Int. J. Vitam. Nutr. Res., 71 (2), 2001, 128–132

Dietary Effects of Marine Food Intake on Intestinal and Hepatic Enzyme Activities in Rats

Marta González, Belén Caride, Asunción Lamas and Cristina Taboada

Department of Physiology, Faculty of Pharmacy, University of Santiago de Compostela, 15706, Spain

Received for publication: July 14, 2000

Abstract: Dietary effects of two diets high in protein from two marine species *(Haliotis tuberculata* and *Anemonia viridis)* as compared to a high-quality patron protein such as casein (or casein supplemented with olive oil) on intestinal and hepatic enzymes were studied.

After 23 days, the two marine species as diet compared to casein increased the disaccharidase and alkaline phosphatase activities. Feeding *Haliotis tuberculata* meal produced a decrease on intestinal leucine aminopeptidase activity. The hepatic gamma-glutamyltranspeptidase activity decreased slightly in animals fed *Haliotis tuberculata* meal. Supplementation of casein with olive oil tended to decrease the intestinal and hepatic enzyme activity.

Key words: Protein, amino acid, casein, *Anemonia viridis, Haliotis tuberculata,* disaccharidases, alkaline phosphatase, leucine aminopeptidase, gamma-glutamyltranspeptidase

Introduction

Marine invertebrates constitute an important food source, supplying high nutritional value, omega-3 fatty acids and a variety of mineral salts [1]. The traditional sea resorts have undergone a change in the last years. Some new or unusual marine animal utilization processes, as protein concentrates, extracts and spread pastes productions are proposed as human food new sources from fish protein. This seafood is characterized by a high digestibility coefficient and biological response in growing rats, that were similar to the traditional sea species [2].

Several investigators have reported that intestinal disaccharidases are localized in the brush border of the intestinal mucosa [3] and might play an important role in terminal digestion and absorption of dietary nutrients [4]. Due to high protein demand from intestinal epithelium, the activities of intestinal enzymes can be altered by the amino acid composition of the dietary protein [5]. Kuusanmäki *et al* [6] pointed out that brush border enzymatic activity can be used to assess the functional capacity of small intestine, whereas Gudmandhoyer and Skovbjerg [7] asserted that variations in the activity of one or more enzymes can cause clinical disorders.

Anemonia viridis and *Haliotis tuberculata* are two marine invertebrates that are not commercially exploited at the present. In this work, we study their amino acid content comparing it to the amino acid content of casein, and the influence of these two sources of protein in the diet on intestinal and hepatic enzymes activity. Isoproteic diets were elaborated in all cases in order to avoid differences due to protein quantity.

Materials and Methods

Recently weared male Sprague-Dawley were maintained in individual metabolic cages in a room at constant temperature and humidity, with a 12:12 h light:dark cycle. The rats were divided into four groups that were fed with the corresponding diet and water *ad libitum* for 23 days.

Diet composition in percentage of dry matter is detailed in Table I. The casein meal was additionally supplemented with 0.6%. DL-methionine, since it is deficient in this amino acid. Dietary fat for the control group was made to 4% with olive oil. The fat content of *Haliotis tuberculata* was also determined and the difference up to 4% was completed with olive oil. Toxicity of *Anemonia viridis* polypeptides was eliminated by a heating procedure using olive oil for frying. After that, we determined the fat content by extraction in a Soxhlet before elaborating the diet and in this trial we used as comparison a control group fed with a casein diet supplemented with the same quantity of olive oil as the *Anemonia viridis* diet. Protein content of *Anemonia viridis* and *Haliotis tuberculata* was determined by the Kjeldahl method. The amino acid content of matter used as protein source was determined by HPLC/DAD.

