Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite

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Protein, generally agreed to be the most satiating macronutrient, may differ in its effects on appetite depending on the protein source and variation in digestion and absorption. We investigated the effects of two milk protein types, casein and whey, on food intake and subjective ratings of hunger and fullness, and on postprandial metabolite and gastrointestinal hormone responses. Two studies were undertaken. The first study showed that energy intake from a buffet meal ad libitum was significantly less 90 min after a 1700 kJ liquid preload containing 48 g whey, compared with an equivalent casein preload (P < 0.05). In the second study, the same whey preload led to a 28% increase in postprandial plasma amino acid concentrations over 3 h compared with casein (incremental area under the curve (iAUC), P < 0.05). Plasma cholecystokinin (CCK) was increased by 60% (iAUC, P < 0.005), glucagon-like peptide (GLP)-1 by 65% (iAUC, P < 0.05) and glucose-dependent insulinotropic polypeptide by 36% (iAUC, P < 0.01) following the whey preload compared with the casein. Gastric emptying was influenced by protein type as evidenced by differing plasma paracetamol profiles with the two preloads. Greater subjective satiety followed the whey test meal (P < 0.05). These results implicate post-absorptive increases in plasma amino acids together with both CCK and GLP-1 as potential mediators of the increased satiety response to whey and emphasise the importance of considering the impact of protein type on the appetite response to a mixed meal.

Dietary protein: Satiety: Cholecystokinin: Glucagon-like peptide-1: Postprandial amino acids

It is well established that protein is more satiating, kJ for kJ, than carbohydrate or fat (Booth et al. 1970; De Castro, 1987; Barkeling et al. 1990; Hill & Blundell, 1990; Johnstone et al. 1996; Stubbs et al. 1996; Vandewater & Vickers, 1996; Porrini et al. 1997; Latner & Schwartz, 1999). However, the satiating influence of protein is variable. One influence is the habitual protein intake that affects the satiating response to a standard protein meal in individuals. Thus, the satiating response to a standard protein meal is less in subjects previously adapted to a high- compared with a low-protein intake (Long et al. 2000). While the mechanism for this has not been demonstrated, one possibility relates to a more rapid postprandial clearance and oxidation of amino acids (AA) in subjects adapted to higher protein intakes. Another influence is the protein itself. There is some evidence that different protein sources may differ in their satiating capacity (Uhe et al. 1992), although not all observers report differences (Lang et al. 1998, 1999). If the extent of postprandial excursions in circulating AA levels influence satiety (the basis of Mellinkoff’s original aminostatic concept (Mellinkoff et al. 1956)), then factors that influence dietary protein digestion and absorption could influence the relative satiating influence of proteins. The digestion and absorption of whey and casein differ in that casein, unlike whey, coagulates in the stomach due to its precipitation by gastric acid (Billeaud et al. 1990; Miller et al. 1990). As a result, overall gastric emptying time for casein is longer and there is a smaller postprandial increase in plasma AA compared with the non-coagulating whey protein. The concept of ‘fast’ and ‘slow’ proteins was introduced by Boirie et al. (1997) to describe these differences in digestion and absorption of whey and casein. It might be predicted therefore that whey, a ‘fast’ protein, would be more satiating than casein.

Abbreviations: AA, amino acid; CCK, cholecystokinin; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; VAS, visual analogue scale.

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The aims of the present study were therefore: (1) to test the hypothesis described earlier; (2) to investigate possible physiological and metabolic bases for any differences in appetite found. The effects on appetite of isoenergetic liquid preloads containing either casein or whey were investigated in normal-weight healthy volunteers, by measuring their relative influence on food intake and subjective appetite ratings. In a separate experiment, postprandial profiles of circulating hormones and metabolites were measured following consumption of the two protein preloads. In particular, the potential roles of cholecystokinin (CCK) and glucagon-like peptide (GLP)-1, two gastrointestinal hormones that have both been previously shown to play an important role in satiety in human subjects (Ballinger & Clark, 1994; Lieverse et al. 1994; Gutzwiller et al. 1999; Verdich et al. 2001), were investigated.

