Plasma Glucagon and Insulin Responses Depend on the Rate of Appearance of Amino Acids after Ingestion of Different Protein Solutions in Humans[1,2]

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ABSTRACT To find out whether the hormonal response to feeding with protein solutions is influenced by the nature and degree of protein fractionation, we examined insulin and glucagon responses after intake of protein solutions containing the same amount of nitrogen (2.9 g each) in three men and three women. Four test meals (600 mL) [glucose (419 kJ/L), pea (PPH) and whey peptide hydrolysates (WPH) (921 and 963 kJ/L, respectively) and a cow's milk solution (MS) containing complete milk proteins (2763 kJ/L)] were tested. Peptide hydrolysates elicited a faster increase in venous plasma amino acids than did MS ($P < 0.05$). Despite the higher carbohydrate content of the MS, the peptide hydrolysates elicited a peak insulin response that was two and four times greater than that evoked by the MS and glucose solutions, respectively ($P < 0.05$). The insulin response was closely related to the increase in plasma amino acids, especially leucine, isoleucine, valine, phenylalanine and arginine, regardless of the rate of gastric emptying. The three protein solutions elicited similar increases of plasma glucagon; however, the response was fastest for both peptide hydrolysates ($P < 0.05$) and more prolonged for the MS ($P < 0.05$). The glucagon response was linearly related to the increase in plasma amino acids, regardless of the rate of gastric emptying or meal composition ($r = 0.93$, $r = 0.96$ and $r = 0.78$, all $P < 0.05$, for the PPH, WPH and MS). Among the plasma amino acids, tyrosine ($r = 0.82–0.98$, $P < 0.05$) and methionine ($r = 0.98$, $P < 0.001$) were most closely related to the plasma glucagon response. This study shows that the glucagon response to feeding with protein solutions depends on the increase in plasma amino acid concentrations. The combined administration of glucose and peptide hydrolysates stimulates a synergistic release of insulin, regardless of the protein source. J. Nutr. 132: 2174–2182, 2002.

KEY WORDS: • *peptide hydrolysate* • *milk protein* • *amino acid kinetics* • *insulin* • *glucagon*

The fate of ingested proteins may depend on the hormonal responses elicited by the whole meal, which in turn are determined by the composition of the meal, the rate of gastric emptying and the digestion and absorption of nutrients. Several studies suggest that insulin and glucagon play a primary role, given that insulin stimulates protein synthesis and decreases protein breakdown (1–5), whereas glucagon enhances amino acid catabolism (6–8). When proteins are administered alone, a large increase in plasma glucagon and a slight elevation in insulin are usually elicited, and when proteins are given with carbohydrates, the insulin release is synergistically potentiated (9,10). In contrast, less is known about the influence that the degree of protein fractionation and amino acid composition have on the insulin and glucagon responses to feeding with protein solutions and how other constituents of the solutions may modulate these responses.

A number of animal and human studies have shown that the same nitrogen load is absorbed faster when delivered as oligopeptides rather than as whole proteins or free amino acids (11–16). Similarly, several studies have reported an increase in nitrogen incorporation into tissue protein in animals fed oligopeptides compared with those receiving the same amount of nitrogen as whole protein or free amino acids (17,18). However, whether a faster absorption rate translates into better nitrogen utilization remains controversial in humans (12,13,16,19–21). For example, almost all of the studies performed with postoperative critically ill patients agree that oligopeptides are more effective than either whole proteins or free amino acids in evoking protein synthesis and reducing protein breakdown, as assessed by changes in visceral protein and 3-methylhistidine levels (13,22,23). In contrast, studies

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[2] A detailed analysis of the gastric emptying of the solutions studied was previously published [Calbet, J. A. & MacLean, D. A. (1997) Role of caloric content on gastric emptying in humans. J. Physiol. 498: 553–559]. The plasma glucose and insulin responses were presented at the Meeting of the Physiological Society held in London in December 1996 and was published as an abstract in the proceedings [Calbet, J. A. & MacLean, D. A. (1996) Rate of gastric emptying and plasma insulin response to peptide hydrolysate solutions in humans. J. Physiol. 491P: 60P–61P (abs.)].

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carried out in normal humans have failed to reveal a similar oligopeptide superiority (16,19). Collin-Vidal et al. (16) reported slightly higher plasma insulin levels (nonsignificant) after the continuous nasogastric infusion of isonitrogenous and isocaloric diets containing the same amino acids as either oligopeptides or whole protein. However, there is a possibility that with a more physiological administration of the meal, a faster absorption rate of amino acids would occur and, subsequently, a faster and more accentuated insulin and glucagon response would be elicited.

In addition, the amino acid profile of the ingested protein itself may play a role in the hormonal response to feeding. For example, some amino acids such as arginine, lysine, phenylalanine, ornithine, alanine, leucine and isoleucine stimulate insulin secretion (24–27), whereas aromatic amino acids stimulate glucagon release (28). Furthermore, a number of amino acids, specifically the three branched-chain amino acids (isoleucine, leucine and valine) selectively escape uptake by the liver (29) and could potentially affect the time course of hormonal release. Thus, both the amino acid makeup of the meal and the form in which it is delivered (i.e., oligopeptides vs. whole protein) may contribute to the overall systemic appearance of amino acids and their effects on hormonal responses.

Therefore, the purpose of the present study was to determine the effects that different protein containing meals (complete vs. peptide hydrolysates) have on the magnitude and direction of insulin and glucagon responses. A further aim was to determine the rate of appearance of amino acids and which specific amino acids have a dominant influence on these hormones.

SUBJECTS AND METHODS

Subjects. Six healthy adults (three men and three women) with no clinical history of gastrointestinal disease participated in this study. Their ages, weights and heights were (means ± SEM) 22.7 ± 0.9 y; 73.2 ± 3.2 kg and 177.3 ± 4.1 cm, respectively. The experimental protocol was approved by the ethical committee for Copenhagen and Frederiksberg Communities and the subjects were informed of the purposes and risks of the study and written consent was obtained.

