Eur J Nutr 39 : 237–243 (2000)

ORIGINAL CONTRIBUTION

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Protein hydrolysate vs free amino acid-based diets on the nutritional recovery of the starved rat

Summary *Background and Aims* To test the hypothesis that a peptide-based enteral product was equivalent to a low-fat, free amino acid-based formula in the nutritional and functional recovery of the starved rat.

Methods Sixteen male Wistar rats were starved for 3 days. Then, rats were randomised to a whey protein hydrolysate-based diet or a free amino acid-based diet and refed for 3 days. The experiment was designed to provide the same energy intake in both groups. The parameters studied included body weight gain, nitrogen retention, plasma free amino acid concentrations, muscle glutamine concentrations and glutathione levels in gut mucosa and liver.

Results Weight gain was statistically higher on the peptide-based diet than on the elemental diet after the refeeding period. This difference in weight gain was associated with a statistically higher nitrogen retention. Plasma and muscle free glutamine concentrations were higher in rats fed the whey protein hydrolysate-based diet than those in rats refed the free amino acid-based diet, even though the glutamine intake was higher in the latter group. Glutathione concentrations in liver and gut mucosa were similar in the groups.

Conclusion We conclude that enteral diets containing peptides were more effective than a diet containing free amino acids in the nutritional recovery of the starved rat.

Key words Protein hydrolysates – free amino acids – rats – enteral nutrition

Received: 17 May 2000
Accepted: 1 September 2000

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Introduction

Over the years, enteral nutrition has become the most effective means of providing nutritional support for patients requiring artificial nutrition when the intestine is functional [1, 2]. Dietary restriction as well as certain gastrointestinal disorders severely affect the morphology and function of the small intestine and other organs [3, 4]. Since the hydrolytic capacities of the digestive tract could also be affected, the molecular form of the nitrogen supply has been the subject of considerable discussion [5, 6]. Semi-elemental and elemental diets (based on hydrolysed proteins and free amino acid mixtures, respectively) are frequently used for the nutrition support of the critically ill patient [7–10]. In growing and severely undernourished rats, it has been previously shown that the molecular form of the alimentary proteins can affect nitrogen balance and small intestine adaptation [11–15]. In fact, it appears that under conditions of discontinuous enteral feeding, a mixture of small peptides is of greater nutritive value than a mixture of free amino acids with a similar composition, due probably to different absorption kinetics, resulting in different metabolic utilisation of amino acids [16]. In addition to quantity, the quality and molecular form of the protein source, investigators are increasingly focusing on specifically enriched amino acid formulations. Glutamine, branched-chain amino acids and arginine-fortified formulas have been proposed to have a wide range of benefits over standard solutions [17]. The objective of the present work was to test the hypothesis that a peptide-based enteral product was equivalent to a low-fat, free amino acid-based formula, rich in glutamine and arginine, on the nutritional and functional recovery of the starved rat.

Material and methods

Diets

The control diet was composed and designated as follows: soy protein isolate, 220 g/kg; sucrose, 120 g/kg; glucose, 50 g/kg; cellulose, 50 g/kg; corn oil, 100 g/kg; mineral mix AIN 76, 35 g/kg [18]; vitamin mix AIN76, 10 g/kg [18]; L-methionine, 3.5 g/kg; choline bitartrate, 2 g/kg; inositol, 0.25 g/kg; corn starch, 409 g/kg. Two complete enteral regimens were used as experimental diets: a free amino acid-based powder formula (AA, = Vivonex Plus®, Novartis Nutrition) and a whey protein hydrolysate-based liquid formula (PEP, = Peptamen®, Nestlé Clinical Nutrition). Both experimental diets was offered to rats in powder form. The chemical composition and the molecular form of the nitrogen source, the micronutrient composition and the amino acid profile of both diets are given in Tables 1–3, respectively. Both formulas were added 5 % cellulose to give adequate fibre content to the usual rat diets.

Experimental design

All experiments were approved by the Ethical Committee of Nestlé Research Center and by the Service Vétérinaire Cantonal (Lausanne, Switzerland).

