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ORIGINAL CONTRIBUTION

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Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans

Summary *Background* The influence of protein fractionation on gastric emptying and rate of appearance of their constituent amino acids in peripheral blood remains unknown. *Aim of the study* To examine the influence of the degree of protein fractionation on gastric emptying, gastric secretion, amino acid absorption and enterogastrone response, after the intragastric administration of complete cow milk proteins or their respective peptide hydrolysates in man. *Methods* Six healthy males were randomized to receive one of the following four solutions: whey whole protein (W), casein whole protein (C), whey peptide hydrolysate (WHY) or casein hydrolysate (CAHY). All solutions were matched for volume (600 mL), nitrogen content (9.3 g/L), energy density (1069–1092 kJ/L), osmolality (288–306 mosmol/kg), pH (6.9–7.0) and temperature (37 °C). *Results* Solutions were emptied at similar rates, with mean half-times of (mean ± SEM) 21.4 ± 1.3, 19.3 ± 2.2, 18.0 ± 2.5 and 19.4 ± 2.8 min, for the WHY, CAHY, C and W, respectively. The rates of intestinal absorption of water and amino acids were similar with the exception of the casein protein solution, for which the speed of intestinal amino acid absorption was slower ($p < 0.05$). The peptide hydrolysates elicited about 50 % more gastric secretion than the whole protein solutions ($p < 0.05$), which was accompanied by higher glucose-dependent insulinotropic polipeptide (GIP) plasma levels during the first 20 min of the gastric emptying process. Similar glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) plasma responses were elicited by the four solutions. *Conclusions* The rate of gastric emptying and the plasma GLP-1 and PYY responses to feeding with cow milk protein solutions in humans are independent of the degree of protein fractionation and are not altered by small differences in the amino acid composition or protein solubility. In contrast, the GIP response is accentuated when milk proteins are delivered as peptide hydrolysates.

Key words protein digestion – oligopeptides – liquid meal – intestinal absorption – humans

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Introduction

It has been reported that plasma amino acid concentration is higher and rises more quickly following the ingestion of peptide hydrolysates than after the ingestion of their corresponding complete proteins [1]. Moughan et al. based their conclusions on the observation that peptide hydrolysate solutions undergo faster gastric emptying and intestinal absorption of their constituent amino acids than the complete proteins from which they derive [1]. However, very little is known about the kinetics of gastric emptying and intestinal absorption of peptide hydrolysates compared with their original protein in humans. The latter is important because a faster rate of gastric emptying may alter the incretin and enterogastrone response, but also may modify the hormonal response to feeding [2] and, hence, the outcome of the

nitrogen ingested. In addition, if peptide hydrolysates are emptied faster from the stomach, these preparations could be a good alternative for patients with volume intolerance [3].

Gastric emptying is regulated depending upon several factors among which the physical and chemical characteristics of ingested meals play a significant role. It has been demonstrated that low pH and temperature as well as high osmolality, viscosity, fiber content and energy density (caloric content) delay gastric emptying [4–6]. Moreover, ingestion of a large volume has been shown to increase the rate of gastric emptying [4]. Among these factors, energy density and volume seem to be the primary determinants of gastric emptying [4–7]. In addition, the particle size and the degree of hydrolysis of meal constituents may play a role [6, 8]. For example, it has been shown that isoenergetic solutions containing glucose or a glucose polymer are emptied at different rates, due to the higher osmolality of the monomeric glucose solutions [6]. In contrast, the rate of gastric emptying is similar for glucose isosmotic and isoenergetic solutions, regardless of the degree of polymerization of the carbohydrate [6].

Milk proteins comprise two major components: soluble whey protein and micellar casein, the latter representing about 80 % of the whole protein present in milk [9]. Some studies support a faster digestion and absorption for whey protein than for casein [10–12]. The latter may be due to the fact that casein precipitates and clots in the stomach forming small fragments that could be emptied from the stomach like solids, i. e., with slower kinetics [9, 12]. If casein is emptied and digested more slowly than other proteins, then an attenuation of the GLP-1 response to feeding should be observed after the ingestion of casein solutions compared with other predigested or more soluble protein solutions. This is particularly important since the rate of gastric emptying could indirectly limit the rate of intestinal absorption of amino acids and peptides [13, 14]. In turn, the chronology of amino acid availability is an important and independent factor modulating nitrogen retention [12, 15, 16], which is regulated by the hormonal response to feeding [15].

Among nutrients, proteins and amino acids are among the most potent gastric secretagogues [17, 18]. It appears that peptides and free amino acids are more potent gastric secretagogues than complete proteins in dogs [17, 18]. Silk et al. reported a higher intestinal secretion for free amino acid meals than for oligopeptides [14]. Thereby, oligopeptide solutions could elicit higher amounts of gastric secretion than the complete proteins from which they originate. The secretagogue effect of amino acids is counterbalanced by gut peptides known as enterogastrones, which are released by enteral endocrine cells and inhibit gastric secretion [19] such as glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polipeptide (GIP) and peptide YY (PYY) [20–26]. Whether the administration of a peptide hydrolysate solution will evoke a higher enterogastrone response than the complete protein from which it originates is not known.

Therefore, there were three main purposes of this study. First, to determine the rate of gastric emptying of isonitrogenous and isotonic amounts of casein and whey protein. Specifically, we sought to ascertain whether the replacement of a protein by its peptide hydrolysate has any influence on either the rate of gastric emptying or gastric secretion, when solutions are matched for energy content, energy density, osmolality, nitrogen content, pH, volume and temperature. Second, to ascertain the role that the rate of gastric emptying *per se* may have in determining the speed of intestinal absorption and, hence, the rate of increase of plasma amino acid concentrations. Third, to verify if the enterogastrone response is modified depending on the degree of fractionation of the protein administered. Compared to previous studies, a unique aspect of the present study is that gastric function, hormone secretion, and plasma amino acid concentrations were measured simultaneously under controlled experimental and dietary conditions in healthy humans.

Materials and methods

Subjects

Six healthy men with no clinical history of gastrointestinal disease volunteered to participate as experimental subjects. Their mean (range) ages, weights and heights were 30 years (27–32 years); 75 kg (68–81 kg) and 179 cm (172–184), respectively. The experimental protocol was approved by the ethical committee for Copenhagen and Frederiksberg communities and the subjects were informed about the purposes and risks of the study, and written consent obtained.