Test solutions. Four different test solutions of 600 mL were used in this study (**Tables 1** and **2**). The glucose solution (control) was composed of glucose (25 g/L) and NaCl (9 g/L) and the three additional solutions were prepared so that they all contained the same quantity of glucose (25 g/L) and protein (0.25 g/kg body mass, equivalent to one third of the daily dietary allowance for protein). However, the protein compounds were derived from different sources. One of the solutions was prepared from a pea protein hydrolysate and the other was prepared from a whey protein hydrolysate (MD Foods, Copenhagen, Denmark). The pea and whey peptide hydrolysate solutions contained different quantities of essential (35.5 and 45.1%, respectively) and nonessential amino acids (64.5 and 54.9%, respectively). The last solution was a complete milk solution containing intact cow's milk proteins, lactose and fat milk. Preliminary experiments showed that similar osmolalities among the three protein solutions were achieved by adding 36.4 mg/kg body weight of NaCl to both the pea and whey peptide hydrolysate solutions. 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 was 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 siphoned and the experiments were only carried out if the washout was clear and free of food residues. An 18-gauge venous catheter was then inserted into an antecubital vein and intermittently flushed with saline to keep it patent. After subjects rested for 30 min in a sitting position, a 10-mL blood sample was obtained and one of the four different test solutions was administered through the nasogastric tube. Time zero was taken as the moment when all of the test solution had been administered. The volume remaining in the stomach was assessed using George's double-sampling technique as applied by Beckers et al. (30). 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. To measure the gastric volume in the stomach, 9 mg of phenol red was added to each

TABLE 1

Composition of the administered solutions[1]

Component	PPH	WPH	MS	Glucose
		g/L		
Protein	29.8 ± 1.3	30.5 ± 1.3	30.5 ± 1.3	—
Nitrogen	4.8 ± 0.2	4.8 ± 0.2	4.8 ± 0.2	—
Glucose	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0
Lactose	—	1.5 ± 0.0	42.8 ± 1.8	—
Fat milk	—	<0.1	29.3 ± 1.3	—
Na	1.3 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	3.3 ± 0.0
Cl	0.8 ± 0.0	0.8 ± 0.0	<0.1	5.3 ± 0.1
Ca	—	0.3 ± 0.0	1.5 ± 0.1	—
		mosml/kg		
Osmolality	367 ± 9*	381 ± 9*	348 ± 12*	418 ± 6
pH	7.0 ± 0.0	7.0 ± 0.0	7.1 ± 0.0	7.1 ± 0.1
		kJ/L		
Energy density	921 ± 17*	963 ± 17*	2763 ± 103*†**	419 ± 3

[1] Values are means ± SEM, $n = 6$ solution samples. PPH, pea peptide hydrolysate; WPH, whey peptide hydrolysate; MS, milk solution.
* $P < 0.05$, vs. glucose; † $P < 0.05$, vs. the WPH solution; ** $P < 0.05$, vs. the PPH solution.

TABLE 2

Amino acid composition of the administered solutions[1]

Amino acid	PPH	WPH	MS
		mmol/L	
Alanine	14.71 ± 0.64	15.41 ± 0.67*	18.13 ± 0.79*†
Arginine	15.22 ± 0.67	4.20 ± 0.18*	4.55 ± 0.20*
Aspartic acid	29.09 ± 1.27	24.98 ± 1.09*	15.34 ± 0.67*†
Cysteine	1.97 ± 0.09	5.29 ± 0.23*	2.01 ± 0.09†
Glutamic acid	43.13 ± 1.89	39.61 ± 1.73*	44.13 ± 1.93*†
Glycine	17.05 ± 0.75	6.91 ± 0.30*	7.30 ± 0.32*
Histidine	5.37 ± 0.24	4.52 ± 0.20*	4.71 ± 0.21*†
Isoleucine	9.31 ± 0.41	13.49 ± 0.59*	13.47 ± 0.59*
Leucine	15.89 ± 0.70	20.93 ± 0.91*	20.91 ± 0.91*
Lysine	16.91 ± 0.74	20.03 ± 0.88*	14.80 ± 0.65*†
Methionine	2.00 ± 0.09	3.89 ± 0.17*	4.49 ± 0.20*†
Phenylalanine	7.93 ± 0.35	4.80 ± 0.21*	8.12 ± 0.36†
Proline	12.15 ± 0.53	16.95 ± 0.74*	26.72 ± 1.17*†
Serine	15.87 ± 0.69	15.68 ± 0.69	15.66 ± 0.69
Threonine	9.75 ± 0.43	18.17 ± 0.79*	10.74 ± 0.47*†
Tryptophan	0.88 ± 0.04	1.64 ± 0.07*	1.94 ± 0.08*†
Tyrosine	5.59 ± 0.24	4.04 ± 0.18*	7.90 ± 0.35*†
Valine	12.21 ± 0.53	14.85 ± 0.65*	16.40 ± 0.72*†

[1] Values are means ± SEM, $n = 6$ solution samples. PPH, pea peptide hydrolysate; WPH, whey peptide hydrolysate; MS, milk solution.

