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Comparison of the absorption of two protein hydrolysates and their effects on water and electrolyte movements in the human jejunum*

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SUMMARY Because of the generally more rapid amino acid absorption and lower osmotic pressure of small peptides compared with free amino acids, it has been suggested that 'elemental' diets should contain both small peptides and free amino acids as the nitrogen source. While studying protein hydrolysates intended for use in such diets we observed surprising differences in the absorption of amino acids, water, and Na^+ during jejunal perfusion of partial enzymic hydrolysates of two proteins (lactalbumin and fish) which contained high and approximately equal amounts of their constituent amino acids in the form of small peptides. Total α amino nitrogen (αNH_2N) absorption from the lactalbumin hydrolysate was greater, and individual amino acid absorption more even, than from equinitrogenous solutions of the fish protein hydrolysate, or from mixtures of free amino acids simulating either hydrolysate. Net water and Na^+ *absorption* occurred during perfusion of the lactalbumin hydrolysate, whereas net water and Na^+ *secretion* occurred during perfusion of the fish protein hydrolysate. These differences were significant ($P < 0.05$ or less). As the differences between the hydrolysates are so marked, we conclude that it is unwise to assume that all protein hydrolysates are equally suitable for use in patients.

The original rationale for the use of chemically defined elemental diets was to provide a low bulk formula of high nutrient content and complete digestibility.[1] Because it was then generally believed that dietary proteins were completely hydrolysed in the intestinal lumen and absorbed entirely as free amino acids, there was little reason to doubt that amino acids were an ideal nitrogen source. Since then, however, our concepts of the intestinal absorption of dietary protein have changed radically.[2–4] The evidence now strongly suggests that specific systems exist which can mediate the transport of intact small peptides derived from the incomplete hydrolysis of dietary protein, and that such peptide transport may comprise a significant fraction of normal α amino nitrogen (αNH_2N) absorption.[2–4] The more rapid and 'even' absorption of amino acid residues from protein hydrolysates than from equivalent mixtures of free amino acids alone[5–7] may promote more efficient use of the amino acid residues for protein synthesis.[8 9] Thus, this evidence suggests that elemental diets should contain small peptides rather than, or as well as free amino acids.

The normal physiological events after the intake of food include stimulation of jejunal water and electrolyte absorption by components of the meal such as glucose,[10] some amino acids[11 12] and some peptides[12 13] It seems reasonable that 'synthetic' diets should do likewise.

While studying the absorption of protein hydrolysates intended for use in elemental diets, we noted surprising differences in the absorption of amino acids, water, and electrolytes induced by two protein hydrolysates containing a high and approximately equal amount of their constituent amino acids in the form of small peptides. This paper reports these differences and contrasts the effects of the peptide-containing hydrolysates with those of equivalent mixtures containing only free amino acics.

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Methods

ENZYMIC HYDROLYSATES AND AMINO ACID MIXTURES

The fish protein hydrolysate was prepared by

Table 1 *Composition of perfusion solutions*

a. *Amino acid content of perfusion solutions*

Amino acid	*Lactalbumin hydrolysate*			*Fish protein hydrolysate*		
	Total mMol/l	*Free*		*Total mMol/l*	*Free*	
		mMol/l	*%*		*mMol/l*	*%*
Aspartic acid and asparagine	5·3	0·9	16·5	6·7	0·1	1
Threonine	4·7	0·4	8	3·5	—	—
Serine	4·0	0·5	11	3·1	—	—
Glutamic acid and glutamine	6·5	0·7	11	9·6	—	—
Proline	2·7	—	—	2·5	—	—
Glycine	2·2	0·15	7	5·2	—	—
Alanine	7·1	1·2	17	6·1	0·2	4
Valine	5·7	1·3	23	5·7	1·8	32
Methioine	2·2	0·8	39	2·8	1·4	49
Isoleucine	4·3	1·1	26	4·3	1·5	36
Leucine	11·7	3·6	31	5·5	0·7	13
Tyrosine	2·4	—	—	1·0	0·5	50
Phenylalanine	2·6	—	—	3·0	2·5	82
Lysine	5·8	—	—	5·9	0·6	10
Histidine	1·2	—	—	1·4	0·3	20
Arginine	1·6	—	—	3·2	1·6	52
Tryptophan	1·3	—	—	0·4	0·4	100