Sixteen male Wistar rats, weighing 200 g, were obtained from Iffa Credo (France). Animals were allocated to Macrolon cages and were maintained at 23 °C with a 12-hour light period. They had free access to the control diet for 3 d. On d 4, a fixed amount of the control diet was offered at 4 pm every day for the following 3 d (269 kJ/d). On d 7, animals were put into metabolic cages and were randomised by weight. All animals were food deprived for 72 h with free access to water only. On d 10, animals were transferred to new metabolic cages, and refed one of the two enteral products, Vivonex Plus® or Peptamen® for 3 d (8 animals in each group). A fixed amount of diet was offered, maintaining a consumption of 269 kJ/d. Previous experiments were done to confirm that the animals would consume all of the feed offered. During this period (d 10–13), faeces and urine from all rats were collected to determine N balance. Metabolic cages specially designed for the separate collection of faces and urine avoiding fecal and urine contamination from food spillage were used. Urine was collected daily in special tubes containing 2 mL 1M HCl and was frozen at –20 °C until analysis. Faces were collected daily and dried in an oven at 80 °C. On d 14, after overnight fasting, rats were anaesthesized using isoflurane and total blood was collected using heparinized tubes. The blood was immediately centrifuged for 6 min at 2000 g, 4 °C and stored at –20 °C until required for the determination of the plasma amino acid concentrations. The liver, muscle tibialis and the small intestine were collected. Jejunum was scraped with a glass slide at nearly 0 °C.

Table 1 Chemical composition of diets (dry matter values)

	Energy kJ/100 g	Protein g/100 g	Fat g/100 g	CHO g/100 g
Control	1972.0	19.02	10.0	67.9
PEP	2059.7	16.41	19.32*	62.88*
AA	1717.1	16.06	2.76*	78.30*
		Protein % energy	Fat % energy	CHO % energy
Control		17.4	20.6	62.1
PEP		13.4	35.4	51.2
AA		16.0	6.2	77.9

* following declaration. *PEP* protein hydrolysate-based diet; *AA* amino acid-based diet. Molecular distribution of the whey protein hydrolysate (Daltons): > 5000 Da: 29 %; 1000–5000 Da: 49 %; 200–10 000 Da: 21 %; < 200 Da: 1 %.

Table 2 Micronutrient composition of experimental diets

	PEP	AA
Vit.A (IU)	1572	1639
Vit. D (IU)	126	115
Vit. E (IU)	9.4	11.5
Vit. K (µg)	16.7	32.8
Vit C (mg)	25.2	57.4
Thiamine (mg)	0.63	0.82
Riboflavin (mg)	0.72	0.98
Niacin (mg)	8.4	11.5
Vit. B_6 (mg)	0.84	1.64
Folic acid (µg)	167	221
Pantothenic acid (mg)	4.2	5.7
Vit. B_{12} (µg)	2.5	3.3
Biotin (µg)	126	164
Ca (mg)	210	328
P (mg)	210	287
Mg (mg)	84	164
Zn (mg)	4.7	5.7
Fe (mg)	3.8	4.9
Cu (mg)	0.42	0.57
Mn (mg)	0.63	1.11
I (µg)	31	41
Na (mg)	230	205
K (mg)	399	513
Cl (mg)	356	410
Cr (µg)	31	16
Mo (µg)	52	49
Se (µg)	21.0	16.4

Values are expressed per 100 g diet. *PEP* protein hydrolysate-based diet; *AA* amino acid-based diet.

A part of the mucosa obtained (100 mg) and a liver sample (100 mg) were immediately homogenised in 2 mL of cold perchloric acid (50 g/L) solution and centrifuged after-

Table 3 Amino acid composition of diets (g/100 g amino acids)

Amino acid	Control	PEP	AA
Ala	4.9	4.9	1.1
Arg	7.3	2.7	11.1
Asp	11.6	10.5	2.5
Cys	0.9	2.5	–
Gln*	9.0*	6.2*	22.2
Glu	10.4	10.7	–
Gly	4.4	1.9	1.1
His	2.5	1.7	2.0
Ile	4.6	5.6	7.5
Leu	8.0	10.3	15.0
Lys	5.8	9.5	6.2
Met	3.1	2.2	5.5
Phe	4.8	3.1	6.6
Pro	5.2	5.3	2.3
Ser	5.4	5.5	1.3
Thr	3.3	7.1	4.2
Trp	1.2	2.1	1.5
Tyr	3.1	3.0	2.4
Val	4.4	5.2	7.5

PEP protein hydrolysate-based diet; *AA* amino acid-based diet.
* Gln was determined after extensive enzymatic hydrolysis.