Test solutions

Four different test solutions of 600 mL were administered in this study (Tables 1 and 2) to each subject. Solutions were given on different days, separated by a 2–4 day period. All solutions were composed of similar amounts of nitrogen (9.3 g/L, equivalent to approximately 60 g of protein/L). Two solutions were made with complete cow whey (Lacprodan DI-9223, Arla Foods, Copenhagen) or casein protein (Midprodan 30, Arla Foods, Copenhagen), respectively. The other two solutions, i. e., the whey (Lacprodan DI-3065 DH, Arla Foods, Copenhagen) and casein peptide hydrolysate (Lacprodan IF-2050 DH) solutions, were prepared as enzymatic

Table 1 Composition of the administered solutions

Component	Whey hydrolysate	Casein hydrolysate	Whey whole protein	Casein whole protein
Volume (mL)	600	600	600	600
Protein (g/L)	60.0	60.0	60.0	60.0
Nitrogen (g/L)	9.3	9.3	9.3	9.3
Glucose (g/L)	–	3.8	3.7	2.5
Lactose (g/L)	4	1.5	0.3	0.2
Lipids (g/L)	< 0.1	< 0.1	< 0.1	0.7
Na (g/L)	2	2	3.3	3.2
Cl (g/L)	2.5	2.5	4.7	4.8
Ca (g/L)	0.60	0.60	0.23	0.78
Osmolality (mosml/kg)	292	288	299	306
pH	7.0	7.0	6.9	6.9
Energy density (kJ/L)	1069	1092	1070	1071

Table 2 L-Amino acid composition of the administered solutions

Amino acid	Whey hydrolysate	Casein hydrolysate	Whey whole protein	Casein whole protein
	mmol/L			
Alanine	30.30	18.86	31.65	17.51
Arginine	8.27	11.71	6.54	11.37
Aspartic acid	49.14	31.56	49.59	28.40
Cysteine	10.40	0.99	11.88	2.48
Glutamic acid	77.91	86.88	68.12	77.50
Glycine	13.58	12.78	13.58	12.78
Histidine	8.89	12.37	7.35	10.44
Isoleucine	26.52	23.78	27.90	22.41
Leucine	41.16	37.96	46.65	39.33
Lysine	39.40	32.83	37.76	28.73
Methionine	7.64	11.66	7.24	10.86
Phenylalanine	9.44	15.98	10.90	16.71
Proline	33.33	52.60	30.21	44.79
Serine	30.83	29.69	28.54	30.26
Threonine	35.74	19.13	36.74	18.12
Tryptophan	3.23	2.94	5.00	3.53
Tyrosine	7.95	17.22	12.25	15.89
Valine	29.21	32.79	30.23	30.74

hydrolysates from the complete cow whey and casein protein by Arla Foods (patent A23J 3/30; WO 92/21248; Copenhagen, Denmark). Briefly, the whey protein isolate (approximately 80 % protein) was first diluted with deionized water to 8 % at 55 °C. The product was pasteurized at 85 °C and pH adjusted to 8.0 with $Ca(OH)_2$. The solution was then submitted to enzymatic hydrolysis during 12 hours at 53 °C starting with 2.2 % Alcalase (Novo Nordisk, Bagsvaerd, Denmark) and when the pH of the solution reached 7.0, 1.1 % Neutrase (Novo Nordisk, Bagsvaerd, Denmark) was added. The latter was followed by ultra-filtration and the permeate heated to 85 °C for 3 min to inactivate the enzymes. Sterile filtration and spray-drying yielded the final product as a powder, which was used as the raw matter to prepare the peptide hydrolysate solution. The manufacturer applied a similar procedure to obtain the casein hydrolysate. The amount of nitrogen present in the test solutions was determined by the manufacturer using the Kjeldahl method (error < 1 %). About 94 % of the nitrogen contained in the whey peptide hydrolysate was in the form of oligopeptide (average chain length of 3.7 residues), while only 6 % of the nitrogen was as free amino acids (determined by the manufacturer using gel permeation chromatography). In turn, the casein peptide hydrolysate contained 93 % of the nitrogen as oligopeptides (average chain length of 3.8 residues) and 7 % as free amino acids. The whey protein solution contained a small quantity of lactose (3.7 g/L) and the casein protein solution a small amount of fat (0.7 g/L). Thus, to equalize energy density between solutions, glucose (2.5–3.8 g/L) was added such that all solutions had a similar energy content and energy density (641–655 kJ; i. e., 1069–1092 kJ/L). Subsequently, solutions were made isotonic by adding NaCl. Each test solution was delivered at 37 °C after adjusting the pH to 7.0–7.1.

Experimental procedures

Subjects reported to the laboratory after an overnight fast and had a gastroduodenal catheter (Levine type, CH 12, 120 cm) placed in the stomach. Residual gastric contents were then aspirated and the pH measured. The stomach was then washed with 400 mL of deionized water administered with a 50 mL syringe through the nasogastric tube. The gastric washout was readily syphoned and the experiments were only carried out if the washout was clear and free of food residues. Then, an 18-gauge venous catheter was inserted into an antecubital vein and was intermittently flushed with saline to maintain patency. After the person had been resting for 30 min in a sitting position, a 10 mL blood sample was withdrawn, and one of the four different test solutions was administered through the nasogastric tube. The volume remaining in the stomach was assessed using George's double-sampling aspiration technique as applied by Beckers et al. [27]. Briefly, this procedure is based on the addition of a known quantity of dye marker, and gastric volume is calculated as a function of the dilution of this marker. However, in the current study tritiated water was used as a marker.