Materials and methods

Two studies were performed both involving a randomised, single-blind, within-subject experimental design. In the first, investigations were limited to study of the appetite responses to casein and whey preloads by means of subjective ratings and measurement of food intake ad libitum during a buffet-style meal. In the second study, the focus was on the physiological and biochemical sequelae of the two protein pre-meals and a subsequent standard meal, although subjective appetite ratings were also measured.

Subjects

All subjects were non-dieting and non-smokers. No subjects had special dietary requirements, significant current or previous medical history, or took medication apart from oral contraceptives. Written informed consent was obtained from all participants and the study protocol was approved by the University of Surrey Advisory Committee on Ethics.

Subjects in study 1 consisted of sixteen lean healthy volunteers (eight female and eight male) who were recruited from the University of Surrey, mean age 22 (SD 2·0) years and mean BMI 22·6 (SD 1·5) kg/m². Subjects in study 2 were nine lean healthy volunteers (eight female and one male) recruited from the University of Surrey, mean age 25 (SD 2·4) years and mean BMI 22·6 (SD 1·5) kg/m². In each case, potential volunteers were excluded if they consumed >20 % energy intake as protein (assessed by 4 d food diary in study 1, and 7 d food diary in study 2) or if their mean score was >3·0 for the Dutch Eating Behaviour Questionnaire (van Strien et al. 1986).

Preloads, meals and measurement of appetite

Casein and whey liquid preloads. Identical isoenergetic high-protein liquid preloads, containing about 1700 kJ and 48 g protein, were used in both studies. The liquid preload was formulated using commercially available powdered whey (Prolab Nutrition, Bloomfield, CT, USA) or casein (Tropicana World Ltd, Birmingham, UK), protein, double cream and maltodextrin made up to 450 ml with water. Macronutrient and AA compositions of the two protein powders are shown in Table 1 and the macronutrient composition and energy content of the final formulated preloads in Table 2. In addition, in study 2, the liquid preloads also contained 1·5 g paracetamol (Pandol; GlaxoSmithKline, Brentford, Essex, UK) in order to monitor gastric emptying (Heading et al. 1973).

Meals and appetite measurement. Food intake after consumption of the preload was assessed in study 1 using a buffet test meal ad libitum (Hill et al. 1995) that enabled subjects to choose from a range of familiar foods, appropriate to the time of day. Subjects were asked to rank various buffet food items in order of preference. They were then offered their second and third choices of sandwich fillings and food types. The total energy available from the buffet ranged from 10 751 kJ to 12 611 kJ (protein 13, fat 38, carbohydrate 49 % energy) depending on subjects’ preferences and the subsequent food items offered. The number and range of food items presented to each subject was identical on each test occasion. The buffet food was weighed before and re-weighed after each subject had finished eating, to allow calculation of energy and macronutrient intake.

A cold lunch was supplied in study 2 based on the subjects’ food choices (obtained during recruitment). This was standardised to supply each subject with 42 kJ/kg body weight, and with each component of the meal making up the same proportion of total energy intake for each individual (calculated on the basis of the nutritional composition of the foods). The meal choices were similar to those offered to subjects in the buffet-style meal used in study 1. Subjects were instructed to consume all the food that was offered.

Subjective assessment of hunger, satiety and desire to eat was made in both studies using subjective visual analogue scales (VAS) (Hill et al. 1995). Each scale consisted of a 100 mm line anchored at either end with...
after the liquid preload. Subjects were instructed to eat
GLP-1, glucose-dependent insulinotropic polypeptide
measurement of plasma paracetamol, AA, glucose, insulin,
and at 5, 15, 35, 45, 60, 75, 90, 120 and 180 min after pre-
state. Subjects consumed the liquid casein or whey preload
and then consumed nothing until the study in order that
ing before and until the study, and in study 2 to refrain
subjects were offered the pre-weighed buffet-style test
for the next 3 h. Ninety minutes after the liquid preload,
ance of the preloads. VAS ratings were completed before
by marking the scale at the point that was most appropriate
to their feeling at that time. Ratings on the scales were con-
ercially dried under low vacuum and the tubes stored at
nol (plasma–ethanol (980 g/l) 1:2, v/v) on the study day,
ached using Waters PICO-TAGTM Amino Acid
Analysis System (Waters, MA, USA), which involves the
s in the black opaque beaker with a lid and ingested through
straw, thus masking any visible difference in the appear-
ance of the preloads. VAS ratings were completed before
and after consumption of the drink, and at 20 min intervals
for the next 3 h. Ninety minutes after the liquid preload,
subjects were offered the pre-weighted buffet-style test meal ad libitum, from which energy and macronutrient
intakes were calculated.

