey-GMP*. 60 min: alpha-lactalbumin/gelatin + TRP > casein/whey/whey-GMP*. 90 min: alpha-lactalbumin/gelatin + TRP > casein/soy/whey/whey-GMP*. 120 min: alpha-lactalbumin/gelatin + TRP > casein/whey/whey-GMP*. 180 min: alpha-lactalbumin/gelatin/gelatin + TRP > casein/soy/whey/whey-GMP*. Area Above the Curve: alpha-lactalbumin > casein/whey/whey-GMP/gelatin*, gelatin + TRP > casein/whey/whey-GMP/gelatin*. D: 60 min: alpha-lactalbumin/gelatin + TRP > casein/whey-GMP*. 120 min: gelatin + TRP > casein/whey-GMP*. 180 min: alpha-lactalbumin/gelatin + TRP > casein/whey/whey-GMP*. Area Above the Curve: soy > gelatin*, alpha-lactalbumin > gelatin*, gelatin + TRP > casein/whey-GMP/gelatin*.

reduced energy intake thus was indeed straightforwardly related to increased satiety. Alpha-lactalbumin, gelatin, and gelatin + TRP were more satiating than casein, soy, whey, and whey-GMP resulting in a decreased energy intake.

A mechanism for the increased satiety and decreased energy intake may be the increased insulin response, a metabolic satiety signal,^{26,27} after a breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP compared with a breakfast with casein or soy. Moreover, there was an increased GLP-1 response after a breakfast with 25% of energy from gelatin + TRP compared with a breakfast with 25% of energy from casein or soy. Previously, GLP-1 has been found to inhibit appetite and reduce food intake in normal-weight men. GLP-1 possibly exerts its effects via a combination of inhibition of gastric emptying and activation of brain GLP-1 receptors that limits food intake.^{21,28} Increased concentrations of amino acids may also contribute to increased satiety since, according to the amino static theory of Melinkoff from 1956, a larger increase in plasma amino acids increases satiety.²⁹ There were several amino acids that were relatively more increased after a breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP than after a breakfast

with casein, soy, whey, or whey-GMP. However, there was no specific amino acid that was more increased after all the three satiating breakfasts compared with the less satiating breakfasts. Therefore it appears that amino acids do play a role in the satiety response but that each protein has its own mechanisms via which satiety is induced.

Responses of essential amino acid concentrations in the blood in general were larger after a breakfast with casein, whey, whey-GMP or alpha-lactalbumin than after a breakfast with gelatin or gelatin + TRP, which is a reflection of the amino acid composition of the proteins used. An 'ideal protein', with all essential amino acids present in the right amounts, would reflect the recommended daily allowances of essential amino acids, being 14 mg/kg per day histidine, 19 mg/kg per day isoleucine, 42 mg/kg per day leucine, 38 mg/kg per day lysine, 19 mg/kg per day methionine + cysteine, 33 mg/kg per day phenylalanine + tyrosine, 20 mg/kg per day threonine, 5 mg/kg per day tryptophan and 24 mg/kg per day valine.³⁰ This means a distribution with 7% of essential amino acids as histidine, 9% as isoleucine, 20% as leucine, 18% as lysine, 9% as methionine + cysteine, 15% as phenylalanine + tyrosine, 9% as threonine, 2%

Table 2A

Changes in glucose (mmol/l h), insulin (mU/l h), GLP-1 (pmol/l h), ghrelin (pmol/l h), and amino acid ($\mu\text{mol/l h}$) concentrations expressed as AUC after consumption of a breakfast with 20% of daily energy requirements with 10 En% from protein with either casein, soy, whey, whey-GMP, alpha-lactalbumin, gelatin, or gelatin + TRP as protein type in 24 subjects (men and women) measured in preceding studies.^{16–18}