b. *Sodium content of perfusion solutions*

Preparation	*Source*	*Na^+ concentration in perfusion solutions mMol/l*	
Hydrolysate	Fish	140·7 ± S.D. 2·3	NS
	Lactalbumin	136·7 ± S.D. 3·7	
Amino acid mixture	Fish	123·1 ± S.D. 2·4	NS
	Lactalbumin	124·8 ± S.D. 1·6	

Syntex Research, Palo Alto, California. The technique of preparation has been described in detail.[14] In brief, a fish protein hydrolysate was incubated for 18 hours at 43·3°C at pH 8·5 with a mixture of bovine and porcine pancreatic enzymes. After digestion the pH was adjusted to 7·0 with aqueous phosphoric acid and the pancreatic enzymes deactivated by heating the mixture to 62°C. The mixture was then filtered and the filtrate containing peptides and amino acids was mixed with particulate activated charcoal and refiltered. The protein hydrolysate was finally recovered in powder form by lypophilisation. The approximate size of the peptides was estimated by gel filtration using Bio-Gel P6 (200–400 mesh; Bio-Rad Laboratories). Twenty per cent of the α amino nitrogen (αNH_2N) in the sample applied to the column eluted in fractions corresponding to molecular weights of less than 150 daltons (free amino acids). Seventy-two per cent eluted in fractions corresponding to molecular weights of 150–500 daltons (di to tetrapeptide). The remaining 8% eluted in fractions corresponding to molecular weights of 500–1500 daltons (peptide chain length of four to approximately 14 amino acid residues).

The hydrolysate of lactalbumin was provided by Nestlé Technical Assistance (Lausanne, Switzerland). Crude lactalbumin obtained from whey during the commercial processing of milk was hydrolysed using commercial bacterial neutral protease (Novo, Denmark). The resulting digest was separated into peptide fractions by ultrafiltration, one of which was supplied for the present study. The approximate size of the peptides obtained was estimated by gel filtration on columns of Sephadex G-10 using the criteria outlined above. We estimate that the peptide mixture was composed of 10–15% pentapeptides and above, 73–79% di to tetrapeptides, and 11·5% free amino acids. The total amino acid concentration and the amount of peptide bound and free amino acid in the perfusion solutions containing the hydrolysates are shown in Table 1a.

The total amino acid content of the hydrolysates was checked by ion exchange chromatography after hydrolysis as described below (Analytical methods) and was found to correspond closely to those of the free amino acid mixtures supplied by the manufacturers.

PERFUSION TECHNIQUE

Fourteen normal healthy white male volunteers aged 21–25 years were intubated with a double lumen perfusion tube incorporating a proximal occlusive balloon as previously described.[15] The study was approved by the Ethical Committee at St. Bartholomew's Hospital and informed consent was obtained from all subjects. The tube was allowed to pass until the infusion orifice was at the duodenojejunal flexure. The final position was checked radiologically so that the 30 cm perfusion segment was positioned in the upper jejunum.

Test solutions were infused at 15 ml per minute. After an equilibration period of 30 minutes, three 10-minute samples were collected from the distal collecting orifice by simple siphonage into a vessel at 4°C and samples were stored at –20°C until required for analysis.