Study protocols

Each study was a randomised, single-blind, within-subject experimental design involving the testing of either whey or
casein liquid preloads on two separate days. Subjects were
instructed to avoid alcohol and high-protein meals the eve-
ning before and until the study, and in study 2 to refrain
from taking any paracetamol tablets or paracetamol-con-
taining products for the previous 24 h.

Study 1. Subjects were instructed to consume their
usual breakfast before 09.30 hours, and to eat the same
cereal-based breakfast on each test occasion. Individuals
were offered a liquid casein or whey preload at 11.30
hours on separate occasions 7 d apart. This was presented
in a black opaque beaker with a lid and ingested through a
straw, thus masking any visible difference in the appearance
of the preloads. VAS ratings were completed before and after consumption of the drink, and at 20 min intervals
for the next 3 h. Ninety minutes after the liquid preload,
subjects were offered the pre-weighed buffet-style test meal ad libitum, from which energy and macronutrient
intakes were calculated.

Study 2. The protocol for the investigation of physiolo-
gical mechanisms was designed to emulate the pro-
cedures in study 1 as closely as possible. On the day of
each study, subjects consumed a standard breakfast consist-
ing of cornflakes and skimmed milk before 08.00 hours,
and then consumed nothing until the study in order that
all volunteers started the study in a similar metabolic state.
Subjects consumed the liquid casein or whey preload at
11.00 hours. Venous blood samples were taken before, and at 5, 15, 35, 45, 60, 75, 90, 120 and 180 min after pre-
load consumption from a cannulated forearm vein, for the
measurement of plasma paracetamol, AA, glucose, insulin,
GLP-1, glucose-dependent insulinotropic polypeptide
(GIP) and CCK. The standard lunch was provided 90 min
after the liquid preload. Subjects were instructed to eat
all that was provided as quickly as was comfortable and
blood sampling was continued for another 90 min follow-
ing lunch.

Biochemical analyses

Blood samples were taken at regular intervals into tubes
containing preservatives. They were kept on ice before centrifugation at 4°C for 10 min at 1550 g to separate the
plasma. Blood was collected into fluoride oxalate tubes
before centrifugation for glucose measurement, and into
lithium heparin tubes for AA, insulin and GIP measure-
ment. Blood was collected into lithium heparin tubes
containing aprotinin (200 kIU/ml blood) for GLP-1 measure-
ment. The separated plasma was frozen at a temperature of −20°C. For CCK measurement, blood was collected
into chilled 5 ml EDTA tubes containing sodium
acetate buffer (1 ml sodium acetate buffer (175 mM-NaCl,
63 mM-NaOH, 30 mM-acetic acid, adjusted to pH 3-6 with
HCl)/4 ml blood). The separated plasma was added to etha-
ol (plasma–ethanol (980 g/l) 1:2, v/v) on the study day,
and then centrifuged at 1550 g for 15 min. The super-
natant fraction was transferred into assay tubes, immediately dried under low vacuum and the tubes stored at
−50°C until assay. Plasma paracetamol and glucose were
measured using commercial assay kits (Cambridge Life
Sciences (Ely, Cambridge, UK) and Randox (Crumlin,
Co. Antrim, UK) respectively) with an automated Cobas
Mira biochemical analyser (Roche Products Ltd., Welwyn Garden City, Hertfordshire, UK). Plasma AA
were measured using Waters PICO-TAGTM Amino Acid
Analysis System (Waters, MA, USA), which involves the
pre-column derivatisation of the sample with phenyli-
sothiocyanate followed by reversed-phase HPLC. Plasma
insulin, GIP, GLP-1 and CCK were analysed using radio-
immunoassay techniques (Morgan et al. 1978; Beardshall
et al. 1992; Elliott et al. 1993; Hampton & Whithey,
1993). Inter-assay CV for metabolites were <15 % for
insulin, GIP, GLP-1 and CCK. Intra-assay CV were smaller.
All samples from an individual subject were analysed
within the same assay.