| | Casein 10% | Soy 10% | Whey 10% | Whey-GMP 10% | Alpha-lactalbumin 10% | Gelatin 10% | Gelatin + TRP 10% | |
|--------------------------------------|---------------|--------------------|-------------------|------------------|-----------------------|------------------|---------------------|------|
| Glucose | 124 ± 14 | 120 ± 21 | 99 ± 17 | 99 ± 14 | 114 ± 16 | 138 ± 13 | 122 ± 15 | |
| Insulin | 6530 ± 621 | 4936 ± 468 | abc 5820 ± 386 | 6847 ± 500 | 6683 ± 711 | a 7391 ± 723 | b 6744 ± 711 | e |
| GLP-1 | 218 ± 78 | 216 ± 94 | 257 ± 71 | 195 ± 72 | 362 ± 88 | 173 ± 63 | 270 ± 103 | |
| Ghrelin | −708 ± 140 | −399 ± 108 | −439 ± 106 | −471 ± 100 | −385 ± 94 | −339 ± 117 | −382 ± 109 | |
| Glutamate | −102 ± 506 | 209 ± 534 | a −1028 ± 442 | b 266 ± 337 | −740 ± 685 | 1660 ± 803 | ab 174 ± 1062 | |
| Asparagine | 2717 ± 263 | abc 5684 ± 238 | def 3925 ± 337 | ghi 3977 ± 313 | 7148 ± 326 | adj −554 ± 827 | behk −1193 ± 245 | cfil |
| Serine | 3574 ± 500 | abc 3669 ± 327 | def 2960 ± 491 | gh 1354 ± 606 | ij 1954 ± 834 | ad 8827 ± 1761 | begi 8005 ± 593 | cfhj |
| Glutamine | 1072 ± 1489 | a 1296 ± 2881 | 2220 ± 1235 | b 1800 ± 1045 | c −4508 ± 2027 | abc −173 ± 2361 | −555 ± 1823 | |
| Histidine | 2069 ± 217 | ab 2054 ± 495 | cd 832 ± 248 | efg 1418 ± 360 | hij 2264 ± 340 | eh −67 ± 569 | acfi −847 ± 357 | bdgj |
| Glycine | −2242 ± 438 | abc 2160 ± 610 | def −2307 ± 666 | gh −2346 ± 663 | ij −1290 ± 796 | ad 55,300 ± 8371 | begi 54,237 ± 3582 | cfhj |
| Threonine | 4414 ± 333 | a 3975 ± 553 | b 12,828 ± 349 | cde 8484 ± 588 | fg 8651 ± 620 | abc 4356 ± 1328 | df 3269 ± 725 | eg |
| Citrulline | −938 ± 134 | a −894 ± 152 | bc −1487 ± 156 | de −919 ± 149 | fg −1043 ± 216 | 33 ± 207 | abdf −457 ± 196 | ceg |
| Arginine | 1845 ± 238 | abc 6248 ± 517 | d 379 ± 279 | efg 1497 ± 421 | hij −1075 ± 349 | adeh 7053 ± 1448 | bfi 6040 ± 483 | cgj |
| Alanine | 30,021 ± 2219 | ab 32,396 ± 2585 | 36,193 ± 1383 | c 31,910 ± 2111 | de 27,812 ± 3480 | c 41,904 ± 4232 | ad 42,795 ± 4634 | be |
| Taurine | −464 ± 117 | abc 307 ± 120 | de −131 ± 80 | fg −70 ± 118 | hi 63 ± 149 | a 1254 ± 219 | bdfh 1129 ± 115 | cegi |
| Alpha-aminobutyric acid | 149 ± 84 | 122 ± 78 | a 571 ± 76 | bcd 507 ± 88 | efg 68 ± 88 | be 135 ± 94 | cf 262 ± 60 | adg |
| Tyrosine | 3676 ± 473 | ab 2439 ± 322 | cd −205 ± 174 | efg 1973 ± 373 | hij 2993 ± 372 | eh −2173 ± 786 | acfi −3248 ± 212 | bdgj |
| Valine | 7877 ± 409 | abc 5696 ± 786 | def 6487 ± 504 | ghi 6786 ± 1125 | jkl 1094 ± 507 | adj −1268 ± 1150 | behk −2292 ± 532 | cfil |
| Methionine | 1799 ± 212 | abc −785 ± 367 | 868 ± 224 | def 1319 ± 171 | ghi −393 ± 198 | adg −525 ± 302 | beh −537 ± 91 | cfi |
| Isoleucine | 4624 ± 292 | abc 5143 ± 326 | def 9387 ± 303 | ghi 7865 ± 465 | jk 7971 ± 494 | adg −1253 ± 1172 | behj −2681 ± 367 | cfik |
| Phenylalanine | 1990 ± 154 | abc 2984 ± 236 | def −178 ± 123 | gh 1193 ± 280 | ijk 1440 ± 186 | adgi −485 ± 402 | bej −1018 ± 179 | cfhk |
| Tryptophan | −216 ± 144 | abc 253 ± 254 | def 1558 ± 180 | ghi 3241 ± 145 | jkl 8562 ± 510 | adj −1202 ± 999 | behk 6640 ± 393 | cfil |
| Leucine | 7027 ± 393 | abc 4948 ± 477 | def 10,219 ± 373 | ghi 16,262 ± 586 | hij 12,007 ± 733 | adj −2469 ± 1833 | behk −4412 ± 608 | cfil |
| Ornithine | 2366 ± 284 | abc 2978 ± 196 | de −700 ± 1398 | fg 1501 ± 217 | hij 26 ± 257 | adh 4527 ± 710 | befi 3755 ± 368 | cgj |
| Lysine | 13,181 ± 725 | abc 8812 ± 1068 | def 16,328 ± 663 | ghi 20,146 ± 909 | jk 20,262 ± 1074 | adg 6734 ± 1902 | behj 3512 ± 722 | cfik |
| Branched-chain amino acids | 19,528 ± 959 | abc 15,787 ± 1492 | def 18,736 ± 6020 | gh 30,914 ± 2087 | ijk 21,073 ± 1643 | adi −7225 ± 2446 | begj −9385 ± 1407 | cfhk |
| Large neutral amino acids | 25,194 ± 1248 | ab 21,211 ± 1995 | cd 25,709 ± 1135 | ef 34,080 ± 2674 | gh 25,505 ± 2065 | −7648 ± 5112 | aceg −13,651 ± 1664 | bdfh |
| Tryptophan/large neutral amino acids | −0.01 ± 0.01 | abc −0.03 ± 0.04 | def 0.06 ± 0.01 | ghi 0.10 ± 0.01 | jk 0.36 ± 0.04 | adj 0.09 ± 0.10 | beh −0.66 ± 0.15 | cfik |
| Sum amino acids | 84,438 ± 5316 | ab 89,695 ± 10,998 | c 91,364 ± 6611 | 108,164 ± 8655 | 95,058 ± 8365 | 122,788 ± 19,326 | a 112,577 ± 10,462 | bc |