Table 3 (cont.) Amino acid composition of diets (residues/100 residues)

Amino acid	Control	PEP	AA
Ala	7.2	7.1	1.7
Arg	5.5	2.0	8.9
Asp	11.3	10.2	2.6
Cys	1.0	2.7	0.0
Gln*	8.0	5.5	21.2
Glu	9.2	9.4	0.0
Gly	7.6	3.3	2.0
His	2.1	1.4	1.8
Ile	4.6	5.5	8.0
Leu	7.9	10.2	15.9
Lys	5.2	8.4	5.9
Met	2.7	1.9	5.1
Phe	3.8	2.4	5.6
Pro	5.9	6.0	2.8
Ser	6.7	6.8	1.7
Thr	3.6	7.7	4.9
Trp	0.8	1.3	1.0
Tyr	2.2	2.1	1.8
Val	4.9	5.8	8.9

PEP protein hydrolysate-based diet; *AA* amino acid-based diet.

wards at 13000 g for 20 min at 4 °C. Supernatants were frozen in liquid nitrogen and stored at – 80 °C until required for glutathione determination.

Growth test

Rats were refed a fixed amount of energy (269 kJ/d) via one of the experimental diets for 3 d. The indices used to estimate the nutritional quality of the protein sources were the following [19, 20]:

- Relative body weight gain (average increase in body weight during the refeeding period per day/rat weight after starvation*100).
- Apparent digestibility: absorbed N/ingested N.
- Apparent biological value (BV): retained N/absorbed N.
- Apparent net protein utilisation (NPU): retained N/ingested N.
- Energy conversion efficiency (ECE): weight gain (g/3 d)/energy intake in kJ (g/3 d).
- Protein efficiency ratio (PER): body weight gain (g/3 d)/ intake of proteins (g/3 d).

Plasma amino acids

Once obtained, 200 µL of plasma was deproteinised by addition of 20 µL of a solution containing sulfosalicylic acid (400 g/L) and vitamin C (60 g/L). After mixing, samples were centrifuged (10000 g 3 minutes) and supernatants were frozen at –80 °C until analysis, after the addition of internal standards (D-glucosaminic acid and S-(2-aminomethyl)-L-cysteine.HCl). The analyses were performed in a Beckman 6300 amino acid analyser (Fullerton, US) using a buffer program for physiological samples. To avoid glutamine degradation, samples were kept at 10 °C before injection. Amino acid concentrations (µM) were calculated from individual peak area, external standard and the two internal standards areas [20].

Muscle glutamine

One hundred mg of muscle were thawed and homogenised in 2 mL of an ice cold solution of trichloroacetic acid (100 g/L). The supernatant was separated from the precipitate by centrifugation at 13 000 g for 10 min at 4 °C. The analyses were performed in a Beckman 6300 amino acid analyser [21].

Glutathione determination in liver and gut mucosa

Reduced glutathione (GSH) and oxidised glutathione (GSSG) concentrations were determined by HPLC (Waters, Milford, US), using fluorometric detection, according to the method of Martin and White [22]. The between and within-day coefficients of variation were 2.2 % and 0.8 % for GSH and 3.8 and 1.7 % for GSSG, respectively.

GSH and GSSG concentrations were calculated from individual peak area, external standard and internal stan-

dard areas, and expressed in nmol/mg protein. Tissue protein concentration was measured according to the the bicinchoninic acid method [23].

Statistical analysis

Data are expressed as mean ± SEM. One-way analysis of variance (factor = group) was done for all rats, including non-starved control rats and starved rats before refeeding. Bonferroni tests were used to detect the presence of significant differences between the PEP and AA groups for all the parameters studied. A difference was considered significant at $p < 0.05$.

Results

Starvation produced an average weight loss of 14.6 %. Rats gradually recovered weight during the refeeding period. Relative body weight gain was significantly higher in animals fed the protein hydrolysate-based diet compared to those fed the elemental diet ($p < 0.05$). Due to the fact that the experiment was designed to have the same energy intake in both groups, protein intake was slightly higher in animals fed the amino acid-based formula than those fed the protein hydrolysate-based formula during the refeeding period (6.9 ± 0.1 g vs 6.3 ± 0.0 g, respectively). PEP showed significantly higher energy conversion efficiency, protein efficiency ratio, nitrogen protein utilisation and biological value levels than AA, although AA exhibited a slightly higher digestibility than PEP (96.5 ± 0.4 vs 94.7 ± 0.3, respectively) (Table 4).