Statistical analysis

Food intake ad libitum from the buffet meal was analysed
using Student’s paired t test. VAS ratings and biochemical
measurements were analysed by repeated-measures analysis of covariance with type of protein and time as

### Table 2. Composition of liquid test meals

<table>
<thead>
<tr>
<th>Test meal</th>
<th>Casein*</th>
<th>Whey†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>Macronutrient (g)</td>
</tr>
<tr>
<td>Protein powder</td>
<td>57</td>
<td>48‡</td>
</tr>
<tr>
<td>Double cream</td>
<td>23</td>
<td>11§</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>24</td>
<td>24‖</td>
</tr>
</tbody>
</table>

* Total energy 1674 kJ.
† Total energy 1695 kJ.
‡ Protein.
§ Fat.
‖ Carbohydrate.
within-subjects factors. Baseline values were treated as covariates or, for plasma AA concentrations, an ANOVA was carried out on the change from baseline values (x - baseline value). All results are expressed as mean values with their standard errors unless otherwise stated. P values < 0.05 were considered statistically significant.

Results

Study 1

Energy intake ad libitum for the buffet meal is shown in Fig. 1, including the proportion of energy consumed as carbohydrate, fat and protein. Total energy intake was significantly lower following the whey protein than following the casein protein preload (3676 (SEM 359) v. 4537 (SEM 528) kJ respectively, P<0.05). This difference was reflected in intakes of all three macronutrients. Subjective appetite ratings (VAS ratings) for hunger, desire to eat and fullness varied significantly over time (P<0.0001). However, no significant differences were found due to protein type (results not shown).

Study 2

Appetite. VAS ratings for hunger, desire to eat and fullness varied significantly with time (P<0.0001). Desire to eat (Fig. 2) was significantly reduced by whey protein compared with casein following the standard lunch (P<0.0005), an effect that failed to reach significance for hunger ratings (P=0.061). Fullness (Fig. 3) was significantly increased following the liquid whey preload compared with casein for the duration of the 180 min (P<0.0005).

Plasma metabolites. AA profiles were measured for thirteen AA. Total AA profiles are shown in Fig. 4. There was a significant preload x time interaction for plasma total AA (P<0.0005) due to higher circulating AA concentrations during the second and third hours following the liquid whey preload compared with casein. Increases in postprandial plasma profiles of the branched-chain AA following the whey compared with the casein preload were particularly marked and highly significant. Increases (%) following whey compared with casein over 180 min were: valine 34 (P<0.001), isoleucine 102 (P<0.00005) and leucine 70 (P<0.0000001), and threonine was increased by 95 (P<0.000005). No significant difference was observed in plasma glucose response following the two different protein liquid preloads.

Plasma hormones. There were significantly greater increases in overall plasma concentrations of GIP (P<0.005), GLP-1 (P<0.05) and CCK (P<0.01) following the whey preload compared with casein (Figs 5, 6 and 7), but postprandial increases in plasma insulin did not differ in response to the two protein preloads.

Gastric emptying. Plasma paracetamol concentrations following casein and whey preloads demonstrated a significant preload x time effect (P<0.05). This reflects a difference in rates of gastric emptying between the two preloads, with a faster initial rise in plasma paracetamol concentrations following the casein meal and then a levelling off compared with the whey preload.

Discussion

The present study extends our interest in aminostatic mechanisms of appetite regulation (Long et al. 2000) to an examination of the differing satiating capacity of various protein sources (Uhe et al. 1992; Lang et al. 1998, 1999). Here we focus on the ‘fast’ and ‘slow’ protein concept introduced by Boirie et al. (1997) to describe the differences between whey and casein in their digestion and absorption. Our present results confirm that casein, as a coagulating protein, exhibits a slower rate of gastric emptying and mediates lower postprandial excursions of plasma AA concentrations compared with the non-coagulating protein whey (Boirie et al. 1997). On the basis of Mellinkoff’s original aminostatic concept (Mellinkoff et al. 1956), a larger rise in plasma AA would increase satiety, and this is demonstrated by our present findings.