Values are means ± SEM. Mann–Whitney *U*-test: the same character within a row indicates a significant difference between two treatments ($p < 0.05$). Concentrations of glucose, insulin, GLP-1 and ghrelin were measured for 3 h, concentrations of amino acids for 4 h.

Table 2B

Changes in glucose (mmol/l h), insulin (mU/l h), GLP-1 (pmol/l h), ghrelin (pmol/l h), and amino acid ($\mu\text{mol/l h}$) concentrations expressed as AUC after consumption of a breakfast with 20% of daily energy requirements with 25 En% from protein with either casein, soy, whey, whey-GMP, alpha-lactalbumin, gelatin, or gelatin + TRP as protein type in 24 subjects (men and women) measured in preceding studies.^{16–18}

| | Casein 25% | | Soy 25% | | Whey 25% | | Whey-GMP 25% | | Alpha-lactalbumin 25% | | Gelatin 25% | | Gelatin + TRP 25% | |
|--------------------------------------|------------------|-----|----------------|-----|-----------------|-----|------------------|-----|-----------------------|------|------------------|------|-------------------|------|
| Glucose | 68 ± 18 | | 122 ± 13 | | 95 ± 11 | | 93 ± 17 | | 84 ± 22 | | 82 ± 13 | | 105 ± 12 | |
| Insulin | 4792 ± 980 | abc | 7520 ± 929 | d | 9159 ± 692 | | 9876 ± 886 | | 9080 ± 988 | ad | 7698 ± 847 | b | 8227 ± 1033 | c |
| GLP-1 | 161 ± 90 | a | 195 ± 72 | b | 425 ± 135 | | 306 ± 103 | | 407 ± 118 | | 438 ± 105 | | 462 ± 105 | ab |
| Ghrelin | −546 ± 184 | | −430 ± 128 | | −721 ± 145 | | −882 ± 176 | | −426 ± 111 | | −619 ± 103 | | −626 ± 124 | |
| Glutamate | 2220 ± 454 | ab | 3264 ± 643 | cd | 3705 ± 517 | ef | 2163 ± 381 | gh | 2962 ± 704 | | 6568 ± 1283 | aceg | 6565 ± 851 | bdfh |
| Asparagine | 7304 ± 428 | abc | 13,958 ± 278 | de | 10,122 ± 382 | fgh | 9195 ± 454 | ijk | 15,415 ± 853 | afi | −809 ± 432 | bdgj | −866 ± 387 | cehk |
| Serine | 7943 ± 754 | ab | 10,277 ± 416 | cd | 9178 ± 889 | ef | 6038 ± 743 | ghi | 8924 ± 640 | g | 21,259 ± 2498 | aceh | 22,768 ± 1112 | bdfi |
| Glutamine | 9993 ± 2288 | | 7818 ± 943 | | 12,156 ± 1655 | a | 7146 ± 1676 | | 5680 ± 2776 | | 6486 ± 2841 | | 6075 ± 1488 | a |
| Histidine | 5448 ± 453 | ab | 4314 ± 241 | cde | 3311 ± 305 | fgh | 3356 ± 260 | ijk | 6776 ± 554 | cfi | 970 ± 408 | adgj | 619 ± 475 | behk |
| Glycine | −476 ± 791 | ab | 6760 ± 675 | cde | −2759 ± 1044 | fgh | −4686 ± 914 | ijk | 1316 ± 1691 | cfi | 115,972 ± 10,058 | adgj | 123,145 ± 6050 | behk |
| Threonine | 13,370 ± 803 | a | 11,500 ± 544 | b | 34,393 ± 1284 | cde | 21,892 ± 1154 | fg | 24,137 ± 1496 | abc | 11,890 ± 1619 | df | 12,488 ± 966 | eg |
| Citrulline | −339 ± 126 | abc | −273 ± 136 | def | −33 ± 136 | ghi | 203 ± 116 | jkl | 966 ± 182 | adgj | 916 ± 290 | behk | 778 ± 309 | cfgl |