Enzyme activities were measured in small intestinal mucosa and liver. The mucosa and liver samples were obtained by the method of Kessler *et al* [8]. 1 g of mucosa or 2.5 g of liver were homogenized in 10 ml of 2 mM TrisHCl buffer (pH = 7.1), containing 50 mM of mannitol. Homogenization was carried out using a Potter-Elvehjem (200–300 r.p.m., 10 stokes) with the teflon point at 4°C. Solid $CaCl_2$ was added to a final concentration of 10 mM. Centrifugation of the calcium-treated homogenates was carried out at 4500 r.p.m. for 10 min. The enzymatic activity was determined in the supernatant.

Disaccharidases and alkaline phosphatase activities were determined according to a modification by Miller [9] of Dahlqvist's [10] method. The results were expressed as specific activities (μmoles of substrate hydrolyzed per minute and per milligram of protein). Protein was determined by the method of Lowry *et al* [11] using crystalline bovine serum albumin as standard.

Leucine aminopeptidase activity was determined according to Goldberg and Ruttenberg [12] (β-naftilamide/mg protein/minute) and hepatic gamma-glutamyltranspeptidase activity was determined by Meister *et al* [13] method (p-nitroaniline/g protein/minute).

Data were subjected to one-way ANOVA, and differences between means were assessed by Dunnett's test and were considered statistically different at $p < 0.05$.

Table I: Composition of the feeds (% dry weight) supplied to the rats in each group

	Casein	Casein + olive oil	*H. tuberculata*	*A. viridis* fried
Protein	12	12	12	12
Cellulose	8	8	8	8
Olive oil	4	14.53	3.29	12.66
Marine oil	–	–	0.71	1.87
Mineral complex[1]	5	5	5	5
Vitamin complex[2]	5	5	5	5
Sucrose	33	33	33	33
Starch	33	33	33	33

In all diets vitamin A and D was 30 Ul/day and 10 Ul/day respectively.

[1] Provided (g/kg diet): 330 sodium chloride, 334 calcium phosphate dibasic, 219 potassium hydrogen carbonate, 72 di-potassium hydrogen phosphate, 38 magnesium carbonate, 1.18 manganese sulphate, 0.16 cupric sulphate, 0.08 aluminium-potassium sulphate, 0.08 calcium chloride, 0.04 potassium iodate, 0.08 sodium fluoride, 0.04 zinc carbonate and 9.30 Iron II sulphate.

[2] Provided (mg/kg diet): 1500 thiamine, 200 riboflavin, 2200 nicotinamide, 200 pyridoxol hydrochloride, 300 calcium pantothenate and until 1000 g with starch.

Results

The composition (%) of marine species are listed in Table II. Fresh *Haliotis tuberculata* has a higher protein content than fresh *Anemonia viridis* but slightly lower than fried *Anemonia viridis* protein content. The composition of the amino acids is shown in Table III. The marine species of the study have a higher content of glycine, arginine and cysteine than casein and a similar content of aspartic acid and alanine. For the rest of amino acids, *Anemonia viridis* and *Haliotis tuberculata* have lower contents than casein.

If we pay attention, the process of *Anemonia viridis* frying has reduced the amino acid content except for serine, histidine, proline methionine and cysteine.

The levels of activities of disaccharidases were higher in intestinal homogenates from *Anemonia viridis* and *Haliotis tuberculata*-containing diet fed rats than in casein fed rats. The results are present in Table IV. Olive oil caused a decrease on the disaccharidases activities in rats fed with casein that was significant for lactase when compared to rats fed casein alone. The maltase, sucrase and lactase activities in the intestinal homogenate of the animals fed casein with olive oil were found to be significantly lower than that in the *Anemonia viridis* group. The

Table II: Composition of marine species (%)

	A. viridis fresh	*A. viridis* fried	*H. tuberculata*
Water	83.82	58.00	72.48
Protein	11.12	27.81	26.61
Fats	1.87	14.53	0.71

Table III: Amino acid content of the protein sources (mg amino acid/g protein), after methionine supplementation