To measure the residual gastric volume in the stomach, 5 μCi of tritiated water was added to each test solution and mixed. Then, a 2 mL sample was taken to de-

termine the initial tritium activity of the solution before administration. Immediately after the administration of the test solution the gastric contents were thoroughly mixed using a 50 mL syringe to aspirate and re-inject 20–30 mL, 10 times; mixing took approximately 1 min. Immediately after mixing, a gastric sample was taken and used to calculate the initial gastric volume. To assess the volume of the gastric contents at each time point, a 5 mL gastric sample was taken followed by the injection of 5 mL of a stock marker solution through the nasogastric tube. The stock marker solution was made by adding 300 μCi of tritiated water to a liter of physiological saline solution. The additional marker added at each sampling point (~1.5 μCi of tritiated water) was mixed with the gastric contents by pumping in-out with the 50-mL syringe for 1 min followed by the aspiration of a 2.5 mL sample. Assuming that the amount of tritium absorbed or secreted by the stomach during the sampling procedure is negligible, that the rate of water absorption by the stomach is too slow to significantly contribute to the total intestinal water absorption, and that gastric emptying does not occur during mixing or sampling, it is possible to successively calculate the gastric volume [27]. Thus, radioactivity was measured twice at each sampling point, before and after the addition of a known quantity of marker. Since each subject participated in four trials the overall radiation dose administered was approximately 68 μCi. This value is equivalent to a body dose of 14.8 mrem (or 1.48 μSv, i. e., about 5 % of the dose administered with a standard chest X-ray).

Gastric samples for the measurement of gastric volume, chloride and pH, as well as blood samples for the determination of tritium activity were taken every 5 min during the first 20 min and every 10 min thereafter during the first hour. Gastric content volumes were corrected for the small difference between the volume sampled (7.5 mL) and the volume injected (5 mL) at each sample time. The pH was measured immediately during the experiment and the aspirated gastric samples were frozen at –80 °C until analyzed. Solutions were administered in random order (Latin square design) and each trial was separated by one week.

The blood samples were all immediately centrifuged and the supernatant was collected and stored at –80 °C. Plasma amino acids were determined in duplicate by prior derivatization with phenyl-isothiocyanate [28] and HPLC (Waters, Millipore, Milford, MA).

Determinations of GLP-1, GIP, PYY

Blood samples and RIAs: blood samples were withdrawn before the administration of the four solutions (0 time sample) and then 5, 10, 15, 20, 30, 60, 90 and 120 min after the administration of the solution. The blood samples were collected into prechilled EDTA tubes containing aprotinin (Trasylol, 400.000 kallikrein-inactivating units/L of blood; Bayer, Leverkusen, FRG), centrifuged at 4 °C for 10 min at 2.000 g and stored at –80 °C for analysis of tritium activity, GLP-1, GIP and PYY.

GIP and GLP-1 concentrations in plasma were measured after extraction of plasma with 70 % ethanol (vol/vol, final concentration). For the GIP radioimmunoassay [29] we used the C-terminally directed antiserum R 65, which cross-reacts fully with human GIP but not with the so-called GIP 8000, whose chemical nature and relationship to GIP secretion is uncertain. Human GIP and 125-I human GIP (70 MBq/nmol) were used for standards and tracer. The plasma concentrations of GLP-1 were measured [30] against standards of synthetic GLP-1 7–36 amide using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore does not react with GLP-1-containing peptides from the pancreas. The results of the assay accurately reflect the rate of secretion of GLP-1 because the assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1 9–36 amide, into which GLP-1 is rapidly converted [31]. For both assays sensitivity was below 1 pmol/L, intra-assay coefficient of variation below 6 % at 20 pmol/L, and recovery of standard, added to plasma before extraction, about 100 % when corrected for losses inherent in the plasma extraction procedure.

PYY-LI was analyzed by means of antiserum code no. 8412–2II (a gift from R. Håkanson, Dept. of Pharmacology, University of Lund, Lund, Sweden) raised in rabbits against synthetic porcine PYY-(136) (Peninsula Europe, Merseyside, UK) as previously described [32] but without conjugation to carrier protein [33]. The antiserum cross-reacts to 100 % with human PYY. The detection limit of the assay was 1 pmol/L, and the coefficient of variation was 5 %.

Validation of the method used to measure the rate of gastric emptying

Tritiated water has not been used previously to determine the rate of gastric emptying. The main advantages of tritiated water over phenol red as a dilution marker are that 1) tritiated water does not interact with the meal constituents, while phenol red may interact with some amino acids; 2) with tritiated water it is possible to assess (indirectly) the rate of intestinal water absorption by following the rate of tritium accumulation in peripheral blood. If the rate of gastric emptying of several solutions with identical osmolality is similar, then an equal rate of water absorption should be expected. Thus, with the use of tritiated water it is possible to assess simultaneously the rate of gastric emptying and intestinal water absorption. To validate this new method based on tritiated water, six healthy men with a mean (range) age, weight and height

of 30 (25–34) years, 81 (73–90) kg and 184 (180–192) cm, agreed to participate in a study in which the rate of gastric emptying was determined with the double-sampling technique using simultaneously phenol red and tritiated water as dilution markers. In the laboratory, a nasogastric tube (Levine type, CH 12, 120 cm) and an antecubital venous catheter (18 gauge) were placed as described in the experimental procedures section. Then, a 600 mL solution containing fructose (66.7 g/L) in deionized water, to which 5 μCi of tritiated water and 9 mg of phenol red were added, was administered through the nasogastric tube. Gastric content samples were withdrawn at 0, 5, 10, 15, 20 and 30 min using the double-sampling technique [27]. In addition, blood and saliva samples were obtained every 5 min during the first 30 min and then every 10 min until the 60th min.

Tritium activity was measured with a beta counter. Phenol red concentration was determined spectrophotometrically as previously described [34].

The osmolality of the test solutions was determined immediately before administration by freezing point depression (osmometer 3W2, Advanced Instruments, Norwood, MA). The gastric samples for each subject were thawed, vortexed and centrifuged (10,730 g for 10 min). The supernatant was then used to determine the pH (691 pH meter, Metrohm, Herisau, Switzerland) and chloride concentration (AVL 983-S, Graz, Austria). A more detailed description of the calculations can be found in the appendix incorporated at the end of this article.

Calculations and statistics

A two-way repeated measure analysis of variance was used to examine the effects of treatment (degree of protein fractionation) and time. The results were corrected for multiple comparisons using the Bonferroni post hoc test. To determine if the peptide hydrolysate solutions elicited a higher gastric secretion during the first 2 hours of the postprandial period than their original complete protein solutions the paired Student's t-test was applied. Statistical significance was accepted at $p < 0.05$. Data are presented as means ± SEM. Analyses were performed with SPSS (SPSS Inc., Chicago, IL). The relationship between variables was assessed using the Pearson's correlation test.