| Arginine | 6638 ± 386 | abc | 17,924 ± 669 | d | 5327 ± 404 | efg | 6292 ± 309 | hij | 2725 ± 496 | adeh | 19,221 ± 1656 | bfi | 19,216 ± 1001 | cgj |
| Alanine | 36,568 ± 1822 | ab | 41,833 ± 2408 | cd | 49,814 ± 2859 | efg | 38,665 ± 3059 | hi | 30,529 ± 4744 | e | 66,482 ± 6954 | acfh | 74,223 ± 4705 | bdgi |
| Taurine | −72 ± 102 | ab | 297 ± 72 | cde | 137 ± 132 | fg | −68 ± 81 | hi | −44 ± 135 | c | 2174 ± 225 | adfh | 2328 ± 191 | begi |
| Alpha-aminobutyric acid | 682 ± 97 | | 443 ± 100 | abc | 1262 ± 111 | def | 793 ± 96 | | 705 ± 83 | ad | 862 ± 157 | be | 888 ± 75 | cf |
| Tyrosine | 11,423 ± 727 | abc | 11,091 ± 509 | def | 6452 ± 565 | ghi | 9980 ± 583 | jkl | 13,739 ± 1017 | adgj | −2116 ± 377 | behk | −1820 ± 275 | cfil |
| Valine | 28,574 ± 1396 | abc | 22,855 ± 870 | def | 34,006 ± 1327 | ghi | 24,916 ± 1072 | jkl | 17,090 ± 1224 | adgj | 6500 ± 1029 | behk | 7392 ± 473 | cfil |
| Methionine | 5470 ± 366 | abc | 954 ± 233 | | 4354 ± 514 | def | 4297 ± 327 | ghi | 765 ± 151 | adg | 458 ± 199 | beh | 678 ± 108 | cfi |
| Isoleucine | 13,811 ± 605 | abc | 18,154 ± 450 | def | 31,195 ± 1133 | ghi | 22,388 ± 1152 | jk | 25,190 ± 1531 | adg | 443 ± 671 | behj | 1128 ± 239 | cfik |
| Phenylalanine | 5416 ± 290 | abc | 8098 ± 285 | def | 3298 ± 203 | ghi | 4379 ± 222 | jkl | 6484 ± 418 | adgj | 799 ± 245 | behk | 1173 ± 211 | cfil |
| Tryptophan | 1947 ± 201 | abc | 2571 ± 197 | def | 7214 ± 281 | ghi | 8408 ± 474 | jkl | 22,243 ± 1493 | adgj | −2478 ± 452 | behk | 17,154 ± 797 | cfil |
| Leucine | 22,578 ± 1038 | abc | 21,071 ± 1393 | def | 40,815 ± 1502 | gh | 46,428 ± 2256 | ijk | 41,790 ± 2462 | adi | 1592 ± 1003 | begj | 2501 ± 529 | cfhk |
| Ornithine | 4735 ± 375 | abc | 7918 ± 411 | def | 3390 ± 382 | ghi | 2967 ± 267 | jkl | 899 ± 251 | adgj | 9929 ± 969 | behk | 10,519 ± 754 | cfil |
| Lysine | 27,251 ± 1139 | abc | 22,530 ± 922 | def | 43,270 ± 1231 | ghi | 46,139 ± 1996 | jk | 50,879 ± 2619 | adg | 13,358 ± 2511 | behj | 15,223 ± 719 | cfik |
| Branched-chain amino acids | 64,963 ± 3002 | abc | 62,081 ± 2476 | def | 106,016 ± 3703 | ghi | 93,733 ± 4377 | jkl | 84071 ± 5066 | adgj | 8535 ± 2571 | behk | 11,021 ± 1191 | cfil |
| Large neutral amino acids | 81,802 ± 3884 | abc | 81,269 ± 3032 | def | 115,766 ± 4172 | gh | 108,092 ± 4992 | ij | 104,294 ± 6262 | ad | 7218 ± 3085 | begi | 10,374 ± 1568 | cfhj |
| Tryptophan/large neutral amino acids | 0.02 ± 0.00 | abc | 0.03 ± 0.00 | def | 0.06 ± 0.00 | ghi | 0.07 ± 0.01 | jkl | 0.22 ± 0.01 | adgj | −0.24 ± 0.14 | behk | 1.92 ± 0.28 | cfil |
| Sum amino acids | 210,435 ± 10,785 | abc | 233,355 ± 8463 | def | 300,607 ± 11430 | | 260,891 ± 11,934 | g | 280,970 ± 15,050 | ad | 282,230 ± 28,288 | be | 322,176 ± 12,994 | cfg |