Plasma free amino acid concentrations of starved rats refed both enteral products for 3 days are shown in Table 5. Total amino acids, total essential amino acids, sulfur amino acids, threonine and glutamine concentrations were significantly higher in plasma of rats fed the whey protein hydrolysate-based diet compared to that of those fed the amino acid-based diet. Rats fed the elemental diet exhibited higher plasma urea concentrations than rats fed the

Table 4 Body weight gain, food, energy and protein intake, food conversion efficiency and protein efficiency ratio, digestibility, net protein utilisation and biological value during the refeeding period.

Variable	PEP	AA
Weight prior to starvation (g)	217.6 ± 3.4	213.5 ± 1.8
Weight after starvation (g)	185.8 ± 3.0	182.2 ± 1.9
Weight after refeeding (g)	215.7 ± 2.7	206.9 ± 1.8†
Relative body weight gain(%/d)	5.4 ± 0.2	4.6 ± 0.2†
Food intake (g/3 days)	39.7 ± 0.0	44.4 ± 0.2†
Energy intake (KJ/3 days)	817.3 ± 0.0	761.2 ± 3.1†
Protein intake (g/3 days)	6.3 ± 0.0	6.9 ± 0.1†
ECE ($x10^3$)*	38.5 ± 0.5	34.2 ± 0.3†
PER*	4.7 ± 0.2	3.6 ± 0.1†
Apparent Digestibility* (%)	94.7 ± 0.3	96.5 ± 0.4†
Apparent BV* (%)	51.1 ± 1.9	40.0 ± 1.5†
Apparent NPU* (%)	53.9 ± 2.0	41.5 ± 1.6†

Values are means ± SEM, n=8.

* *ECE* Energy conversion efficiency (weight gain in g/energy intake in kJ).

PER Protein efficiency ratio (weight gain in g/protein intake in g).

Digestibility (%) (absorbed nitrogen/ingested nitrogen)×100 D= ((I-F)/I)*100

BV(%) (retained nitrogen/absorbed nitrogen)×100 BV=((I-F-U)/(I-F))*100

NPU (%) (retained nitrogen/ingested nitrogen)×100 NPU= (I-F-U)/I)*100 where *I* ingested nitrogen; *F* faecal N excretion; *U* urine N excretion.

PEP protein hydrolysate-based diet; *AA* amino acid-based diet.

† Significantly different from PEP group ($p < 0.05$).

Table 5 Plasma amino acid concentrations of non starved control rats and starved rats refed PEP and AA for 3 days

Amino acid	Non-starved	PEP	AA
Lys	507.2 ± 45.2	443.2 ± 34.7	307.0 ± 15.6*
Ile	70.1 ± 6.3	51.2 ± 1.9	57.4 ± 4.2
Leu	116.9 ± 9.7	97.6 ± 18.7	114.6 ± 3.4
Val	148.4 ± 11.2	116.5 ± 9.5	111.5 ± 4.8
Met	49.9 ± 2.5	72.1 ± 1.9	33.3 ± 1.1*
Cys	12.6 ± 1.3	12.2 ± 1.0	5.7 ± 0.8*
Tau	134.1 ± 17.0	154.8 ± 27.7	222.5 ± 25.6
Phe	63.1 ± 4.2	50.0 ± 4.5	53.2 ± 4.1
Tyr	80.7 ± 3.3	39.3 ± 3.7	92.7 ± 3.5*
Thr	259.7 ± 6.0	406.4 ± 16.0	196.1 ± 6.5*
Trp	74.0 ± 3.8	52.5 ± 4.5	55.9 ± 1.2
Asp	16.7 ± 2.4	18.4 ± 1.4	16.9 ± 2.6
Asn	67.5 ± 6.0	63.3 ± 7.0	49.6 ± 5.2
Glu	90.0 ± 7.3	104.8 ± 8.3	89.0 ± 5.8
Gln	829.1 ± 75.2	1025.6 ± 54.4	738.7 ± 39.1*
Arg	134.2 ± 3.3	65.8 ± 2.3	69.5 ± 2.9
Orn	49.9 ± 4.1	44.3 ± 3.8	68.1 ± 3.6*
Cit	86.6 ± 6.9	83.6 ± 8.3	78.9 ± 5.4
His	62.7 ± 5.5	64.8 ± 5.0	61.9 ± 2.3
Pro	167.9 ± 10.9	143.3 ± 11.2	153.4 ± 13.6
Ser	267.2 ± 27.0	273.0 ± 37.5	229.2 ± 19.1
Gly	359.5 ± 19.8	332.2 ± 17.7	270.3 ± 25.8
Ala	640.6 ± 82.1	711.7 ± 103.1	784.2 ± 51.6
Total	4288.6 ± 152.4	4454.0 ± 141.8	3854.0 ± 215.8*
Essential	1352.0 ± 24.4	1341.2 ± 26.4	1027.8 ± 22.8*
BCAA	335.4 ± 15.2	266.0 ± 12.4	283.5 ± 8.3
Sul	62.5 ± 3.7	84.4 ± 4.1	39.0 ± 0.4*
Urea	3548.4 ± 128.9	2627.0 ± 145.5	3161.8 ± 108.2*