Results

Validity of the method used to measure gastric emptying

The volume of the gastric content was similarly estimated when using phenol red and tritiated water as volume markers ($r = 0.93$, $p < 0.001$). A good agreement was also observed between the secretion values obtained by both methods ($r = 0.86$, $p < 0.01$). Tritium activity in saliva and plasma remained always below 2 % of the activity measured at the same time in the gastric contents, minimizing the effects of label re-circulation.

Gastric emptying, secretion and pH

The rate of gastric emptying for all solutions was found to fit an exponential pattern ($r = 0.92$–1). Solutions were emptied at similar rates, with half-times of (mean ± S. E. M.) 21.4 ± 1.3, 19.3 ± 2.2, 18.0 ± 2.5 and 19.4 ± 2.8 min, for the whey hydrolysate, casein hydrolysate, casein and whey protein, respectively.

During the first hour of the gastric emptying process, the peptide hydrolysates elicited about 50 % more gastric secretion than the whole protein solutions (935 ± 187, 869 ± 205, 543 ± 103, 644 ± 105 mL, for the whey hydrolysate, casein hydrolysate, casein and whey protein, respectively, $p < 0.05$, paired Student's t-test) (Fig. 1).

The pH of all the solutions was adjusted to 6.9–7.0 prior to their administration. After delivering the solutions the pH of the gastric content decreased as time progressed ($p < 0.001$). As illustrated in Fig. 2, this decrease was more accentuated for the complete proteins than for their peptide hydrolysates as shown by the sig-

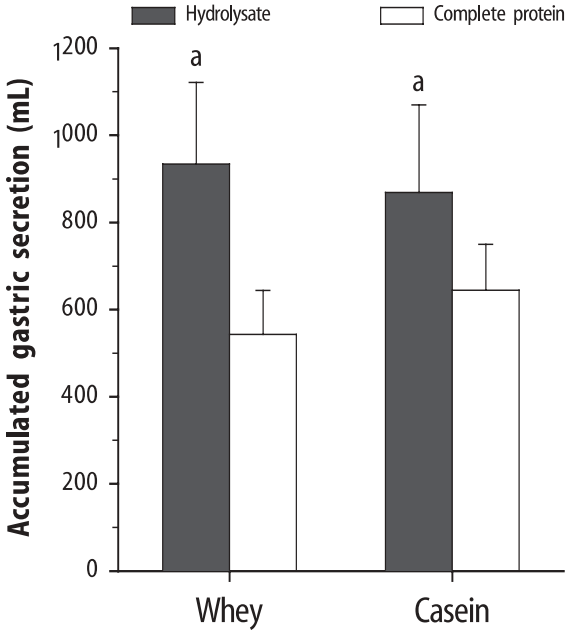

Fig. 1 Gastric secretion accumulated during the first 60 min of the postprandial period, in six men after the ingestion of four different solutions (600 mL each), which contained 9 g/L of nitrogen delivered as whey protein, whey peptide hydrolysate, casein and casein peptide hydrolysate, respectively. [a] Different from its respective complete protein, $p < 0.05$. Values are means ± SEM, n = 6

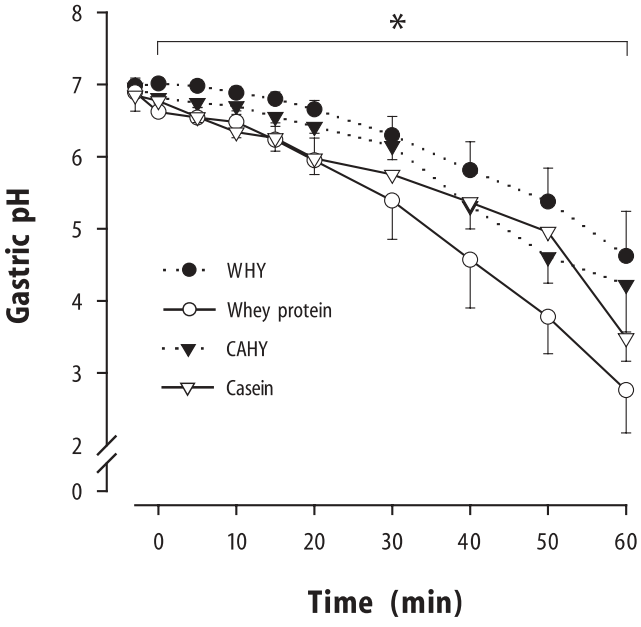

Fig. 2 Gastric content pH in six men following the ingestion of four different solutions (600 mL each), which contained 9 g/L of nitrogen delivered as whey protein, whey peptide hydrolysate (WHY), casein and casein peptide hydrolysate (CAHY), respectively. The pH elicited by the peptide hydrolysates was significantly lower than that elicited by the complete proteins, * $p < 0.05$ for the comparison between the two peptide hydrolysates and the two complete protein solutions (ANOVA). Values are means ± SEM, n = 6

nificant interaction observed in the ANOVA analysis ($p < 0.01$).

Water absorption

The rate of water absorption for all solutions, as reflected by the increase in plasma tritium activity, was found to fit a parabolic pattern (the correlation coefficient ranged between r = 0.74 and r = 1). Water was absorbed at similar rates, with half-times of 31.1 ± 2.4, 31.6 ± 2.3, 32.9 ± 2.0 and 34.1 ± 3.2 min, for the whey hydrolysate, casein hydrolysate, casein and whey protein, respectively.

Plasma amino acids

Essential (EAA), branched chain (BCAA) and total plasma (TAA) amino acid concentrations were enhanced just 5 min after the administration of the solutions ($p < 0.05$) and remained elevated for 90–120 min (Fig. 3). In general, peak plasma amino acid concentrations were attained between the 20th and the 60th min of the postprandial period. No significant differences in rate of increase of BCAA, EAA and TAA were observed between the whey protein and the two peptide hydrolysate solutions. In fact, the increase in plasma amino acid concentrations elicited by the whey protein and whey peptide hydrolysate was nearly identical. However, the casein solution elicited lower BCAA, EAA and TAA plasma amino acid increases than its matched casein hydrolysate solution during the first 20 min of the postprandial period ($p < 0.05$).