Values are means ± SEM. Mann–Whitney *U*-test: the same character within a row indicates a significant difference between two treatments ($p < 0.05$).

Concentrations of glucose, insulin, GLP-1 and ghrelin were measured for 3 h, concentrations of amino acids for 4 h.

as tryptophan and 11% as valine. From the proteins we used, casein comes closest to this amino acid composition whereas gelatin is the protein with the worst quality. Gelatin is an incomplete protein and it may be hypothesized that the oxidation of gelatin has high energy costs. This may induce an increased satiety, since a positive relationship was observed between energy expenditure and satiety by Westerterp-Plantenga et al.³¹ Hochstenbach–Waelen indeed showed an increased energy expenditure and a decreased hunger and desire to eat after a high gelatin diet compared with a normal gelatin diet for 36 h.³² An increased energy expenditure may be the mechanism for gelatin to induce an increased satiety and reduce subsequent energy intake. Alpha-lactalbumin is a relatively complete protein, nevertheless it also increased satiety compared with other types of protein, so other mechanisms are also involved in protein-induced satiety.

Our results show that with breakfasts with different protein types a significant difference in energy intake at lunch is likely to be achieved if the difference in induced satiety is considerably; 15–25 mm on a Visual Analogue Scale a ~40% increased satiety. Apparently when differences were smaller it was not enough to induce significant effects on energy intake.

Timing of the moment when an *ad lib* meal is offered is important in evaluating the satiating properties of protein.⁸ Hall et al. report a significantly lower energy intake following a whey protein preload compared with a casein preload.³ However, the buffet meal was offered at 90 min after the preloads, when effects of casein have not been fully developed, and therefore probably is too soon to be a realistic and sensitive moment to measure differences in energy intake. On the other hand, it should be prevented that differences in appetite ratings or 'satiety' hormone levels have become extinguished over time. Despite appetite ratings suggesting that gelatin was more satiating than casein, Lang et al. did not observe significant differences in energy intake and macronutrient intake at dinner or over 24 h after a test lunch with casein, gelatin, or soy protein.⁷ However, dinner was offered 8 h after lunch, so the differences in satiety may have diminished by this time. We therefore determined the most sensitive time point to offer lunch in preceding experiments.^{16–18}