Our experimental design involved an initial study of appetite responses alone (study 1) followed by attempts to identify physiological and biochemical responses (study 2). We assumed that the serial blood sampling might make it more difficult to identify appetite responses, so we made no attempt to measure food intake ad libitum in study 2, limiting our measures to observations of subjective differences in appetite (VAS scores). The relative palatability of the casein and whey preloads was not measured by VAS or any other methodology. However, the presentation of the two meals in black opaque beakers with lids and straws minimised the visual and olfactory stimulus, and the protein powder formulations used were both fruit-flavoured, so there was no marked difference between the sensory qualities of the preloads. If there was a difference in palatability, the buffet-style ad libitum test meal in study 1 was 90 min after ingestion, and any influences on satiety resulting from the palatability of the preloads would have been minimal by then. In study 2,
Fig. 2. Study 2: effects of 1700 kJ preloads (48 g casein or whey) on desire to eat ratings (visual analogue scale). ■, Casein; □, whey. The liquid test meal was given at 0 min and the standard lunch at 90 min. For details of liquid test meals, subjects and procedures, see Tables 1 and 2 and p. 240. Values are means for nine subjects with their standard errors shown by vertical bars. There was a significant reduction in desire to eat after the whey preload following the standard lunch (analysis of covariance 90–180 min, \( P<0.005 \)).

Fig. 3. Study 2: effects of 1700 kJ preloads (48 g casein or whey) on fullness ratings (visual analogue scale). ■, Casein; □, whey. The liquid test meal was given at 0 min and the standard lunch at 90 min. For details of liquid test meals, subjects and procedures, see Tables 1 and 2 and p. 240. Values are means for nine subjects with their standard errors shown by vertical bars. There was a significant increase in fullness after the whey preload (analysis of covariance 0–180 min, \( P<0.05 \)).
Fig. 4. Study 2: effects of 1700 kJ preloads (48 g casein or whey) on plasma total amino acids. ●, Casein; □, whey. The liquid test meal was given at 0 min and the standard lunch at 90 min. For details of liquid test meals, subjects and procedures, see Tables 1 and 2 and p. 240. Values are means for nine subjects with their standard errors shown by vertical bars. There was a significant increase in total amino acid concentration following the whey preload (ANOVA 1–180 min, treatment × time interaction, \( P < 0.005 \)).

Fig. 5. Study 2: effects of 1700 kJ preloads (48 g casein or whey) on glucose-dependent insulinotropic polypeptide (GIP). ●, Casein; □, whey. For details of liquid test meals, subjects and procedures, see Tables 1 and 2 and p. 240. Values are means for nine subjects with their standard errors shown by vertical bars. There was a significant increase in plasma GIP after the whey preload (analysis of covariance 0–180 min, \( P < 0.005 \)).
the largest differences in subjective appetite ratings were also observed following the standardised lunch at 90 min.

The 19% reduction in food intake ad libitum following a liquid whey preload compared with the casein in study 1 clearly demonstrated that a whey-based liquid meal has a greater satiating efficiency than a casein-based liquid meal. Although no differences in subjective measures of appetite were shown in study 1, in study 2 significant
differences were observed. Thus, the whey preload resulted in increased fullness for the duration of the study and suppression of both hunger and desire to eat each following the standardised lunch-type meal. We interpret our inability to detect differences in the VAS scores in study 1 as reflecting the difficulty of quantifying this measure of appetite (Rogers, 1993; Hill et al. 1995; Green & Blundell, 1996; Flint et al. 2000; Stubbs et al. 2000). While all subjects appeared motivated and compliant with the experimental procedures, the subject groups in the two studies did differ to some extent. Subjects in study 2 were slightly but significantly older (mean age 25 v. 22 years, P<0·037). In study 2, unlike study 1, subjects were compensated financially for their time and the cannulation procedures. We do not know whether these factors influenced the subjects’ use of the VAS ratings. However, the largest differences in subjective appetite ratings found in study 2 were recorded following the standardised lunch. These differences are likely to have been obscured in study 1 since subjects were allowed to eat ad libitum and therefore ate different amounts at each study session. The differences in hunger and desire to eat were mainly evident following the standard lunch, whereas subjective ratings of fullness were different throughout the 180 min. This observation reflects the fact that ‘hunger’ and ‘satiety’ are distinct sensations and should not be regarded as simply the two extremes of a continuum. For example, the initiation of an eating episode does not wholly rely on hunger sensations, since the sensory properties of a food can stimulate food intake even when satiety signals are present. The fact that the protein preloads were administered as a covert liquid meal rather than a more customary solid meal may have meant that the cognitive and sensory stimulus that normally inhibits desire to eat was repressed until the consumption of a more familiar solid meal, the standard lunch. Sensations of fullness, however, are strongly determined by sensations of gastric distension, and this may account for the apparently consistent difference in satiation between the two preloads throughout the whole 180 min of the study.