Amino acids	Casein	*H. tuberculata*	*A. viridis fried*	*A. viridis fresh*
Aspartic acid	27.52	29.95	25.98	35.82
Glutamic acid	122.98	64.67	54.12	73.50
Serine	29.00	18.18	18.24	23.35
Glycine	8.95	27.72	45.62	61.39
Histidine	32.08	12.17	18.61	24.37
Threonine	58.05	36.07	12.80	37.03
Alanine	20.56	29.62	22.08	31.38
Arginine	26.73	59.50	31.55	44.80
Proline	64.42	17.94	21.73	23.14
Tyrosine	46.47	32.92	15.40	28.76
Valine	67.22	26.94	11.76	32.30
Methionine	31.82	23.66	11.56	19.53
Cysteine	2.90	4.29	4.67	4.26
Isoleucine	53.98	31.32	9.92	25.92
Leucine	94.89	63.14	18.16	45.60
Phenylalanine	52.26	26.28	13.65	43.60
Lysine	62.88	36.48	43.79	58.74

Table IV: Disaccharidases activity in intestinal homogenates of different groups (µ-moles of liberated glucose/g of protein/minute)

	Casein	Casein + olive oil	*H. tuberculata*	*A. viridis* fried
Maltase	116.8 ± 24.1	95.0 ± 10.9[b]	127.9 ± 17.3	148.9 ± 20.7
Sucrase	24.4 ± 5.9	16.6 ± 5.8[b]	27.1 ± 8.0	39.2 ± 12.0
Lactase	21 ± 3.2	12.1 ± 2.5[a,b]	26.3 ± 6.1	31.8 ± 9.7

Values are means ± S.D.
[a] Significantly different from corresponding casein group.
[b] Significantly different from corresponding *Anemona viridis* group ($P < 0.05$).

alkaline phosphatase activity was similar in both groups that consumed casein. *Anemonia viridis* and *Haliotis tuberculata* meal showed a higher specific enzymatic activity of this enzyme.

The intestinal leucine aminopeptidase activity was lower when the rats were fed casein with olive oil or *Haliotis tuberculata* than when they were fed casein or *Anemonia viridis.* The hepatic gamma-glutamyltranspeptidase activity in rats fed casein with olive oil was significantly lower than when they were fed casein alone or *Anemonia viridis.* In the case of *Haliotis tuberculata* the activity of this enzyme decreased slightly.

Discussion

Haliotis tuberculata has a higher protein content than fresh *Anemonia viridis.* The friend Anemonia viridis protein content was high due to loss of water by a heating procedure using olive oil for frying. Fresh *Anemonia viridis* has a higher fat concentration than *Haliotis tuberculata.*

Haliotis tuberculata and fried *Anemonia viridis* are rich in arginine, glycine and cysteine, and they have a similar content in aspartic acid and alanine than casein. The heating procedure using olive oil for frying could be the cause of the loss of amino acid content but if we pay attention, the amino acid content of fresh *Anemonia viridis* is similar to *Haliotis tuberculata.* Fresh *Anemonia viridis* is rich in aspartic acid, serine, glycine, alanine, arginine and cysteine.

Enzymatic adaptations occur in intestinal mucosa. Some of these changes may be due to nutritional effects on the metabolism, growth and differentiation of intestinal epithelial cells. Others may be due to accompanying changes in pancreatic function or by the trophic action of gastrin, which is necessary for the maintenance of the structural integrity of the gastrointestinal tract. Korman *et al* [14] indicated that dietary protein and amino acids produce significant increases in serum gastrin levels, and dietary protein source affects pancreatic and jejunal trypsin and chymotrypsin activities [15].

Takase and Goda [16] showed that feeding diets containing long-chain triglycerides, causes reduction of jejunal sucrase activity. This phenomenon may relate to the increase of bile production induced by high-fat diets [17]. In this work, the animals fed the olive oil casein diet exhibited a reduced maltase, sucrase and lactase activities but the high-fat content caused no changes in alkaline phosphatase value. Alkaline phosphatase is known to be intimately associated with the hydrophobic core of the membrane. Such an intrinsic enzyme may have different sensitivities to changes in the membrane ultrastructure compared with the extrinsic enzymes sucrase, maltase and lactase. Goda and Takase [18] suggested that the dietary manipulation, even when the diets are complete in terms of the content of energy, protein, and micronutrients, can modify the structure of the microvilli of small intestinal epithelial cells, leading to alterations of the digestive-ab-