Plasma GLP-1, GIP and PYY

Fig. 4 shows the time course of the rise of GLP-1, GIP and PYY plasma concentrations. Similar GLP-1 and PYY plasma responses were elicited by the four solutions. However, the peptide hydrolysates elicited a greater plasma concentration of GIP during the first 20 min of the postprandial period and a lower concentration during the last 60 min, as shown by the significant interaction in the ANOVA analysis.

GLP-1 increased 3.5-fold, from 8.8 ± 0.6 pmol/L just before the delivery of the solutions to a maximum of 31.0 ± 1.7 pmol/L ($p < 0.001$). The GIP concentration was enhanced from 1.7 ± 0.5 pmol/L to a peak concentration of 28.4 ± 2.2 pmol/L ($p < 0.001$). In general, the GLP-1 and GIP concentration reached its maximum between the 15th and the 20th min of the postprandial period. In turn, PYY plasma concentration rose more progressively from 4.9 ± 0.5 pmol/L to 8.3 ± 1.3 pmol/L at the 60th of the postprandial period ($p < 0.05$). No significant relationship was observed between the enterogastrone responses and the gastric secretion.

Discussion

In the present study it has been shown that both whey and casein protein, as well as their respective peptide hydrolysates are emptied from the stomach at similar rates following a mono-exponential pattern. In accordance, the plasma tritium and amino acid concentrations, as indices of the rate of intestinal absorption of water and amino acids, were similar with the exception of the casein protein solution, for which the speed of intestinal amino acid absorption was slower. In addition, it has been demonstrated that peptide hydrolysates elicited a greater volume of gastric secretion, which was associated with higher plasma concentrations of GIP than the complete protein solutions.

Gastric emptying

To the best of our knowledge this is the first time that the rate of gastric emptying of complete proteins and peptide hydrolysates has been determined, controlling for other variables that may *per se* influence the gastric

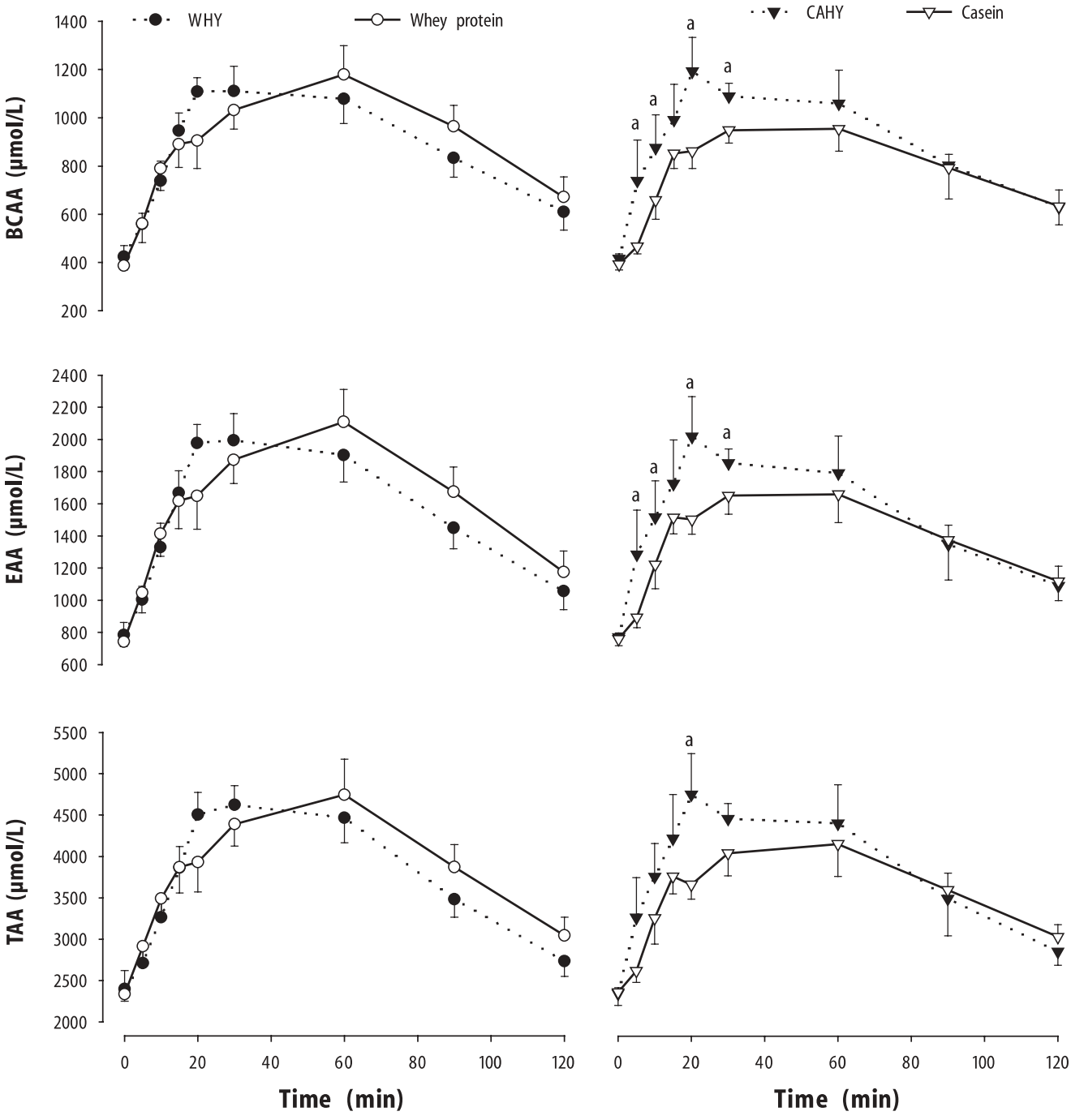

Fig. 3 Plasma amino acid concentrations after the ingestion of four different solutions (600 mL each), which contained 9 g/L of nitrogen delivered as whey protein, whey peptide hydrolysate (WHY), casein and casein peptide hydrolysate (CAHY), respectively. [a] Different from its respective complete protein, $p < 0.05$. Values are means ± SEM, n = 6

emptying process. Since no lag phase was detected in the gastric emptying process for any of the four solutions, the rate of gastric emptying was calculated by fitting a simple exponential model, as it has been applied to describe the gastric emptying of liquid meals [5, 6, 27]. In contrast, solids are emptied from the stomach after a lag phase, following a slower linear pattern [5]. It must be considered that while whey protein and the peptide hydrolysates remain soluble in the stomach, casein clots [9, 35]. Therefore, casein protein solutions may be emptied