* $P < 0.05$, vs. the PPH solution; † $P < 0.05$, vs. the WPH solution.

test solution as the marker. Immediately after the administration of the test solution, the gastric contents were mixed (using a 50-mL syringe to aspirate and reinject 20–30 mL) 10 times, which took approximately 1 min. Immediately after mixing, a gastric sample was taken and used to calculate the initial volume of the gastric residue. 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 marker (phenol red 500 mg/L). The additional marker added was mixed with the gastric contents by pumping in and out with the syringe for 1 min followed by the aspiration of a 2.5-mL sample. Assuming that the amount of dye absorbed or secreted by the stomach during the sampling procedure is negligible, that no water is absorbed by the stomach and that gastric emptying does not occur during mixing or sampling, it is possible to successively calculate the gastric volume (30). Thus, phenol red was measured twice at each sampling point, before and after the addition of a known quantity of marker. Gastric samples for the measurement of gastric volume were taken every 10 min during the first hour and every 20 min thereafter until the stomach was emptied. During the 3 h that each experiment lasted, a 10-mL blood sample was obtained every 20 min for the assessment of plasma glucose, lactate, free amino acids, insulin and glucagon as well as serum free fatty acid (FFA[5]) concentrations. The selection of test solution was randomized by a Latin square design and each trial was separated by 1 wk.

Analysis and calculations. The osmolalities of the solutions were determined with an osmometer [by freezing-point depression using a 3W2 osmometer (Advanced Instruments, Norwood, MA)]. The gastric samples for each subject were thawed and centrifuged (10.730 g for 10 min) and the supernatant filtered (Minisart NML, 0.2 μm pore size; Sartorius, Switzerland). The concentration of phenol red in the filtrate was analyzed spectrophotometrically (Beckman Instruments, Fullerton, CA) after dilution (1:20) with $NaOH/NaHCO_3$.

Gastric emptying curves were constructed and tested for linearity using linear regression. Because 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.

Blood samples for plasma were drawn with syringes treated with heparin and immediately centrifuged and the supernatant analyzed for glucose and lactate using an automatic glucose-lactate analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Meanwhile, blood samples for serum were drawn with untreated syringes and left to stand on melting ice for 60 min followed by centrifugation. Both serum and the remaining plasma samples were stored at −80°C for further analyses. Serum was analyzed in duplicate for FFA (Wako FFA kit no. 990-75401, Wako Chemicals, Richmond, VA) using a Beckman Du-70 spectrophotometer (Beckman Instruments). Plasma amino acids were determined in duplicate by prior derivatization with phenyl-isothiocyanate (31) and HPLC (Waters, Millipore, Milford, MA). Plasma insulin and glucagon were measured in blood samples collected in tubes containing aprotinin (Aprotinin Novo, 500,000 kallikrein-inactivating units/L of blood; Novo Nordisk, Copenhagen, Denmark) using commercially available radioimmunoassay kits (Novo Nordisk kit no. 7350104, Copenhagen, Denmark and Linco Research kit no. GL-32K, St. Charles, MO).

Statistics. A two-way repeated measure analysis of variance was used to examine the effects of treatment and time. If significance was indicated, a Tukey's honestly significant difference post hoc point-to-point comparison test was used to determine where the differences occurred. Analysis of covariance (ANCOVA) was used to determine whether there existed significant differences between solutions after adjusting for the rate of gastric emptying. The relationship between variables was assessed using the Pearson's correlation test. Finally, stepwise multiple linear regression was used to determine the best predictors of the insulin and glucagon responses. Statistical significance of differences was accepted at $P < 0.05$. Data are presented as means ± SEM.

RESULTS

Rate of gastric emptying. A detailed analysis of the rate of gastric emptying for these solutions was previously published (32). Briefly, the rate of gastric emptying for the glucose solution (control) was the fastest, with a half-time of 9.4 ± 1.2 min ($P < 0.05$). In contrast, the milk protein solution, which had the highest energy density, was emptied the slowest, with a half-time of 26.4 ± 10.0 min ($P < 0.05$) compared to that of the other solutions. Meanwhile, the pea and whey peptide hydrolysate solutions were emptied at similar rates (16.3 ± 5.4 and 17.2 ± 6.1 min, respectively).

Plasma glucose and free fatty acids. At 20 min after the ingestion of the glucose and milk solutions the plasma glucose concentration was increased from 4.9 ± 0.1 and from 4.8 ± 0.1 mmol/L at basal conditions, to 6.5 ± 0.3 and 6.1 ± 0.4 mmol/L, respectively ($P < 0.01$). In contrast, 60 min after the subjects had consumed the pea and whey peptide hydrolysates a nadir was reached, where the plasma glucose concentrations had decreased from 4.8 ± 0.1 and from 4.9 ± 0.1 mmol/L at basal conditions, to 3.8 ± 0.3 and to 3.6 ± 0.3 mmol/L, respectively ($P < 0.05$) (**Fig. 1**). No relationship was found between the mean rate of gastric emptying and the peak plasma glucose concentration measured after the consumption of the glucose, pea protein hydrolsate (PPH), whey protein hydrolysate (WPH) and milk solutions.

After the consumption of the four solutions, serum FFA concentrations decreased during the first 60 min of the postprandial period and increased progressively during the last 2 h (Fig. 1). Nadir serum FFA concentrations were attained 60 min after the consumption of the glucose, PPH and WPH

[5] Abbreviations used: BCAA, branched-chain amino acids; EAA, essential amino acids; FFA, free fatty acids; MS, milk solution; NEAA, nonessential amino acids; PPH, pea peptide hydrolysate; TAA, total amino acids; WPH, whey peptide hydrolysate.

