Acute effects of breakfasts containing alpha-lactalbumin, or gelatin with or without added tryptophan, on hunger, 'satiety' hormones and amino acid profiles.

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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

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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 ± SEM BMI: 24.8 ± 0.5 kg/m^2; 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 ~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 (~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 ~40% more satiating than other proteins (casein, soy, whey, whey-GMP) induce a related ~20% reduction of subsequent energy intake.

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]

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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 plasma[15] 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/m^2, 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 $1 \leq 9$), not using medication apart from oral contraceptives and at most moderate alcohol users ($\leq$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/m^2). 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 – Alphalactalbumin™, 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 ± 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

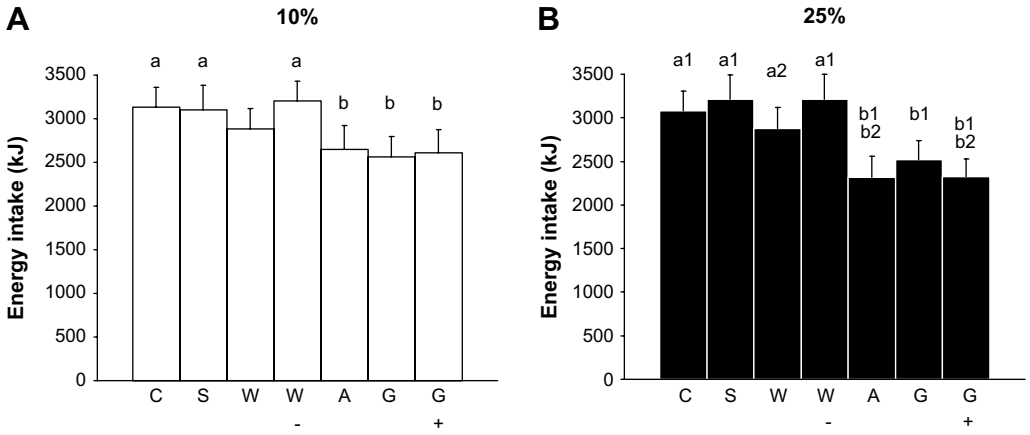

Fig. 1. *Ad lib* energy intake (kJ) at lunch after consumption of a breakfast with 20% of daily energy requirements with either 10 En% (A) or 25 En% (B) from protein with either casein, soy, whey, whey-GMP, alpha-lactalbumin, gelatin, or gelatin + TRP as protein type in 24 subjects (men and women). Values are means ± 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 ~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;

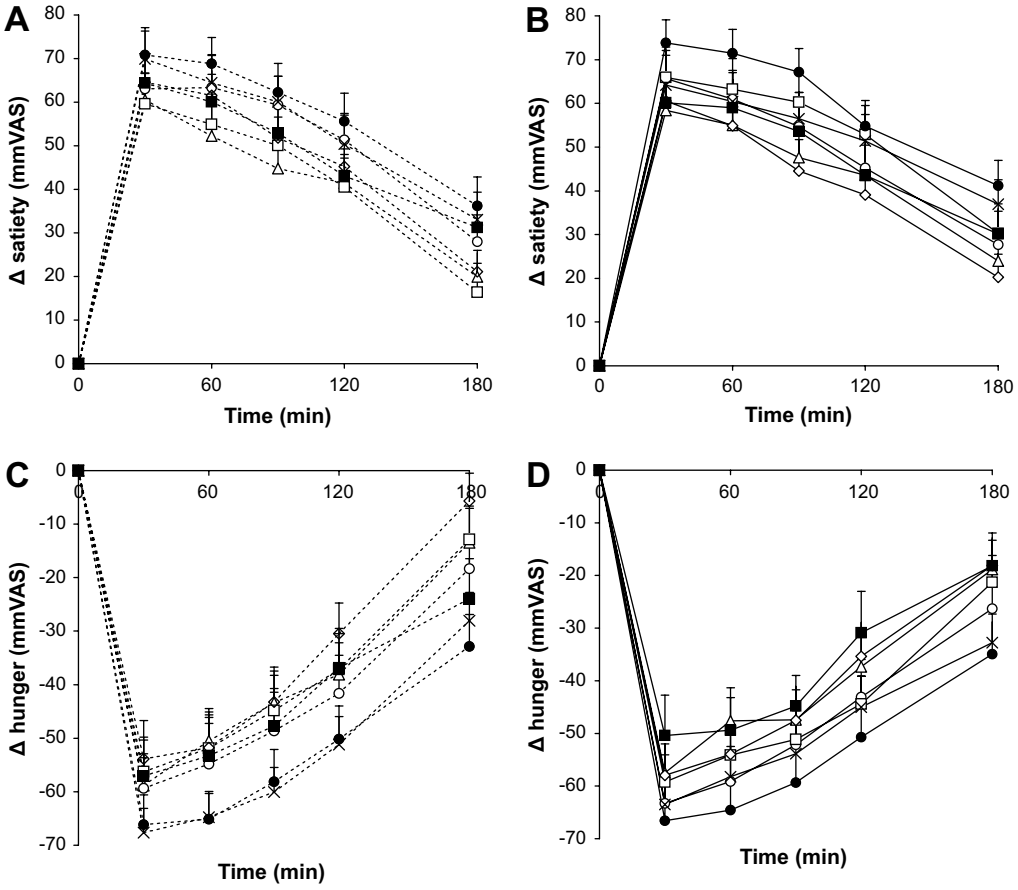

Fig. 2. Changes in satiety and hunger (mmVAS) after consumption of a breakfast with 20% of daily energy requirements with either 10 En% (A + C) or 25 En% (B + D) from protein with either casein, soy, whey, whey-GMP, alpha-lactalbumin, gelatin, or gelatin + TRP as protein type in 24 subjects (men and women). Values are means ± SEM, ANOVA repeated measures with Fisher's PLSD correction. —△ 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%. 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*. 2C: 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 (μmol/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	a	114 ± 16		138 ± 13	a	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	jkl	7148 ± 326	adgj	−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	adgj	−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	adgj	−1202 ± 999	behk	6640 ± 393	cfil
Leucine	7027 ± 393	abc	4948 ± 477	def	10,219 ± 373	ghi	16,262 ± 586	jkl	12,007 ± 733	adgj	−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	adgj	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 (μmol/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 breakfast[17] 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.

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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.

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