In the experiments presented here, a difference in food intake or subjective satiety ratings was observed during a set period of 180 min following the casein and whey preloads. It is not clear from these present studies however, whether these differences would persist beyond that time period, or in fact whether the ‘fast’ protein would prove to be less satiating in the longer term than the ‘slow’ protein. Subjective fullness ratings and plasma CCK concentrations were still significantly increased following the whey at 180 min (analysis of covariance, P<0·01 and P<0·05 respectively), which could potentially impact satiety at the next meal. This suggests that the satiating capacity of whey could be maintained over a longer time-period than included in the current work. Longer-term studies are needed to address the question of whether whey protein is more satiating than casein over a day, or whether there would be a compensatory increase in food intake at the next meal, in which case there may be no difference in total food intake.

As judged by the plasma paracetamol profiles, an index of gastric emptying, differences in the gastric emptying profiles of the liquid casein and whey preloads occurred, although the nature of these differences was complex. Initial paracetamol absorption was faster following casein, but peak circulating concentrations were higher with whey. The most likely explanation of this pattern is that after the casein protein had precipitated into curds in the stomach, the solubilised paracetamol in the liquid compartment of the meal was emptied and absorbed rapidly, but the slow emptying of the solid casein curds maintained paracetamol absorption with a plateau in plasma concentrations. Indeed, it is possible that the whey meal emptied homogeneously at a steady rate, with a more even dispersion of the lipid and aqueous phases containing the carbohydrate and protein in the whey liquid meal, whereas the casein meal emptied in a biphasic manner due to its transformation to a solid–liquid meal, with the liquid component emptying earlier. Despite the apparent differences in gastric emptying rates of the two preloads, the time course of plasma glucose and insulin concentrations were not significantly different, suggesting that carbohydrate was emptied at a near uniform rate with the liquid component of the casein meal. This is in contrast to previous reports of differences in plasma insulin levels following different meal proteins (Gannon et al. 1988; Hubbard et al. 1989; Lang et al. 1999). Both insulin and glucose have been implicated in the control of appetite (Woods & Porte, 1983; Campfield & Smith, 1990); however, in the present study, it is unlikely that the observed differences in appetite could have been mediated by either glucose or insulin levels. This identifies variation in either protein- or fat-mediated mechanisms of satiety as responsible for the satiating effect of whey.

The marked differences in circulating postprandial AA levels observed following the two protein preloads point to some aminostatic mechanism of appetite regulation as a likely explanation of the differences in appetite observed with the two proteins. Postprandial peripheral AA profiles are likely to reflect rates of digestion, absorption and first-pass metabolism in the enterocyte and liver in addition to the AA composition of the proteins. The greatest differences between whey and casein AA composition (g/kg) were for arginine, methionine, phenylalanine, proline and tyrosine, in each case >50% more in casein compared with whey. Just under half of the plasma AA measured (five out of thirteen) showed an overall greater plasma response to the whey preload. The remainder showed no significant differences in their profiles throughout the study with the exception of proline, which significantly increased following the casein meal, probably reflecting the higher proline content of casein protein. Phenylalanine, whose effects on satiety are thought to be potent (Gibbs & Smith, 1977; Muurahainen et al. 1988; Ballinger & Clark, 1994; Rogers et al. 1995), showed no significant differences in plasma levels following the two test meals, again probably due to the greater phenylalanine content of casein. Tryptophan, an AA frequently linked with appetite regulation (Wurtman et al. 1981; Hrbicky et al. 1985; Hill & Blundell, 1988), could not be measured using our methodology.

The gastrointestinal hormones CCK and GLP-1 are secreted in response to protein ingestion (Hopman et al.
that might be useful in clinical nutrition as sip feeds for elderly or sick patients where increased food intake is desired.

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