Table V: Enzymatic activity of intestinal and hepatic homogenates of different groups

	Casein	Casein + olive oil	*H. tuberculata*	*A. viridis* fried
Alkaline phosphatase	264.1 ± 40.1	262.2 ± 82.1	349.1 ± 59.0	349.1 ± 108.1
LAPase intestinal	78.3 ± 7.0	50.0 ± 9.8[a]	57.5 ± 6.8[a]	72.3 ± 9.9
GGTase Hepatic	556.4 ± 115.2	357.5 ± 58.5[a,b]	517.3 ± 98.2	545.4 ± 84.1

Values are means ± S.D. [a] Significantly different from corresponding casein group. [b] Significantly different from corresponding *Anemona viridis* group ($P < 0.05$).

sorptive surface area of villus cells which can accommodate microvillar glycoproteins. These changes in the structure of microvilli might be related to the rapid and profound alterations of activities of some microvillar membrane stalked disaccharidases induced by dietary manipulation of isoenergic diets.

However, the *Anemonia viridis* meal, in spite of containing the same fat quantity that casein supplemented with olive oil, increased the specific activities of the enzymes studied. So, fat level in the diet is not the only responsible factor of enzymatic alterations. An explanation may relate the enhanced disaccharidase activity to *Anemonia viridis* composition, so it contains a proteinase inhibitor for elastase [19]. Shiozaki *et al* [20] suggested that elastase was the most potent protease for releasing disaccharidases from the brush border.

In the microvillar membranes of small intestinal epithelial cells, various glycoproteins, including digestive enzymes and transporters are localized and exert critical roles in digestion and/or absorption of nutrients [21]. In general the different diets produced specific changes in the activities of various enzymes, mainly affecting alkaline phosphatase in the duodenum, disaccharidases in the jejunum and peptide hydrolases in the ileum. The activity of intestinal brush border hydrolases is adaptative to the dietary levels of carbohydrates and proteins [22] and quantity or quality of dietary protein may affect the structural proteins in the intestinal mucosa since the enzymatic activity is expressed in terms of units per gram of mucosal protein. The findings of Kimura *et al* [23] supported the idea that the segmental sucrase activity of rats was affected by quality and content of amino acids and protein in the diet. Intestinal maltase is also influenced by the quality and quantity of dietary protein [5].

On the other hand, the alkaline phosphatase activity did not show significant differences between the groups of animals, although the highest activity was in groups fed marine protein. An explanation may be the phospholipidic fat content of the studied species [24, 25]; so it is known that alkaline phosphatase may play an important physiological role in the metabolism of phospholipids and other dietary components [26]. Indeed, dietary levels of fat are also well known to affect alkaline phosphatase activity in intestine and serum. In addition, the presence of phenylalanine and leucine inhibits the enzyme activity [27] and the content of these amino acids is lower in both marine species.

The intestinal leucine aminopeptidase activity showed a significantly decrease when the rats were fed *Haliotis tuberculata* face to rats fed casein. Previous studies have shown that dietetic amino acids may modify the proliferating mucosa cells together with a direct effect on the villus cell intestinal formation [28]. Hayakawa and Lee [29] have shown that leucine is the substrate preferentially hydrolyzed by leucine aminopeptidase. Kimura *et al* [23] have reported that diets supplemented with methionine, threonine and glutamic acid increased the leucine aminopaptidase activity. Casein meal is rich in these amino acids. The leucine aminopeptidase activity decreased in the group fed casein supplemented with olive oil in similar form than disaccharidases activity. The high-fat diet can modulate the enzymatic activities in small intestinal microvilli of rats [30]. In addition, high-fat diets seem to be involved on the alterations of some luminal factors affecting degradation of microvillar enzymes i.e: proteinases and bile acids [31]. The increased leucine aminopeptidase activity in the group fed *Anemonia viridis* may be favoured by the presence of an elastase inhibitor in *Anemonia viridis* composition. This elastase inhibitor may have favoured the permanence of certain brush border enzymes on small intestine.