Apart from the experiments by Hall and Lang mentioned above^{3,7} only a limited number of human studies describe a comparison of different protein types with respect to their effects on energy intake or satiety. A comparison of beef, chicken, and fish protein revealed that fish protein increased satiety compared with the other protein types; food intake afterwards was not measured.³³ In a series of preceding studies, we showed that energy intake at lunch was decreased after a breakfast with whey compared with a breakfast with whey-GMP,¹⁸ that whey was more satiating than casein or soy protein at a level of 10% of energy from protein in a breakfast¹⁷ and that hunger was more suppressed after a breakfast with 10% of energy from alpha-lactalbumin compared with a breakfast with 10% of energy from gelatin or gelatin + TRP.¹⁶ Lang and colleagues did not observe significantly different effects of egg albumin, casein, gelatin, soy, pea, or wheat gluten on appetite scores or energy intake, probably because of the presence of other proteins.⁶ A study by Bowen et al. evaluated the effect of casein or whey protein preload on indicators of appetite and food intake, however, no differences in appetite or food intake between casein and whey were observed.⁴ In another study of Bowen et al. no difference was found in appetite ratings and energy intake after whey, soy, or gluten preload.⁵

The results of this study may be used in a weight-loss diet. When people feel less hungry and desire to eat is suppressed, it is easier for them to comply with a diet because they really feel an effect of the diet and then they will actually eat less, as has been

previously shown in experiments by Skov et al.³⁴ and Weigle et al.² Alpha-lactalbumin and gelatin (+TRP) were more satiating than the other types of protein and thus may help to feel subjects to feel less hungry and comply with their weight-loss diet. Gelatin is an incomplete protein and can not be offered as the single protein type in a diet, however addition of this protein to a diet with other high quality proteins present may have beneficial effects on the compliance to the diet.

Summarizing, alpha-lactalbumin, gelatin, or gelatin + TRP containing breakfasts caused a ~20% lower energy intake at lunch than a casein, soy, or whey-GMP breakfast, both at the level of 10 and 25% of energy from protein. Alpha-lactalbumin and gelatin + TRP breakfasts also reduced energy intake compared with a breakfast with whey at the level of 25% of energy from protein. The reduced energy intake of 20% was related to a ~40% reduction in appetite. In conclusion, different proteins (alpha-lactalbumin, gelatin, and gelatin + TRP) that are 30–50% more satiating than other proteins (casein, soy, whey, and whey-GMP) induce a related 17–24% reduction of subsequent energy intake at the following meal.

Conflict of interest

None of the authors had a personal or financial conflict of interest.

Acknowledgements

MABV, AGN, AH-W, KRW, MPKJE, RJMB, NEPD, and MSW-P designed the study. MABV and AH-W collected and analyzed the data. MABV wrote the manuscript and AGN, KRW, MPKJE, NEPD, and MSW-P contributed to interpretation of the data and reviewed the manuscript. The study was executed under supervision of AGN, KRW, and MSW-P.

References

1. Westerterp-Plantenga MS, Luscombe-Marsh N, Lejeune MP, et al. Dietary protein, metabolism, and body-weight regulation: dose-response effects. *Int J Obes (Lond)* 2006;**30**(Suppl. 3):S16–23.
2. Weigle DS, Breen PA, Matthys CC, et al. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *Am J Clin Nutr* 2005;**82**:41–8.
3. Hall WL, Millward DJ, Long SJ, Morgan LM. Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *Br J Nutr* 2003;**89**:239–48.
4. Bowen J, Noakes M, Trenerry C, Clifton PM. Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. *J Clin Endocrinol Metab* 2006;**91**:1477–83.
5. Bowen J, Noakes M, Clifton PM. Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake. *J Clin Endocrinol Metab* 2006;**91**:2913–9.
6. Lang V, Bellisle F, Oppert JM, et al. Satiating effect of proteins in healthy subjects: a comparison of egg albumin, casein, gelatin, soy protein, pea protein, and wheat gluten. *Am J Clin Nutr* 1998;**67**:1197–204.
7. Lang V, Bellisle F, Alamowitch C, et al. Varying the protein source in mixed meal modifies glucose, insulin and glucagon kinetics in healthy men, has weak effects on subjective satiety and fails to affect food intake. *Eur J Clin Nutr* 1999;**53**:959–65.
8. Anderson GH, Tecimer SN, Shah D, Zafar TA. Protein source, quantity, and time of consumption determine the effect of proteins on short-term food intake in young men. *J Nutr* 2004;**134**:3011–5.
9. Spaaij CJ, Pijls LT. New dietary reference intakes in the Netherlands for energy, proteins, fats and digestible carbohydrates. *Eur J Clin Nutr* 2004;**58**:191–4.
10. 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.
11. Dangin M, Boirie Y, Garcia-Rodenas C, et al. The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am J Physiol Endocrinol Metab* 2001;**280**:E340–8.
12. Dangin M, Boirie Y, Guillet C, Beaufrere B. Influence of the protein digestion rate on protein turnover in young and elderly subjects. *J Nutr* 2002;**132**:3228S–33S.