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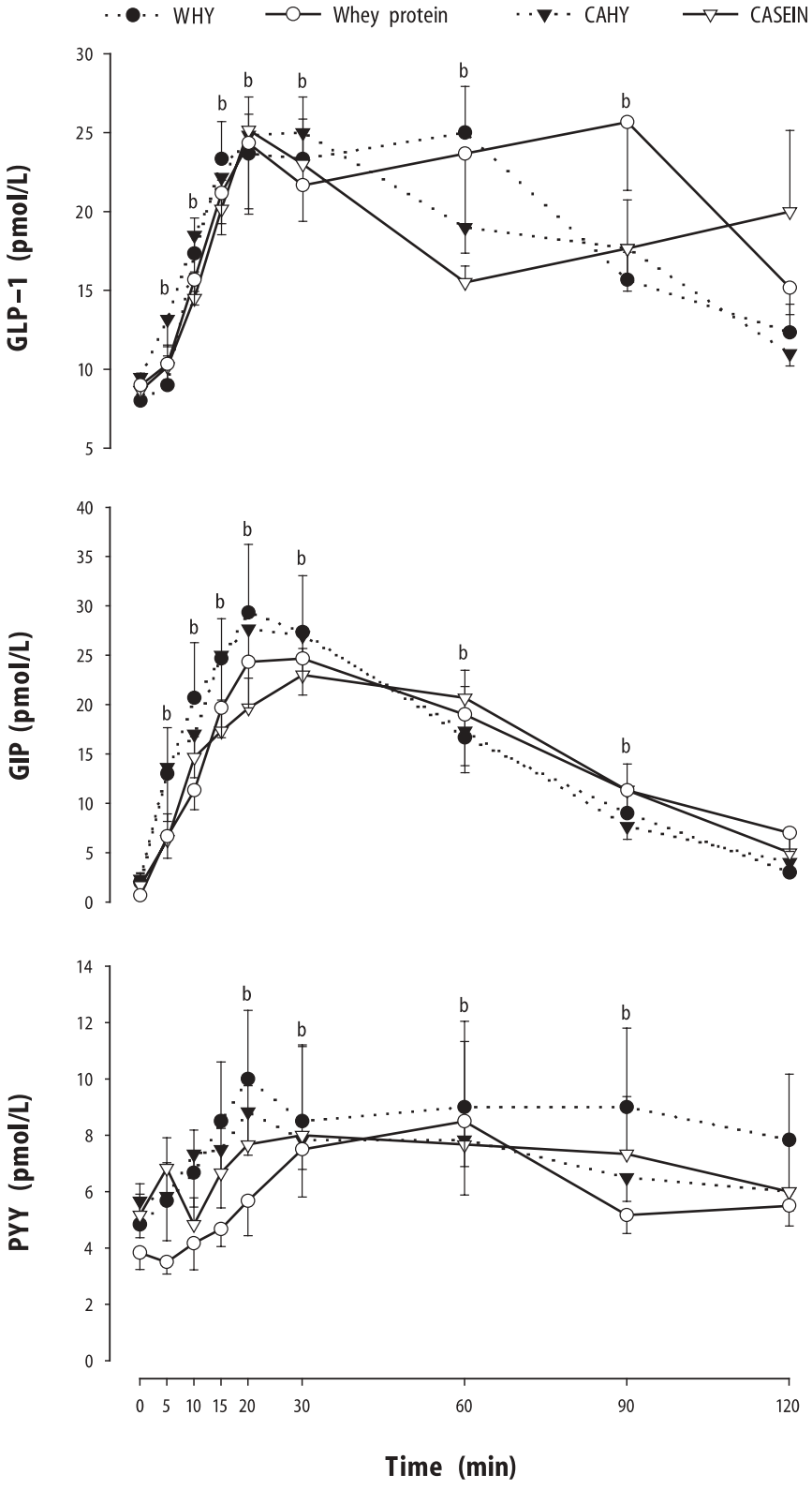

Fig. 4 Plasma concentrations of glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polipeptide (GIP) and peptide YY (PYY) after the ingestion of four different solutions (600 mL each), which contained 9 g/L of nitrogen delivered as whey protein, whey peptide hydrolysate, casein and casein peptide hydrolysate, respectively. [b] Different from time "0", $p < 0.05$. Values are means ± SEM, n = 6

from the stomach in two phases: a slow solid phase and a faster liquid phase [5, 35]. Consequently, we can not rule out a slower delivery of nitrogen to the intestine after the administration of the casein protein solution. Nonetheless, the latter seems unlikely since casein was fragmented is small particles, as observed while handling the gastric samples, and the gastric emptying of small solids suspended in liquids follows the behavior of the liquid phase [5, 36]. However, some casein could have formed large clots in the stomach which will follow the slower gastric emptying pattern of solids [5, 35, 36]. It should be highlighted that the difference in rate of appearance of amino acids in the peripheral circulation between whole casein protein and the casein hydrolysate, though small, may have important physiological implications for the disposal of the amino acid load [37, 38]. The small differences observed in the present study could have been more accentuated if the solutions were delivered directly into the intestine or with other meal components with potential delaying effects on the rate of protein digestion [37, 38].

The half-emptying times observed in this study (i. e., 18–21 min) were lower than those reported for soy-protein solutions (36–38 min) [39], skimmed milk (25 min) [35] and a complex solution of milk protein, fat and carbohydrates (26 min) [34], but slightly higher than those reported for solutions composed of 15–16 g of glucose and peptide hydrolysate (17 min) [34]. In agreement with our results, a half-time close to 20 min has been reported for carbohydrate solutions of a similar volume, osmolality and energy density to that of our protein solutions [34, 40].