The small intestines of six subjects were perfused in random order with a test solution containing the partial enzymic hydrolysate of lactalbumin and a solution containing an equivalent free amino acid mixture. Eight other subjects were perfused with a test solution containing the partial enzymic hydrolysate of fish protein and a solution containing an equivalent free amino acid mixture in random order. All solutions contained 70 mMol αNH_2N/l and were made iso-osmotic with plasma (290 mosm/kg) by the addition of sodium chloride.‡ Solutions contained polyethylene glycol (PEG) 5 g/l labelled with 1 μCi (37 kBq) ^{14}C PEG per litre (New England Nuclear Corporation, Boston, Massachussetts)[16] and were adjusted to pH 7 before infusion. The Na‡ concentration in the perfusion solutions was: (1) fish protein hydrolysate 140·7± SD 2·3 mMol/l‡, (2) lactalbumin hydrolysate 136·7±SD 3·7 mMol/l‡, (3) fish protein aminoacid mixture 123·1±SD 2·4 mMol/l‡, (4) lactalbumin amino acid mixture 124·8 ±SD 1·6 mMol/l* (Table 1a).

ANALYTICAL METHODS AND CALCULATION OF RESULTS

Aliquots of the perfusion solutions and their respective intestinal aspirates were hydrolysed under reflux conditions at 110°C for 24 hours with 6N hydrochloric acid in the presence of norleucine as internal standard to correct for hydrolysis losses. After cooling the amino acid content of the hydrolysed sample was estimated (after appropriate dilution) by ion exchange chromatography using a Locarte automatic loading amino acid analyser (Locarte Company, London, UK). The PEG content of the test solutions and intestinal aspirates was measured by scintillation counting,[16] and the sodium content by flame photometry using an EEL 227 integrating flame photometer (EEL Ltd., Halstead, Essex UK). Rates of sodium, water, and amino acid absorption were calculated from previously described formulae.[10] Luminal disappearance of individual amino acid residues (absorption) was expressed as a percentage of the perfused load. The significance of differences between the mean values was assessed by the paired or unpaired Student's *t* test where appropriate.

‡At first sight the Na^+ concentration in the perfusion solutions appears too high for the stated total osmolality. This is because NaCl does not dissociate completely into osmotically active Na^+ and Cl^- at this concentration, and therefore does not exert its full theoretical osmotic pressure. For example a solution of 156 mMol/l NaCl has a measured osmolality of 290 mosm/kg—that is, less than the 312 mosm expected if complete dissociation had occurred.

Results

PROTEIN HYDROLYSATES

Total αNH_2N absorption (calculated from the sum of the individual amino acid absorption rates) was 42% faster from the lactalbumin hydrolysate (33·8±SEM 5·1 mMol/h/30 cm) than from the fish protein hydrolysate (23·8±SEM 3·0 mMol/h/30 cm, $p<0·05$, Table 2). Significantly more water (70·9± 29·8 ml/h/30 cm) and sodium (6·33±SEM 4·69 mMol/h/30 cm) was absorbed from the lactalbumin hydrolysate than the fish protein hydrolysate (H_2O, –12·8±SEM 20·4 ml/h/30 cm, $p<0·05$; Na^+–3·2±SEM± 2·85 mMol/h/30 cm, $p<0·025$). The sodium content of the perfusion solutions containing the two hydrolysates was similar (Table 1b) and this therefore cannot explain the observed differences in absorption rates of αNH_2N, water, or sodium ion.

AMINO ACID MIXTURES

There was no difference in total αNH_2N absorption between the two amino acid mixtures (Table 2).

Table 2 *Total α amino nitrogen (αNH_2N) absorption, and absorption of sodium and water during perfusion of protein hydrolysates and their equivalent amino acid mixtures*

	n	*αNH²N absorption mMol h⁻¹ 30 cm⁻¹*	*Na⁺ absorption mMol h⁻¹ 30 cm⁻¹*	*Water absorption ml h⁻¹ 30 cm⁻¹*
Amino acid mixture	8	21·91±2·69[a]	–0·62±4·11[a]	20 ±28·2[a]
Pancreatic hydrolysate of fish		23·79±3·01[b]	–3·2±2·85[b]	–12·8±20·4[b]
Lactalbumin hydrolysate	6	33·76±5·05[c]	6·33±4·69[d]	70·9±29·8[e]
Equivalent amino acid mixture		19·61±3·12	3·78±3·3	67·3±21·8