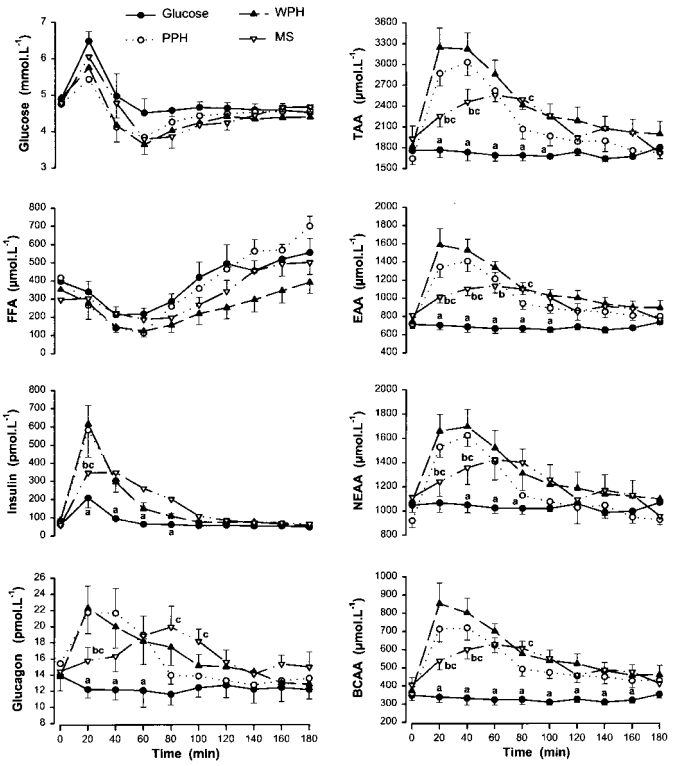

FIGURE 1 Plasma concentrations of glucose, free fatty acids, insulin, glucagon and amino acids during the postprandial period after subjects consumed the glucose, the pea peptide hydrolysate (PPH), the whey peptide hydrolysate (WPH) and the milk solutions (MS). Values are means ± SEM ($n = 6$). $^{a}P < 0.05$, vs. the glucose solution (compared with the other three solutions); $^{b}P < 0.05$, vs. the WPH solution; $^{c}P < 0.05$, vs. the PPH solution. TAA, EAA, NEAA and BCAA are the total, essential, nonessential and branched-chain amino acid concentrations, respectively.

solutions ($P < 0.05$), and 80 min after the consumption of the milk solution ($P < 0.05$).

Total plasma amino acids. As depicted in Figure 1, total plasma amino acid (TAA) concentration increased after the administration of the three nitrogen-containing solutions, but remained constant after the administration of the glucose solution. The increase in the plasma TAA concentration was greatest when the nitrogen compounds were given in the form of peptide hydrolysates rather than milk solution ($P < 0.05$). The plasma TAA concentration was increased to a similar extent 20 min after the subjects consumed the pea (75%, $P < 0.001$) and whey (78%, $P < 0.001$) peptide hydrolysates. In contrast, the administration of the milk solution resulted in a slower rise in plasma TAA concentration.

When the whole postprandial period (180 min) was considered, the whey peptide hydrolysate solution elicited the greatest increase in plasma TAA levels compared to those of the pea peptide hydrolysate and milk solutions ($P < 0.05$). This difference was attributed to the rapid increase in plasma TAA evoked during the first 40 min of the digestive period, during which the increase was approximately 25 and 37% greater after the ingestion of the pea and whey peptide hydrolysate solutions, respectively, than that after ingestion of the milk solution ($P < 0.001$). A detailed description of the plasma concentrations of individual amino acids is presented in **Figures 2** and **3**.

Essential amino acids. The plasma essential amino acid (EAA) concentration increased after the administration of the protein solutions ($P < 0.001$), but remained unchanged after the administration of the glucose solution (Fig. 1). In addition, during the first hour of the postprandial period, the plasma EAA concentration increased to a greater extent when the subjects consumed both peptide hydrolysates than when they consumed the milk solution ($P < 0.05$). The highest concentration was elicited by the whey peptide hydrolysate solution (at 20 min), which increased the plasma EAA level from 738 ± 75 to 1586 ± 178 µmol/L ($P < 0.001$), whereas the pea peptide hydrolysate solution produced a less-dramatic increase from 720 ± 49 to 1344 ± 116 µmol/L ($P < 0.001$). Similarly, 20 min after the administration of the milk solution, plasma EAA levels increased from 814 ± 67 to 1013 ± 66 µmol/L ($P < 0.001$), a value that was lower than that observed after the administration of both peptide hydrolysates ($P < 0.01$). Thereafter, the plasma EAA concentrations followed a decreasing pattern for both the pea and whey peptide hydrolysate