Higher rates of gastric emptying have been reported for whey protein than for casein in animal studies [10, 11] and in short-bowel patients [41]. However, these results conflict with previous studies showing that isoenergetic amounts of fat, protein and carbohydrates are emptied at a similar rate [4, 42]. In this regard, similar rates of gastric emptying for casein and whey protein solutions have been reported by Thorkelsson et al. in preterm infants [43]. It must be highlighted that the latter authors matched their solutions for energy density, osmolality, pH, volume and nitrogen content. Similarly, we have observed in adults that isoenergetic amounts of glucose and peptide hydrolysate from animal and vegetal source (whey and pea, respectively) are emptied at the same rate [34], leading to a similar rate of appearance of amino acids in peripheral blood [2]. In addition, the present study shows that soluble proteins are emptied, essentially, at the same rate as their peptide hydrolysates when administered in isoenergetic solutions of similar energy density, independent of the amino acid compositions.

GLP-1, GIP and PYY effects on gastric emptying

The GLP-1 and GIP response patterns elicited by the protein solutions administered in this study are similar to that previously reported for glucose solutions [44], that is a fast increase in both incretins peaking 20 min after the ingestion. So far it has not been definitively established which is the main gut hormone regulating gastric emptying during the postprandial period, nonetheless, some experimental evidence suggests an important role for cholecystokinin (CCK), GLP-1, GIP and peptide YY in this regard.

Amino acids, peptones and fatty acids appear to be the most potent stimulators of CCK secretion. Cholecystokinin regulates gut motility, gall bladder contraction, pancreatic enzyme secretion and appetite. Additionally, it potentiates the amino acid-induced release of insulin and glucagon [45] and slows gastric emptying [46]. Although CCK was not measured in the present investigation, the fact that the four solutions tested were emptied at the same rate, inducing similar glucagon and insulin responses (data not shown), suggests a similar CCK response to feeding with complete milk proteins and their peptide hydrolysates in our experimental conditions.

Since the release of GLP-1 is stimulated by oligopeptides [47] we expected a faster release of GLP-1 after the administration of peptide hydrolysates. However, the release of GLP-1 was not influenced by the degree of protein fractionation. This finding is compatible with our observation of a similar rate of gastric emptying for complete proteins and their respective peptide hydrolysates. Our results also suggest that the complete protein solutions studied were hydrolyzed so fast in the intestine that at the end the rate of appearance of amino acids in peripheral blood and the hormonal response elicited was independent of the degree of fractionation of the protein ingested.

Several studies have demonstrated that GLP-1 administered intravenously, subcutaneously, and as buccal tablets, in general resulting in supraphysiological plasma levels, inhibits gastric emptying of liquid and solid meals by prolonging the lag phase and/or reducing the emptying rate [20–22, 48, 49]. Schirra et al. have shown that GLP-1 release seems to be controlled by gastric emptying in such a manner that the greater the kJ/min delivered into the duodenum, the larger the release of GLP-1 [44]. In the present study, no significant differences were observed in the rate of gastric emptying and, in concordance, the GLP-1 responses were also similar for the four isoenergetic protein solutions.

Peptide YY has inhibitory effects on gastrointestinal absorption, secretion and motility [50]. Mixed nutrients infused into the ileum or colon (1 kJ/min) have been shown to stimulate PYY release with different potency depending on the composition of the solution. Peptides (casein hydrolysates) stimulated PYY release to a greater

degree than lipids or carbohydrates [50]. Our data further show that protein solutions of similar caloric density elicit a similar PYY response which is hardly influenced by the degree of fractionation or the amino acidic composition.

Gastric secretion

The values of gastric secretion obtained here are similar to those previously reported for hypertonic carbohydrate solutions [51]. It is worthy to note that the peptide hydrolysates elicited more gastric secretion than the complete protein solutions, which is consistent with classical investigations providing evidence for proteins, peptides and free amino acids as potent triggers of gastric secretion [17, 18]. In addition, it must be taken into account that the pH of gastric content is a major determinant of gastric secretion [52]. It has been shown when the pH of the antral contents drops below 3, that gastrin release, which is the most important stimulus for parietal cell acid secretion [52], is completely suppressed [18]. The latter concords with our findings, since the drop in gastric pH was more accentuated for the complete protein solutions probably due to the lower buffer capacity of complete protein solutions compared with their peptide hydrolysates (unpublished observations). The fact that the rate of tritium accumulation in plasma was similar for the four solutions suggests that the excess of water movement into the stomach elicited by the peptide hydrolysate solutions was compensated for by increased net intestinal absorption of water.

Gastric secretion may be inhibited by GLP-1, GIP and PYY [23–26]. For example, the intravenous administration of GLP-1 eliciting plasma concentration 3 times greater that those observed in this study have been shown to attenuate pentagastrin stimulated acid secretion [25]. Although GIP can also inhibit pentagastrin stimulated gastric secretion, its potency is much lower compared with GLP-1 and it seems to have a minimal influence on acid gastric secretion at postprandial physiological concentrations [53]. Peptide YY is a powerful inhibitor of pentagastrin [23] and meal stimulated gastric secretion [54]. However, none of the aforementioned gut peptides seems to account for the differences in gastric secretion observed in this investigation. GLP-1 and PYY were similarly elevated during the 2 hours following the ingestion of the four protein solutions tested in this study. Only a small, but significant, effect was observed in GIP suggesting that this gut peptide may act as an enterogastrone in humans. However, Nauck et al. [55] studied the effects of physiological doses of synthetic human GIP alone, and in co-infusion with human GLP-1 in humans. In this study, neither GIP nor GLP-1 inhibited gastric acid secretion under physiological conditions. In contrast, the co-administration of GIP and GLP-1 resulted in a slight, but significant reduction in gastric secretion [55]. It is likely, then, that the secretagogue superiority of peptide hydrolysates compared to their original proteins relies on a direct effect of the peptide hydrolysates on the gastric mucosa that is not counteracted by the release of increased amounts of enterogastrones. In addition, our data suggest that the effect of physiological plasma concentrations of GIP and GLP-1 on gastric acid secretion in humans is negligibly low and unable to overcome the secretagogue influence of the meals administered.