All preparations contained 70 mMol αNH_2N. Values are mean ±1 SEM. – indicates secretion.
[a] $p>0·1$ between pancreatic hydrolysate of fish protein and equivalent amino acid mixture.
[b] $p<0·05$ between pancreatic hydrolysate of fish protein and lactalbumin hydrolysate.
[c] $p<0·02$ between lactalbumin hydrolysate and equivalent amino acid mixture.
[d] $p>0·5$ between lactalbumin hydrosylate and equivalent amino acid mixture.
[e] $p>0·5$ between lactalbumin hydrolysate and equivalent amino acid mixture.

Table 3 *Jejunal absorption (% perfused load) of amino acid residues* from a pancreatic hydrolysate of fish protein and equivalent amino acid mixture each containing 70 mMol/l αNH_2N*

	Hydrolysate	*P*	*Amino acid mixture*
Methionine	57·1±4·8	NS	54·0±4·5
Leucine	52·1±5·1	NS	47·8±4·2
Isoleucine	49·9±5·4	NS	46·8±5·3
Arginine	44·1±5·1	NS	45·1±4·7
Proline	32·9±4·7	NS	42·5±5·0
Valine	45·2±5·3	NS	33·5±4·0
Alanine	38·2±4·9	NS	30·0±4·9
Lysine	38·0±5·3	NS	28·2±6·8
Phenylalanine	47·8±3·9	<0·005	27·8±2·9
Glutamic acid and glutamine	22·8±3·8	NS	26·6±4·1
Tyrosine	37·9±5·1	<0·01	23·2±3·9
Glycine	24·2±4·1	NS	20·2±4·3
Aspartic acid and asparagine	20·9±4·0	NS	17·2±4·2
Threonine	45·8±5·0	<0·01	15·1±6·7
Serine	45·7±4·9	<0·005	15·0±3·0
Histidine	27·5±3·7	<0·01	5·9±5·2

Values are mean of eight studies ± SEM.
*In this and Table 4, the amino acids are ordered according to the rates of absorption from the free amino acid mixture.

Table 4 *Jejunal absorption (% perfused load) of amino acid residues from a hydrolysate of lactalbumin and equivalent amino acid mixture each containing 70 mMol/l αNH_2N*

	Hydrolysate	*P*	*Amino acid mixture*
Methionine	54·3±4·9	NS	64·2±8·3
Isoleucine	45·5±4·1	NS	44·6±5·1
Leucine	55·1±3·7	<·05	39·8±7·1
Arginine	46·1±9·8	NS	37·5±5·0
Proline	37·0±9·7	NS	37·0±5·1
Valine	47·1±3·2	<·05	34·6±5·0
Alanine	40·8±3·8	NS	30·1±4·7
Phenylalanine	47·1±4·9	<·005	29·2±5·1
Tyrosine	42·1±4·8	<·02	27·8±6·1
Lysine	37·1±6·0	<·05	26·0±7·5
Aspartic acid and asparagine	30·8±7·5	<·02	17·1±5·9
Serine	43·2±5·9	<·01	15·3±4·1
Glycine	33·1±4·1	<·05	15·0±4·2
Glutamic acid and glutamine	37·5±5·9	<·02	13·2±3·4
Threonine	36·1±5·7	<·01	11·8±2·6
Histidine	36·8±4·2	<·001	6·7±2·5

Values are mean of six studies ± SEM

There was likewise no significant difference in αNH_2N absorption between the fish protein hydrolysate and its equivalent amino acid mixture. However, total αNH_2N absorption from the amino acid mixture simulating the lactalbumin hydrolysate was only 58% of that seen during perfusion of the lactalbumin hydrolysate (Table 2, $P < 0{\cdot}02$). Thus the lactalbumin hydrolysate containing small peptides showed a marked kinetic advantage in total αNH_2N absorption over its equivalent amino acid mixture.