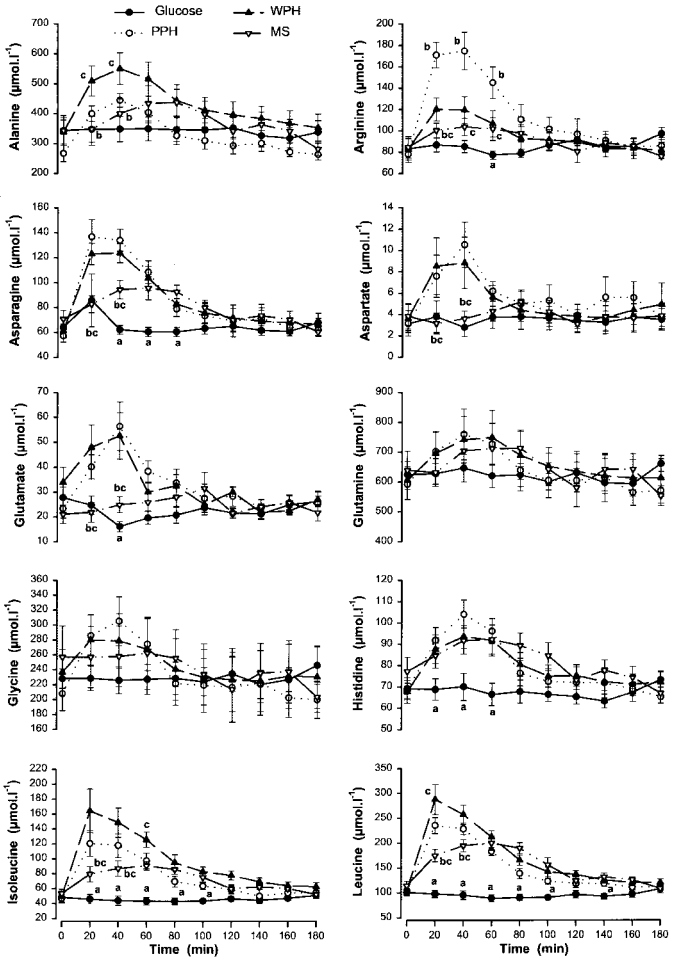

FIGURE 2 Plasma concentrations of single amino during the postprandial period after the administration of the glucose, the pea peptide hydrolysate (PPH), the whey peptide hydrolysate (WPH) and the milk solutions (MS). Values are means ± SEM ($n = 6$). $^{a}P < 0.05$, vs. the glucose solution (compared with the other three solutions); $^{b}P < 0.05$, vs. the WPH solution; $^{c}P < 0.05$, vs. the PPH solution. TAA, EAA, NEAA and BCAA are the total, essential, nonessential and branched-chain amino acid concentrations, respectively.

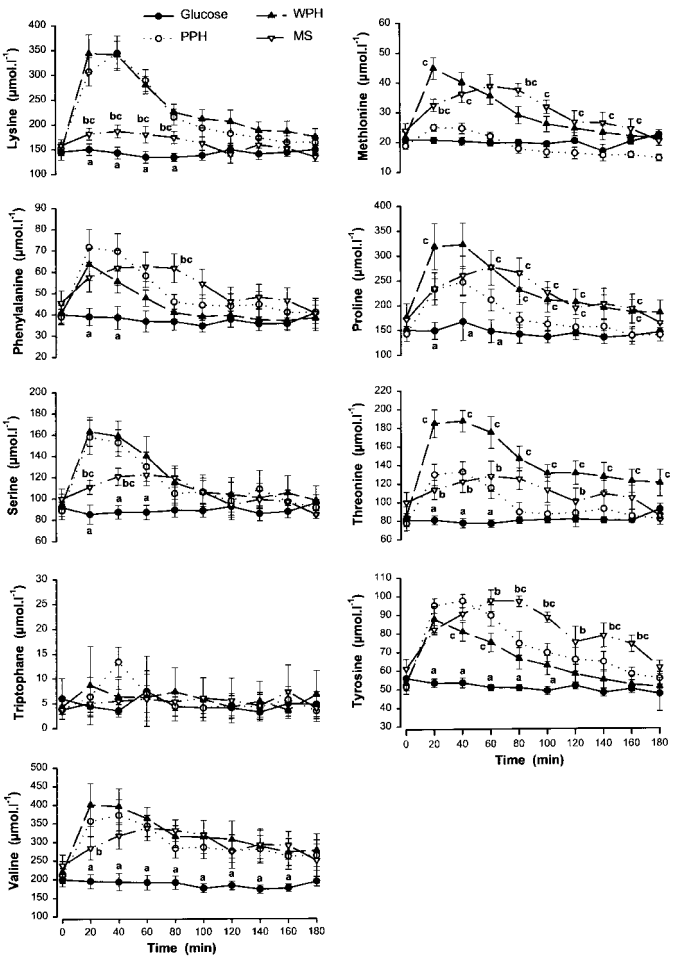

FIGURE 3 Plasma concentrations of single amino during the postprandial period after the administration of the glucose, the pea peptide hydrolysate (PPH), the whey peptide hydrolysate (WPH) and the milk solutions (MS). Values are means ± SEM (n = 6). [a]$P < 0.05$, vs. the glucose solution (compared with the other three solutions); [b]$P < 0.05$, vs. the WPH solution; [c]$P < 0.05$, vs. the PPH solution. TAA, EAA, NEAA and BCAA are the total, essential, nonessential and branched-chain amino acid concentrations, respectively.

solutions, reaching basal values after 80 and 140 min, respectively.

Branched-chain amino acids. The plasma branched-chain amino acid (BCAA) concentration followed a similar pattern to that described for the EAA (i.e., increasing only after the administration of the protein solutions) (Fig. 1). Nevertheless, this increase was more dramatic after the administration of both peptide hydrolysates than after the milk solution ($P < 0.001$). In addition, during the first 20 and 40 min, the greatest increase in the plasma concentration of BCAA was produced by the whey peptide hydrolysate solution compared with the other three solutions ($P < 0.05$). No relationship was found between the rate of gastric emptying and the peak plasma concentration of TAA, EAA, BCAA and nonessential amino acids (NEAA).

Insulin, glucagon and insulin/glucagon responses. Insulin and glucagon responses as well as the insulin/glucagon ratio were significantly different among the protein solutions and the glucose solution (Fig. 1). The highest plasma insulin responses were observed after the ingestion of the pea and whey peptide hydrolysate solutions (basal 79 ± 7 and 87 ± 8 pmol/L to 582 ± 150 and 615 ± 104 pmol/L at the 20th min, respectively, $P < 0.05$). Both peptide hydrolysates stimulated an increase in plasma insulin that was two and four times greater than that produced by the milk solution (388 ± 51 pmol/L, $P < 0.05$) and glucose solution (208 ± 53 pmol/L, $P < 0.05$), respectively. Thereafter, plasma insulin decreased to basal levels 40, 60 and 80 min after the administration of the glucose, peptide hydrolysates (both pea and whey) and milk solution, respectively (Fig. 1). Even though the amount of carbohydrate in the milk protein solution was nearly three times greater than that in both peptide hydrolysates, the areas under the plasma insulin concentration curves were similar for these solutions.