Values are expressed in µM, as means ± SEM for 8 animals. *Essential* essential amino acids; *BCAA* branched chain amino acid; *Sul* Sulfur amino acids; *PEP* protein hydrolysate-based diet; *AA* amino acid-based diet.

* Significantly different from PEP group ($p < 0.05$).

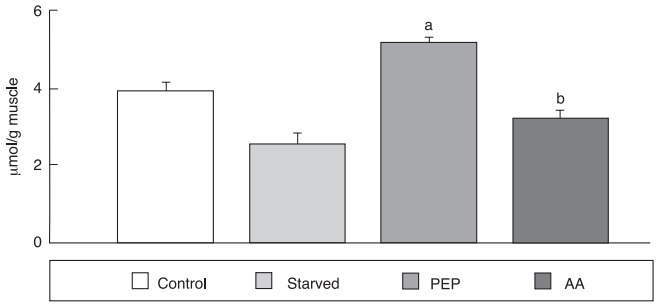

Fig. 1 Muscle glutamine concentrations of non-starved control rats, starved rats and starved rats refed PEP and AA for 3 d. Values are in µmol/g (mean ± SEM, n=8). Values not sharing a common letter letter are significantly different. *PEP* protein hydrolysate-based diet; *AA* amino acid-based diet.

peptide diet. Starvation resulted in a significant decrease in the muscle tibialis free glutamine concentration (Fig. 1). Rats refed PEP showed an average muscle glutamine concentration that was significantly higher than that observed in rats fed AA.

Table 6 shows the liver and gut mucosa glutathione concentrations of starved rats refed PEP or AA. There were no differences in tissue glutathione contents due to the different nutritional treatments.

Discussion

Severe starvation produces a series of metabolic changes that led to reductions in body weight, depression of immunocompetence, and alteration of digestive system functions, particularly of the liver and small intestine [13, 24]. In the present study, rats fed the protein hydrolysate-based diet showed a higher body weight gain during the refeeding period than those fed the elemental diet (10.0 ± 0.3 g/d vs 8.3 ± 0.3 g/d, respectively). These body weight gains are very high compared with that of normal non-starved 200 g rats, about 4–5 g/d [20]. These differences cannot only be due to a higher net protein utilisation or biological value, and they probably resulted from cell incorporation of water [25] and by the intestinal content coming from undigested food during the refeeding period [20]. Therefore, energy conversion efficiency and protein efficiency ratio values are, somehow, overestimated. Cell incorporation of water can be considered as growth, but not intestinal retention of fibre or partly digested material. Nevertheless, in the present study, both groups of rats followed the same experimental design and all these variables affected both groups of animals the same way.

Energy conversion efficiency, protein efficiency ratio and nitrogen retention were significantly higher when the protein hydrolysate-based diet was offered instead of the free amino acid-based diet ($p < 0.05$). These results confirmed the data of Poullain et al. [14] who observed that nitrogen retention was higher in rats after a 3 day starvation period and then refed a whey protein hydrolysed based diet, compared to those refed either a native protein- or free amino acid-based diet. In the Poullain et al. study, all diets have the same chemical composition and amino acid profile, differing only in the molecular form of the nitrogen source. They based this higher nitrogen retention in a lower urinary nitrogen excretion, as a result of better protein synthesis and lower ureagenesis.