Comparison of the data presented in Tables 3 and 4 shows that with the exception of glutamic acid, which is absorbed to a greater extent from the amino acid mixture simulating the fish protein (26·6±SEM 4·1%) than from the lactalbumin amino acid mixture (13·2±SEM 3·4%), the remainder of the amino acid residues were absorbed to a similar extent during perfusion of the two free amino acid solutions. Six amino acids were absorbed more rapidly from the fish protein hydrolysate than from its equivalent amino acid mixture (Table 3). The kinetic advantage in absorption of individual amino acids from the lactalbumin hydrolysate as compared with its amino acid mixture was, however, even more striking. No less than 11 amino acids were absorbed more rapidly from the hydrolysate (Table 4). The sum of these increases in rate, of course, gives rise to the previously-mentioned increment in total αNH_2N absorption. The extent to which amino acids were absorbed form the free amino acid mixture simulating the fish protein hydrolysate varied by almost 10-fold (Table 3), ranging from 54·0±SEM 4·5% for methionine to 5·9±SEM 5·2% for histidine. The variation in the extent to which the amino acid residues were absorbed from the fish protein hydrolysate was considerably less (less than three-fold). This reduction in variation in extent of absorption is due to significantly greater absorption of those amino acids which were absorbed poorly from the amino acid mixture. Similarly there was an almost 10-fold variation in the extent to which the amino acid residues were absorbed from the free amino acid mixture simulating the lactalbumin hydrolysate. The extent of absorption varied from 64·2±SEM 8·3% for methionine to 6·7±SEM 2·5% for histidine. In contrast the lack of variation in extent of absorption of individual amino acid residues from the lactalbumin hydrolysate was striking (Table 4). The nine amino acid residues which were least efficiently absorbed from the free amino acid mixture (phenyalanine, tyrosine, lysine, aspartic acid, serine, glycine, glutamic acid, threonine, and histidine) were all absorbed to a significantly greater extent from the lactalbumin hydrolysate ($P < 0{\cdot}05$ or less). In addition, leucine and valine were absorbed to a significantly greater extent from the lactalbumin hydrolysate ($P < 0{\cdot}05$).

Absorption rates of sodium and water were the same from the two amino acid mixtures (Table 2).

Discussion

The present experiments have demonstrated marked differences in the jejunal handling of two protein hydrolysates in respect of total αNH_2N and individual amino acid absorption, and their effects on

water and sodium absorption. Furthermore, amino acid absorption from the hydrolysates was more 'even' than from the equivalent amino acid mixtures. This last is not a new observation but is more striking in the case of the lactalbumin hydrolysate than has previously been reported.[6 7] Absorption of amino acids from *individual* di- and tripeptides is usually more rapid than from the equivalent free amino acids, particularly at high luminal concentration.[3] The phenomenon of more rapid amino acid absorption from individual peptides and more even absorption from *complex mixtures* of peptides than from the equivalent free amino acids is now recognised to be due to the absorption into the enterocytes of intact small peptides by transport systems not available to free amino acids.[4 17] Because the intestinal lumen after a normal meal contains a complex mixture of free amino acids and peptides[3 18] and the time required for complete intraluminal protein digestion followed by absorption of the liberated free amino acids is too great for this sequence to account for normal protein absorption,[3 19] the transport of intact di- and tripeptides is now considered likely to be of major importance in the assimilation of dietary protein.[3 4 17]