Plasma glucagon concentrations increased after the administration of the three protein solutions but remained constant after the ingestion of the glucose solution (Fig. 1). Furthermore, similar peak glucagon responses were observed 20 min after the administration of both peptide hydrolysates, with the peak increase being 41% (from 15.4 ± 1.9 to 21.7 ± 2.6 pmol/L) and 62% (from 13.8 ± 1.7 to 22.3 ± 2.8 pmol/L) when the subjects consumed the pea and whey peptide hydrolysate solutions, respectively. On the other hand, the plasma glucagon response after the ingestion of the milk solution was less accentuated but more prolonged, increasing from 14.5 ± 1.5 to 19.1 ± 2.6 pmol/L 80 min after the administration of the solution ($P < 0.05$). Glucagon values returned to basal levels 40, 60 and 120 min after the administration of the pea, whey and milk solutions, respectively (Fig. 1). The areas under the plasma glucagon curves elicited by the three protein solutions did not differ.

The insulin and glucagon responses during the 180-min postprandial period were related to the plasma concentration of TAA, EAA, BCAA, NEAA and gluconeogenic amino acids of the administered protein solutions (**Table 3**). Although all three protein solutions stimulated the release of both glucagon and insulin, the release of insulin was higher, resulting in an increase in the insulin/glucagon ratio ($P < 0.001$). The greatest response was elicited by the pea and

TABLE 3

Relationship between plasma amino acids, insulin, glucagon and the insulin/glucagon ratio during the postprandial period given as the Pearson's correlation coefficient[1]

	TAA	EAA	BCAA	NEAA	Gluconeogenic
			r		
Pea peptide hydrolysate					
Insulin	0.80	0.81	0.83	0.80	0.79
Glucagon	0.93	0.93	0.91	0.93	0.93
Insulin/glucagon	0.80	0.80	0.82	0.79	0.78
Whey peptide hydrolysate					
Insulin	0.82	0.83	0.84	0.80	0.77
Glucagon	0.96	0.96	0.96	0.96	0.94
Insulin/glucagon	0.85	0.85	0.86	0.84	0.80
Milk solution					
Insulin	0.76	0.80	0.76	0.72	0.70
Glucagon	0.78	0.77	0.81	0.78	0.74
Insulin/glucagon	0.68	0.72	0.67	0.63	0.62

[1] Abbreviations represent the plasma amino acid concentrations of total amino acids (TAA), essential amino acids (EAA), branched chain amino acids (BCAA) and nonessential amino acids (NEAA), respectively. All correlations were significant.

whey peptide hydrolysate solutions (5.8 ± 2.9 to 29.6 ± 21.8, and 6.6 ± 1.6 to 29.1 ± 11.8, respectively, $P < 0.001$). The milk protein solution elicited a less-dramatic, but more sustained increase in the insulin/glucagon ratio (from 4.4 ± 1.5 to 23.3 ± 11.8, $P < 0.001$).

Plasma amino acid concentrations must reach a certain level before any increase in plasma insulin can be detected, regardless of the rate of gastric emptying or meal composition. After the administration of the glucose solution, plasma insulin concentrations were closely related to plasma glucose concentrations (**Fig. 4**) ($r = 0.98$, $P < 0.001$). However, after ingestion of the peptide hydrolysate solutions, plasma insulin concentrations were more closely related to plasma phenylalanine concentrations ($r = 0.89$ and $r = 0.96$, both $P < 0.001$) than to plasma glucose concentrations ($r = 0.55$, $P = 0.1$ and $r = 0.67$, $P < 0.05$ after the ingestion of the pea and whey peptide hydrolysates, respectively). Multiple regression analysis showed that the plasma concentration of both arginine and glucose accounted for 99 and 96% of the variability in plasma insulin concentrations after the administration of the pea and whey peptide hydrolysate solutions, respectively. On the other hand, after the administration of the milk solution, plasma insulin concentrations were more closely related to plasma arginine concentrations ($r = 0.90$, $P < 0.001$). Meanwhile, multiple regression analysis showed that the plasma concentration of both arginine and glutamine could explain 92% of the variability in plasma insulin concentrations after the ingestion of the milk protein solution.

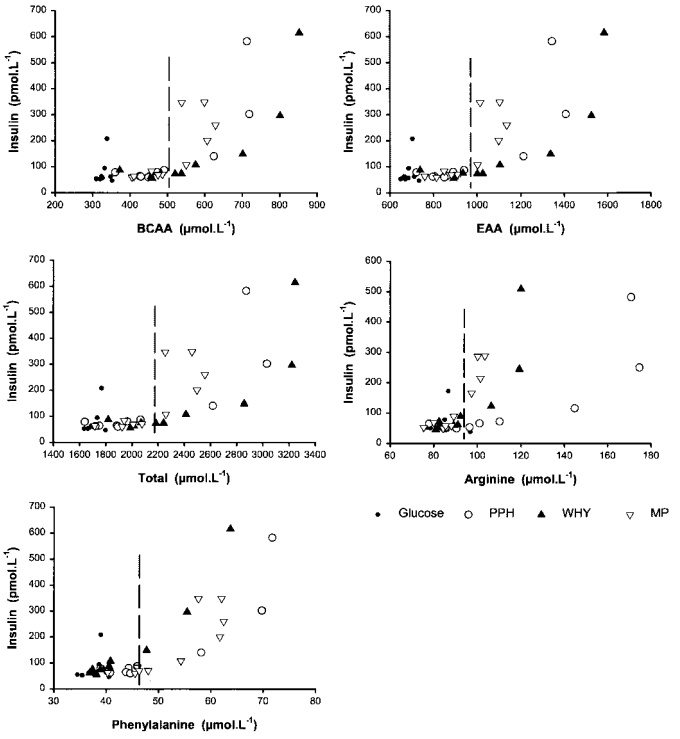

FIGURE 4 Relationships between plasma insulin and plasma amino acid concentration after subjects consumed the glucose, the pea peptide hydrolysate (PPH), the whey peptide hydrolysate (WPH) and the milk solutions (MS). Values are means at each sample point for each solution ($n = 6$). TAA, EAA, NEAA and BCAA are the total, essential, nonessential and branched-chain amino acid concentrations, respectively. The dashed vertical line indicates the point at which an increase in the plasma insulin concentration is detectable.