Another explanation for these results could be due to the different amounts of micronutients that rats received depending on the diet they were fed (Table 2), especially with regard to vit K, vit C, vit B_6, Ca, P, Mg, Mn and Cr. These micronutrients are essential for the correct metabolism of the macronutrients and, to some extent, they could have affected the results of the present study.

Rats fed PEP exhibited higher total plasma essential amino acid concentrations and a higher non-essential/essential amino acid ratios than those rats fed AA, despite the fact that AA fed animals received the highest intake of essential amino acids (Table 3). A high plasma urea concentration (coming from amino acid oxidation) has been observed in rats refed AA compared to those refed PEP. Therefore, these results affirm that an important part of amino acids coming from AA were used to provide energy, rather than for protein synthesis. The amino acid profile and the molecular form of the nitrogen source of the amino acid-based formula were not ideal for protein deposition, at least, in starved adult rats. There are other several factors that could have affected these results. These are

- The relative insolubility of some amino acids in the intestinal environment [26].
- The interference with amino acid transporters
- The very rapid absorption of free amino acids that provokes an increase in liver amino acid oxidation. In this sense, Boirie et al. [27] have studied the influence of the

Table 6 Tissue glutathione concentrations of starved rats refed PEP and AA for 3 days

	Gut mucosa		Liver	
	GSH	GSSG	GSH	GSSG
PEP	30.41 ± 2.31	0.15 ± 0.02	37.46 ± 5.23	0.70 ± 0.10
AA	26.66 ± 2.17	0.13 ± 0.02	40.01 ± 4.37	0.53 ± 0.08

Values are expressed in µmol/g protein, as means ± SEM for 8 animals
PEP protein hydrolysate-based diet; *AA* amino acid-based diet.

rate of appearance of amino acids into plasma after protein ingestion and its effect on protein accretion. The so-called "fast proteins" would produce after absorption a dramatic increase in plasma amino acid concentrations, stimulating insulin release, amino acid oxidation and protein synthesis and it could affect also protein breakdown,
- The different availability of free amino acids to the gut flora compared to peptide-derived amino acids [28]
- Differences in osmolarity that can affect gastric emptying and then, nutrient utilisation [29].

Plasma glutamine concentrations correlated with the muscle intracellular free glutamine concentration. In a previous experiment [20], starvation led to a decrease in muscle glutamine levels. Refeeding was able to restore muscle glutamine stores, although PEP rats showed higher muscle glutamine concentrations than those of rats refed AA, despite their higher glutamine intake.

In humans, during the stress associated with injury, sepsis and inflammation, there is a marked increase in glutamine consumption by the gastrointestinal tract, immunologic cells, inflammatory tissue, and kidney. A relative state of glutamine deficiency may develop if tissue glutamine requirements are not adequately met by dietary glutamine provision and/or endogenous glutamine production. Thus, in these situations, the intracellular pools of glutamine in muscle are markedly reduced [30]. Next, as tissue stores become depleted, plasma or whole blood glutamine concentrations fall. Finally, as the deficiency state is manifest, alterations in tissue function or morphologic changes are observed, and these changes are associated with alterations in the protein economy (e. g., negative protein balance) [31]. In the present study, starved rats refed for 3 days the whey protein hydrolysate diet restored the plasma and muscle glutamine pools, whereas rats refed the glutamine and arginine enriched-elemental diet did not.

Plasma threonine concentrations were significantly higher in rats refed PEP compared to those refed AA, due to the higher threonine content in whey protein than in the elemental diet. Threonine is one of the amino acids more abundant in positive acute phase proteins [32]. The production of these proteins is enhanced in situations of metabolic stress and the supply of threonine (from the diet) for this function may become extremely important. Without an exogenous supply, the major source of amino acids used in acute-phase protein synthesis is the skeletal muscle. Apart from that, threonine accounts for about 25 % of the amino acid composition of mucoproteins [33]. This can account for 60 % of the daily threonine requirements [34]. One of the most important functions of the mucoproteins is the cytoprotection of mucosal cells against toxins, bacteria, bile, digestive enzymes, etc. In patients suffering from gastrointestinal diseases or under metabolic stress, the integrity of the intestinal barrier becomes crucial to avoid the passage of antigenic molecules through the intestine to reach the systemic circulation.