The reasons for the differences in kinetic advantage displayed by the two hydrolysates might lie either in qualitative or quantitative differences in their peptide constitution, or in the basic amino acid composition of the two starting proteins or in their methods of preparation. Because of the potentially enormous numbers of individual peptides in the hydrolysates and the overlap in molecular weights of peptides of different chain lengths it is impossible to estimate accurately the amount in the hydrolysates of any individual peptide or peptides of any given chain length. Thus, although we have estimated on the basis of molecular weights that both hydrolysates contain approximately the same amount of αNH_2N as di- to tetrapeptides, it could be that one contains a high proportion of dipeptides and the other a high proportion of tetrapeptides. As only one[20] of the four studies of tetrapeptide absorption in mammals[20–23] has shown a kinetic advantage of tetrapeptide over free amino acids (and that only at the highest concentration studied), one could postulate that a high content of tetrapeptide in the fish protein hydrolysate might account for the relatively meagre kinetic advantage of this hydrolysate over its equivalent amino acid mixture. However, until more studies of tetrapeptide absorption and more sophisticated methods of peptide sizing are available such an hypothesis cannot be tested. Likewise, the effects of qualitative differences in the peptide constitution of the hydrolysates remain to be examined.

In terms of the use of protein hydrolysates for oral feeding as components of elemental diets, the present results may have important consequences, in that it has been suggested that the more 'even' absorption of amino acids from protein hydrolysates containing small peptides may induce more efficient protein synthesis than when the amino acids are presented to the tissues at widely differing rates,[8 9 21 24] such as may occur during absorption of a mixture of free amino acids. The present results, however, demonstrate that it is dangerous to generalise about the absorption of peptide mixtures, as their absorption characteristics may differ so markedly.

The feeding of peptides to normal people seems unlikely to have nutritional advantage over whole protein, both because of the enormous functional reserve capacity of the normal intestine, and because normal absorption in any case probably takes place from a mixture of amino acids and small peptides produced in the intestine from whole protein by the action of endogenous pancreatic proteases and brush border peptidases.[17 18] However, if the phenomena seen during our perfusion experiments hold true after oral ingestion of these diets a suitable protein hydrolysate containing small peptides may have significant advantages over both whole protein or amino acid mixtures in patients with exocrine pancreatic insufficiency or loss of absorptive surface area due to disease or intestinal resection. Again, however, we suggest that each peptide preparation needs testing in its own right.

The products of digestion of a normal meal stimulate the absorption of ingested and secreted water and sodium ion.[10–13] Compared with isotonic saline—from which little net water or sodium absorption occurs from solutions perfused in the human jejunum[10 12 13 25 26]—glucose,[10] amino acids,[11 12] peptides[12 13] and bicarbonate ion[26] in solutions made isotonic by adding sodium chloride stimulate jejunal water and sodium absorption. The importance and magnitude of this 'solute-associated' water and sodium absorption is illustrated by the reversal of the net intestinal secretion in cholera by enteral administration of glucose or amino acids, but not by enteral isotonic saline.[27] The lactalbumin preparation had the expected stimulatory effect on water and sodium absorption but, surprisingly, this was not shared by the fish protein preparations. We cannot, at present, explain the different effects of the two *hydrolysates* on water and electrolyte absorption. Differences in either the methods of preparation, the peptide or free amino acid composition of the hydrolysates or the amino acid composition of the starting proteins could be responsible. Thus, the fish protein hydrolysate contains greater amounts of glutamine, asparagine, glycine, and arginine con-

taining peptides than the lactalbumin hydrolysate; this raises the possibility that some of these peptides may be inhibitory to intestinal function. However, it is interesting that water and sodium absorption induced by the two hydrolysates is paralleled by that induced by the mixtures of free amino acids, but, possibly because the amino acid mixtures were not compared 'within-subject', the differences fail to reach statistical significance.

In practical terms, however, it would seem only sensible to choose for use in patients with reduced absorptive capacity or excessive enteric loss of endogenous secretions a synthetic diet which stimulates significant intestinal water and sodium absorption, rather than one which does not do so.

Additional studies are being performed to establish whether the phenomena seen during our perfusion studies are reproduced in a more physiological situation, and whether such considerations as we have outlined are of importance in either the eventual use of the absorbed amino acids for protein synthesis or in the water and sodium balance of patients in whom physicians may consider giving elemental diets.

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