13. Marshall K. Therapeutic applications of whey protein. *Altern Med Rev* 2004;**9**:136–56.
14. Pedersen NL, Nagain-Domaine C, Mahe S, Chariot J, Roze C, Tome D. Casein-omacropptide specifically stimulates exocrine pancreatic secretion in the anesthetized rat. *Peptides* 2000;**21**:1527–35.
15. Beulens JW, Bindels JG, de Graaf C, Alles MS, Wouters-Wesseling W. Alpha-lactalbumin combined with a regular diet increases plasma Trp-LNAA ratio. *Physiol Behav* 2004;**81**:585–93.
16. Nieuwenhuizen AG, Hochstenbach-Waelen A, Veldhorst MA, et al. Acute effects of breakfasts containing alpha-lactalbumin, or gelatin with or without added tryptophan, on hunger, 'satiety' hormones and amino acid profiles. *Br J Nutr* 2008;1–8.
17. Veldhorst MAB, Nieuwenhuizen AG, Hochstenbach-Waelen A, et al. Effects of complete whey-protein breakfasts versus whey without GMP-breakfasts on energy intake and satiety. *Appetite*, in press.
18. Veldhorst MAB, Nieuwenhuizen AG, Hochstenbach-Waelen A, et al. Dose-dependent satiating effect of whey relative to casein or soy. *Physiology & Behavior*, in press.
19. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J Psychosom Res* 1985;**29**:71–83.
20. 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.
21. Adam TC, Westerterp-Plantenga MS. Nutrient-stimulated GLP-1 release in normal-weight men and women. *Horm Metab Res* 2005;**37**:111–7.
22. Munro HN. Second Boyd Orr Memorial Lecture. Regulation of body protein metabolism in relation to diet. *Proc Nutr Soc* 1976;**35**:297–308.
23. Harris JA, Benedict FG. *A biometric study of basal metabolism in man*. Washington, DC, USA: Carnegie Institution of Washington; 1919.
24. Westerterp KR, Kester AD. Physical activity in confined conditions as an indicator of free-living physical activity. *Obes Res* 2003;**11**:865–8.
25. Senn S. Cross-over trials in Statistics in Medicine: the first '25' years. *Statist Med* 2006;**25**:3430–42.
26. Woods SC, Chavez M, Park CR, et al. The evaluation of insulin as a metabolic signal influencing behavior via the brain. *Neurosci Biobehav Rev* 1996;**20**:139–44.
27. VanderWeele DA. Insulin is a prandial satiety hormone. *Physiol Behav* 1994;**56**:619–22.
28. Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 1998;**101**:515–20.
29. 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.
30. (FNB) FaNB. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients), *A Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes*. Washington, D.C: Institute of Medicine of the National Academies the National Academies Press; 2005.
31. 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.
32. Hochstenbach-Waelen A, Veldhorst MAB, Nieuwenhuizen AG, Westerterp-Plantenga MS, Westerterp K.R. Effects of gelatin in a 25 vs 10 energy% single protein diet on energy expenditure, substrate balances and appetite, personal communication.
33. Uhe AM, Collier GR, O'Dea K. A comparison of the effects of beef, chicken and fish protein on satiety and amino acid profiles in lean male subjects. *J Nutr* 1992;**122**:467–72.
34. Skov AR, Toubro S, Ronn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *Int J Obes Relat Metab Disord* 1999;**23**:528–36.