Grimble et al. [35] have suggested that sulphur amino acids, especially, cysteine, play a key role in the amino acid economy of the body under inflammatory conditions. The main metabolic uses of cysteine are the glutathione synthesis and the synthesis of specific acute phase proteins, very rich in cysteine [36]. Several studies have shown that sulphur amino acid requirements are increased in stress situations [37, 38] and that cysteine supplementation of the diet of septic rats had beneficial effects on recovery [38]. Authors suggested that these beneficial effects are associated with an increase in glutathione synthesis, since glutathione turnover may account for more than 50 % of cysteine flux in healthy men [39]. Plasma sulphur amino acids were significantly higher in rats fed PEP than those fed AA. However, these differences did not affect the gut mucosa and liver glutathione concentrations.

In conclusion, the administration of the diet based on a whey protein hydolysate (PEP) resulted in a higher growth rate, higher nitrogen retention and higher glutamine stores (plasma and muscle concentrations) than the administration of an elemental-based diet rich in essential amino acids, glutamine and arginine (AA).

Acknowledgments We would like to thank the contribution of J. C. Maire, P. Cailler and R. Muñoz-Box to the statistical analysis, P. A. Finot and B. German for helpful discussion.

References

1. ASPEN, Board of Directors (1993) Guidelines of the use of parenteral and enteral nutrition in adults and pediatric patients. JPEN 17: 7SA–9SA
2. Ziegler F, Nitenberg G, Coudray-Lucas C, Lasser P, Giboudeau J, Cynober L (1998) Pharmacokinetic assessment of an oligopeptide-based enteral formula in abdominal surgery patients. Am J Clin Nutr 67:124–128
3. Jambunathan LR, Neuhoff D, Younoszai MK (1981) Intestinal disaccharidases in malnourished infant rats. Am J Clin Nutr 34: 1879–1894
4. Prosper J, Murry RL, Kern F (1968) Protein starvation and the small intestine. Gastroenterology 55: 223–227.
5. Klein S, Kinney J, Jeejeebhoy K, Alpers D, Hellerstein M, Murray M, Twomey P (1997) Nutrition support in clinical practice: review of published data and recommendations for future research directions. Clin Nutr 16: 193–218
6. McClave SA (1995) Do peptide-based enteral formulas provide any benefit over intact protein diets? Nutrition 11: 395–397
7. Borlase BC, Bell SJ, Lewis EJ, Swails W, Bistrian BR, Forse RA, Blackburn GL (1992) Tolerance to enteral tube feeding diets in hypoalbuminanaemic critically ill geriatric patients. Surg Gynecol Obstet 174: 181–188
8. Meredith JW, Ditesheim JA, Zaloga GP (1990) Visceral protein levels in trauma patients are greater with peptide diet than intact protein diet. J Trauma 30:825–829
9. Rodriguez DJ, Clevenger FW (1993) Successful enteral refeeding after massive small bowel resection. Western J Med 159:192–194