On the other hand, rat experiments have shown that the GIP response to the intragastric administration of peptide hydrolysates is blunted by omeprazole (an inhibitor of gastric acid secretion), while the intraduodenal infusion of HCl resulted in an increase of circulating GIP [56]. In agreement, we observed a greater GIP release following the administration of the peptide hydrolysates which was accompanied by increased gastric secretion. The latter is consistent with the hypothesis that GIP release may be in part mediated through the acid stimulatory properties of hydrolyzed protein [56]. Alternatively, the greater GIP response after the ingestion of the peptide hydrolysate solutions may just reflect a higher intraduodenal peptide concentration during the first 20 min of the postprandial period.

Clinical implications

Some clinical implications may be derived from our findings. First, that in terms of rate of appearance of amino acids in peripheral blood, there is no difference between the administration of whey complete protein, whey peptide hydrolysate or casein hydrolysate, while casein complete protein results in lower plasma amino acid concentrations. Thus, the complete casein solution could be less efficient in assuring a large and fast supply of amino acids to peripheral tissues. Second, the fact that milk peptide hydrolysate solutions elicit greater release of GIP than the complete protein from which they are derived may be interesting in clinical situations where there is insulin resistance and glucose intolerance. Finally, in patients with volume intolerance or dispepsia submitted to enteral nutrition, it may be preferable to prepare the enteral formulas with soluble complete proteins rather than with their peptide hydrolysates.

Conclusions

This study shows that in the adult healthy human, whey and casein protein solutions are emptied from the stomach at similar rates and comparable GLP-1, GIP and PYY plasma responses are elicited. The casein solution, however, elicits lower BCAA, EAA and total plasma amino

acid increases than its matched casein hydrolysate solution; this may be due to the formation of casein clots, which could empty at a slower rate from the stomach. Moreover, the rate of gastric emptying of soluble milk proteins is similar to that of their peptide hydrolysate solutions, when solutions are matched for energy density, osmolality, pH, nitrogen content and volume. Milk peptide hydrolysates elicit higher quantities of gastric secretion and greater GIP release than the complete proteins from which they were derived. Taken together, these results suggest that the GLP-1 and PYY responses to feeding with proteins in humans seem to be independent of the degree of protein fractionation and are not altered by small differences in the amino acid composition or protein solubility. Further studies are required to determine whether other protein solutions, of different nature and concentration, elicit similar hormonal responses and similar effects on gastrointestinal motility and intestinal absorption than their respective peptide hydrolysates.

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Appendix

Calculation of gastric volume and gastric secretion

In the present investigation the double sampling technique of George, as modified by Beckers et al. was applied to determine the gastric volume, gastric secretion and the meal remaining in the stomach [27]. The following equations and abbreviations were used:

V_1A_1 Volume and tritium activity (dpm/mL) in the stomach before administering the test solution; V_2A_2 Volume (600 mL) and tritium activity of the test solution; V_3A_3 Volume and tritium activity of the stomach contents after administering the test solution; V_4A_4 Volume and tritium activity of the stomach contents 5 min after administering the test solution, just before adding tritiated water stock solution; V_5A_5 Volume administered (5 mL) and tritium activity of the tritiated water stock solution; V_6A_6 Volume and tritium activity after adding the tritiated water from the stock solution.

Gastric volumes are then calculated applying the following equation:

$$\text{Time 0: } V_1 = \frac{V_2 \cdot (A_2 - A_3)}{A_3 - A_1} \quad (1)$$

$$\text{Time 5: } V_4 = \frac{V_5 \cdot (A_5 - A_6)}{A_6 - A_4} \quad (2)$$

Being the general equation to calculate the gastric volume at a given time n:

$$\text{Time n: } V_n = \frac{V_5 \cdot (A_5 - A_{na})}{A_{na} - A_{nb}} \quad (3)$$

Where V_n is the volume of the gastric contents at time n, V_5 is the volume of stock solution administered, A5 is the tritium activity (dpm/mL) in the stock solution, A_{na} is the tritium activity after adding the 5 mL of tritiated water (stock solution), and A_{nb} is the tritium activity in the gastric contents sample just before adding the 5 mL of tritiated water (stock solution).

The volume of test solution remaining in the stomach at 5 min (Vt_5) can be calculated applying the equation:

$$\text{Time 5: } Vt_5 = \frac{V_2 \cdot (V_4 - A_4)}{V_3 - A_3} \quad (4)$$

The general equation to apply to subsequent measuring times is:

$$Vt_n = \frac{(V_{nb} - A_{nb})}{(V_{(n-1)a} \cdot A_{(n-1)b})} \cdot \frac{(V_{(n-1)b} \cdot A_{(n-1)b})}{(V_{(n-2)b} \cdot A_{(n-2)b})} \cdot \frac{(V_{2b} \cdot A_{2b})}{(V_{1a} \cdot A_{1a})} \quad (5)$$

Where "nb" and "na", respectively, represent the gastric samples taken before and after adding the tritiated water (stock solution) at the measuring interval "n", "(n-1)a" and "(n-1)b" represent these values for the previous time point, "1a" and "1b" are the first time points of the test, "Vt_n" is the volume of test solution remaining in the stomach at time "n", and "V_n" and "A_n" are the volume of the gastric contents and the corresponding tritium activity.

The difference between the gastric volume and volume of meal remaining in the stomach was considered to be the gastric secretion. In so doing, it was assumed that the amount of saliva swallowed was negligible or similar in all trials. The rate of gastric secretion was calculated by dividing each gastric secretion value by its corresponding collection time interval. Then, the volume of gastric secretion (Vs_n) can be calculated by subtracting the volume of test solution remaining in the stomach from the total gastric volume, so:

$$\text{Time n: } Vs_n = V_n - Vt_n \quad (6)$$

Determination of the rate of gastric emptying

Gastric emptying curves were constructed and tested for linearity using linear regression. Since the best fit was obtained when a logarithmic transformation of time was used, a linear function equation was derived $y = a + bx$, where "y" is the volume remaining in the stomach and "x" is the logarithm of time. The time taken to empty one half of the initial gastric volume ($t_{1/2}$) was derived solving this equation.

Half-time of tritium accumulation in plasma

To calculate the half-time of tritiated water accumulation in plasma, the time course of tritium activity in plasma was fit to a parabola. Then the half-time of tritium accumulation was calculated by solving the equation for $^1/_2$ of the maximum.

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