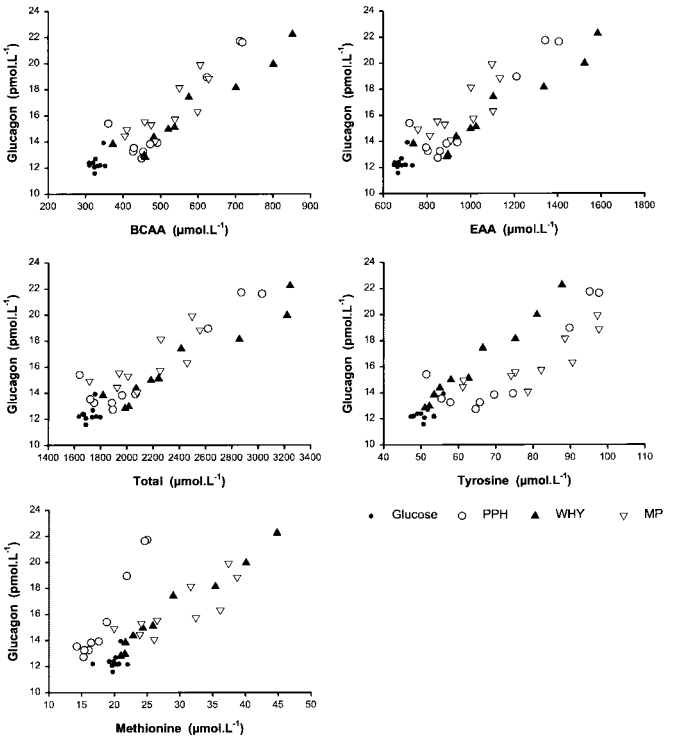

FIGURE 5 Relationships between plasma glucagon and plasma amino acid concentration after subjects consumed the glucose, the pea peptide hydrolysate (PPH), the whey peptide hydrolysate (WPH) and the milk solutions (MS). Values are means at each sample point for each solution ($n = 6$). TAA, EAA, NEAA and BCAA are the total, essential, nonessential and branched-chain amino acid concentrations, respectively.

Plasma glucagon concentrations were linearly dependent on plasma amino acid concentrations and were not influenced by the rate of gastric emptying, the nature of the protein administered (vegetable or animal) or other constituents of the solutions (**Fig. 5**). Multiple regression analysis showed that the plasma concentration of methionine accounted for 96% of the variability in the glucagon response to the pea peptide hydrolysate solution ($r = 0.98$, $P < 0.001$). On the other hand, tyrosine was the amino acid most closely related to the glucagon responses after the administration of the whey peptide hydrolysate ($r = 0.98$, $P < 0.001$) and the milk solution ($r = 0.81$, $P < 0.05$), accounting for 97 and 66% of the variability in plasma glucagon during the whole postprandial period, respectively.

DISCUSSION

The present study clearly shows that the combined oral administration of peptide hydrolysates and glucose acts synergistically to increase plasma insulin concentrations. Despite the fact that the amount of glucose was similar in all four solutions (15 g of glucose) and that the carbohydrate content was nearly three times higher in the milk protein solution (i.e., 15 g of glucose and 26 g of lactose), peak plasma insulin concentrations were two and four times higher after the ingestion of the peptide hydrolysate solutions than after the milk and glucose solutions, respectively. These differences may be explained as follows. First, an accelerated rate of gastric emptying, similar to that observed for the glucose solution, could

have promoted a faster release of insulin as previously suggested (33). Although the rate of gastric emptying was related to the insulin response after the administration of the glucose solution, no relationship was found between the rate of gastric emptying and the insulin response across solutions. In addition, when the plasma insulin concentrations were adjusted for the rate of gastric emptying, differences in insulin responses were still significant. Therefore, other factors such as the composition of the meal or the form in which the protein was administered (i.e., as a complete protein or as a peptide hydrolysate) might have accounted for the differences observed in the plasma insulin responses.

It has been shown that the combination of glucose and proteins acts synergistically to promote insulin release (9,10,27,32,34), but when proteins are given alone, plasma insulin barely changes (9,35). Moreover, this study shows that the synergistic effect of a mixture of glucose and peptide hydrolysates on plasma insulin was even higher than when whole proteins and glucose were combined. This latter point is supported by the fact that the milk solution contained the same amount of nitrogen, but nearly a threefold greater amount of carbohydrates, and elicited a similar plasma glucose response to that observed after both peptide hydrolysates. Meanwhile, the peak plasma insulin response was only half that produced by the ingestion of both peptide hydrolysate solutions. However, the insulin response to the milk protein solution was more prolonged compared to that observed for both peptide hydrolysate solutions and may be attributed to a slower rate of gastric emptying and/or intestinal absorption (36).

Gastric emptying might play an important role in regulating the kinetics of nitrogen absorption from highly digestible proteins such as milk (35,37). In this study the milk solution was emptied the slowest as a result of its high energy content (38). Thus plasma amino acids increased at a substantially slower rate after the administration of the milk protein solution than after that of both peptide hydrolysates. Comparable findings were reported by Van Loon et al. (27,39) in response to feeding with different carbohydrate–protein solutions of an energy density similar to that of our milk solution. On other hand, both peptide hydrolysate solutions were emptied at similar rates. Despite the lower digestibility of pea nitrogen (36,40), the fact that the rate of appearance of amino acids in plasma was similar for those amino acids that were present in similar concentrations in both hydrolysate solutions indicates that both peptide hydrolysates were digested and absorbed at similar rates.