10. Brinson RR, Pitts VL, Taylor AE (1989) Intestinal absorption of peptide enteral formulas in hypoproteinemic (volume expanded) rats: a paired analysis. Crit Care Med 17:657–660
11. Boza JJ, Jiménez J, Baró L, Martínez O, Suárez MD, Gil A (1996) Effect of native and hydrolyzed whey protein on intestinal repair of severe starved rats at weaning. J Pediatr Gastroenterol Nutr 22:186–193
12. Boza JJ, Martínez-Augustin O, Baró L, Suárez MD, Gil A (1995) Protein versus enzymatic protein hydrolysates. Nitrogen utilization in starved rats. Br J Nutr 73:65–71
13. Boza JJ, Martínez O, Baró L, Suárez MD, Gil A (1995) Influence of casein and casein hydrolysate diets on nutritional recovery of starved rats. JPEN 19: 216–221
14. Poullain MG, Cezard JP, Roger L, Mendy F (1989) Effect of whey proteins, their oligopeptide hydrolysates and free amino acid mixtures on growth and nitrogen retention in fed and starved rats. JPEN 13:382–386
15. Poullain MG, Cezard JP, Marche C, Marcy L, Roger L, Grasset E, Broyart JP (1991) Effect of dietary whey proteins, their peptides or amino acids on the ileal mucosa of normally fed and starved rats. Clin Nutr 10: 49–54
16. Monchi M, Rérat AA (1993) Comparison of net protein utilization of milk protein mild enzymatic hydrolysates and free amino acid mixtures with a close pattern in the rat. JPEN 17: 355–363
17. Saunders C, Nishikawa R, Wolfe B (1993) Surgical nutrition: a review. J R Coll Surg Edinb 38: 195–204
18. American Institute of Nutrition (1977) Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. J Nutr 107: 1340–1348
19. Boza JJ, Jiménez J, Martínez O, Suárez MD, Gil A (1994) Nutritional value and antigenicity of two milk protein hydrolysates in rats and guinea pigs. J Nutr 124: 1978–1986
20. Boza JJ, Moënnoz D, Vuichoud J, Jarret AR, Gaudard-de-Weck D, Fritsche R, Donnet A, Schiffrin EJ, Perruisseau G, Ballévre O (1999) Food restriction and refeeding influence on growth, nutrient retention and functional recovery of rats. J Nutr 129:1340–1346
21. Jeevanandam M, Holaday NJ, Petersen SR (1995) Altered brain and muscle amino-acid levels due to remote injury during glutamine supplementation. Clin Nutr 14: 365–372
22. Martin J, White INH (1991) Fluorometric determination of oxidised and reduced glutathione in cells and tissues by high-performance liquid chromatography following derivatization with dansyl chloride. J Chromatog 568: 219–225
23. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150: 76–85
24. Gorostiza E, Poullain MG, Marche C, Gobert JG, Broyart JP, Macry J, Cezard JP (1985) Effect of fasting and refeeding on small intestinal adaptation in the rat. Gastroeneterol Clin Biol 9: 790–796
25. Blommart PJE, Charles R, Meijer AJ, Lamers WH (1995) Changes in hepatic nitrogen balance in plasma concentration of amino acids and hormones and in cell volume after overnight fasting in perinatal and adult rats. Pediatr Res 38: 1018–1025
26. Heird WC (1998) Amino acids in pediatric and neonatal nutrition. *Curr Opin Clin Nutr Metab Care* 1:73–78
27. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrère B (1997) Slow and fast dietary proteins differently modulate pospandrial protein accretion. Proc Nat Acad Sci USA 94: 14930–14 935
28. Ferguson A (1994) Immunological functions of the gut in relation to nutritional state and mode of delivery of nutrients. *Gut* 35:S10-S12
29. Low AG (1990) Nutritional regulation of gastric secretion, digestion and emptying. Nutr Res Rev 3: 229–235
30. Wilmore DW, Black PR, Muhlbacher F (1983) Injured man: trauma and sepsis. In : Winters RW, Green HL (Eds) Nutritional support of the Seriously Ill Patient. Academic Press: New York, pp 33–52
31. Hall JC, Heel K, McCauley R (1996) Glutamine. Br J Surg 83: 305–312
32. Reeds PJ, Fjeld CR, Jahoor F (1994) Do the differences between the amino acid composition of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? J Nutr 124: 906–910
33. Roberton AM, Rabel B, Harding CA, Tasman-Jones C, Harris PJ, Lee SP (1991) Use of the ileal conduit as a model for studying human small intestinal mucus glycoprotein secretion. Am J Physiol 261: G728-G734
34. Fuller MF, Milne A, Harris CI, Reid TM, Keenan R (1994) Amino acid losses in ileostomy fluid on a protein-free diet. Am J Clin Nutr 59: 70–73
35. Grimble RF (1992) Dietary manipulation of the inflammatory response. Proc Nutr Soc 51: 285–294
36. Malmezat T, Breuillé D, Pouyet C, Mirand PP, Obled C (1998) Metabolism of cysteine is modified during the acute phase of sepsis in rats. J Nutr 128: 97–105
37. Breuillé D, Arnal M, Rambourdin F, Rosé F, Obled C (1996) Increased cysteine requirements induced by sepsis. Clin Nutr 15:S21
38. Breuillé D, Arnal M, Buffière C, Denis P, Pouyet C, Obled C (1997) Beneficial effect of cysteine supplementation in response to sepsis. Clin Nutr 16: S17
39. Fukagawa NK, Ajami AM, Young VR (1996) Plasma methionine and cysteine kinetics in response to an intravenous glutathione infusion in adult humans. Am J Physiol 270: E209-E214