It should be noted that when the whole postprandial period (180 min) was examined, the whey peptide hydrolysate solution evoked a higher TAA concentration, mainly because of the higher BCAA concentration of this solution. However, the postprandial amino acid concentration in blood depends on both intestinal absorption and hepatic/tissue uptake. Although the role played by each of these processes cannot be delineated with our experimental approach, other studies have found that BCAA selectively escape uptake by the liver (14,29,41). Consequently, the changes in plasma BCAA primarily reflect the rate of intestinal absorption and solution content.

A close relationship between plasma amino acid concentrations and plasma insulin levels has also been shown (4,13,39). Our data support this finding, given that the interdependency between the rate of plasma amino acid increase and insulin release was accentuated when delivered as oligopeptides rather than as whole proteins. In other words, we found a closer relationship between the plasma TAA concentration and the insulin response after the administration of both peptide hydrolysates than after administration of milk protein. In agreement, higher insulin responses have also been reported when amino acids were delivered as oligopeptides rather than as whole proteins (13) or free amino acids (15).

Amino acids stimulate insulin release, although their potency is lower than that of glucose (34,42). The individual amino acids that were most closely related to the insulin response were phenylalanine after the administration of both peptide hydrolysate solutions, and arginine after the administration of the milk protein solution. Several in vitro studies have shown marked insulinotropic actions of phenylalanine and arginine (43–45). In addition, previous studies showed that the intravenous administration of arginine and phenylalanine combined with glucose results in a more powerful stimulation of insulin secretion than that of other amino acids (34,42). The present study extends these observations to protein and oligopeptide solutions administered orally. Furthermore, it shows that the insulin secretory action of some amino acids appears to be dependent on the plasma glucose level.

The high insulin levels plus low FFA after the ingestion of both peptide hydrolysates create the appropriate environment for increased glucose disposal (4,46,47). In fact, the present study shows that despite the presence of glucose in both peptide hydrolysate solutions, plasma glucose approached hypoglycemic levels 1 h after their administration. Furthermore, two subjects reached mild nonsymptomatic hypoglycemic values after ingestion of the whey peptide hydrolysate solution (2.6 and 3.2 mmol/L, respectively).

Glucagon plays a major role in counteracting insulin-induced hypoglycemia (48). However, plasma glucagon levels peaked before a decrease in plasma glucose occurred. Therefore, other factors must have been influencing this glucagon response. It has been shown that glucagon release depends on the protein-to-carbohydrate proportion of the meal; thus, when this ratio is high, glucagon release is stimulated. Conversely, if the ratio is low, glucagon release is suppressed (49,50). Nevertheless, the protein-to-carbohydrate ratio was higher for both peptide hydrolysates than that for the milk protein solution, which had the lowest ratio; nevertheless, the glucagon response (as shown by the area under the glucagon curves during the 180 min postprandial period) was similar after intake of all these solutions. Therefore, other mechanisms must have been contributing to the regulation of the plasma glucagon levels.

Another interesting finding from this study was that the glucagon response to protein feeding was linearly related to the plasma amino acid concentration, regardless of the nature of the protein, the degree of fractionation or the amino acid profile of the protein ingested. Furthermore, when plasma insulin, glucose and all the amino acids were used in a multiple linear regression model to examine glucagon responses, the best predictor of plasma glucagon was plasma methionine after the administration of the pea peptide hydrolysate, and plasma tyrosine after the ingestion of the whey peptide hydrolysate and milk solution. This is most likely attributable to the known stimulatory effects that aromatic amino acids have on pancreatic A cells (28). On the other hand, the relationship between glucagon and methionine may be attributed to the potent stimulatory effect that glucagon has on liver methionine uptake (7). Unger et al. (51) have proposed that amino acids stimulate glucagon release to increase hepatic glucose production, thus to avert hypoglycemia resulting from concomitant insulin secretion. Chiasson et al. (52) have shown

that glycogenolysis is approximately five times more sensitive than gluconeogenesis to suppression by insulin. In contrast, both amino acids and glucagon enhance gluconeogenesis (8,53), an effect that is achieved by stimulating the hepatic uptake of amino acids and not by increasing whole-body proteolysis (6,7). Thus, it is more likely that glycogenolysis rather than gluconeogenesis was suppressed in our study. However, despite the simultaneous increase in plasma glucagon and amino acid concentrations, plasma glucose followed a decreasing pattern 40 min after the administration of both peptide hydrolysate solutions. This indicates that hepatic glucose production and intestinal absorption did not match the increased glucose disposal caused by the high insulin levels. Moreover, as insulin inhibits glycogenolysis, hepatic glucose production depends on gluconeogenesis, which seems to be insufficient to offset the decrease in plasma glucose during the hyperinsulinemic state that followed the administration of both peptide hydrolysate solutions.

In summary, this study showed that in healthy humans the ingestion of solutions containing similar amounts of glucose and peptide hydrolysate (~15 g of each) results in a synergistic and fast increase in plasma insulin. This is followed by a temporary decrease in plasma glucose, which approaches hypoglycemic levels, despite a large increase in plasma glucagon. This insulin response is mainly determined by the increase in the plasma concentration of both phenylalanine and glucose. In addition, peptide hydrolysates are absorbed at a faster rate from the small intestine than are whole milk proteins delivered as a milk solution, as reflected by the rapid increase in the plasma concentration of BCAA in peripheral blood (even after accounting for differences in rate of gastric emptying). The whey peptide hydrolysate elicited the greatest availability of amino acids during the 3-h postprandial period. The association of high levels of plasma amino acids and insulin might explain a superiority of peptide hydrolysates over whole proteins in promoting better nitrogen utilization, especially when administered in combination with glucose. Finally, plasma glucagon levels increase as a linear function of plasma amino acid concentrations.

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