The gamma-glutamyltranspeptidase activity in the liver showed a significantly decrease when the rats were fed casein supplemented with olive oil compared to rats fed casein alone or *Anemonia viridis.* Several authors have reported that the mechanism of release of this enzyme from the liver into the plasma was partially attributed to bile salts [32] and also to the modification of phospholipid composition of plasma membranes [33]. Moreover, the gamma-glutamyltranspeptidase has been shown to be inhibited by bile acids [34]. Consequently, it is possible that fat excess can cause variations in this factors related with enzyme activity. In contrast, rats fed the *Anemonia viridis* meal showed higher activity of this enzyme, that may be due to the high content of glycine and cysteine, good substrates for this enzyme.

Acknowledgement

This work was supported by Grant XUGA, Xunta de Galicia, Spain.

References

1. Soriguer, F., Serna, S., Valverde, E., Hernando, J., Martin-Reyes, A., Soriguer, M., Pareja, A., Tinahones, F. and Esteva, I. (1997) Lipid, protein, and calorie content of different Atlantic and Mediterranean fish, shellfish, and molluscs commonly eaten in the south of Spain. Eur. J. Epidemiol. 13, 451–463.
2. Goodman-Lowe, G. D., Carpenter, J. R., Atrinson, S. and Ako, H. (1999) Nutrient, fatty acid, amino acid and mineral analysis of natural prey of the Hawaiian monk seal, Monachus schauinslandi. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 123, 137–146.

3. Van Beers, E. H. (1998) Pediatric duodenal biopsies: mucosal morphology and glycohydrolase expression do not change along the duodenum. J. Pediatr. Gastroenterol. Nutr. 26, 186–193.
4. Mizuma, T. and Awazu, S. (1998) Intestinal metabolism and transport of alpha-disaccharide conjugates: the role of disaccharidase in the Na^+/glucose cotransporter-mediated transport. Res. Commun. Mol. Pathol. Pharmacol. 100, 43–52.
5. Rebolledo, E., Andrés, D., González, A., Taboada, C., Lamas, M. and Fernandez, P. (1986) Effects of fish meal as source of protein on intestinal maltase activity and intestinal and renal leucine aminopeptidase activity on growing rats. Ann. Nutr. Metab. 30, 365–368.
6. Kuusanmäki, P., Lauronet, J., Paavonen, T., Pakarinen, M., Savilahti, E., Hayry, P. and Halttunen, J. (1996) Brush border enzymes in evaluation of acute small bowel rejection. Scand. J. Inmunol. 43, 566–573.
7. Gudman-Hoyer, E. and Skoubjerg, H. (1996) Dissacharidase digestion and maldigestion. Scand. J. Gastroenterol. Supl. 216, 111–121.
8. Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M. and Semenza, G. A. (1978) Modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport systems. Biochim. Biophys. Acta 506, 136–154.
9. Miller, D. L. (1981) Disaccharidase activities. Am. J. Nutr. 34, 1153–1170.
10. Dahlqvist, A. (1984) Assay of intestinal disaccharidases. Scand. J. Clin. Lab. Invest. 44, 169–172.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 67–75.
12. Goldberg, J. A. and Ruttenberg, A. M. (1958) The colorimetric determination of leucine aminopeptidase in urine and serum of normal subjects and patients with cancer and another diseases. Cancer 2, 283–291.
13. Meister, A., Tate, S. S. and Ross, L. L. (1976) Membrane-Bound γ-glutamyltranspeptidase. In: Enzymes of Biological Membranes (Martosoni, A. N., ed.) pp. 33–39, vol. 3, Plenum, New York.
14. Korman, M. G., Soveny, C. and Hansky, J. (1971) Effect of food on serum gastrin evaluated by radioimmunoassay. Gut. 12, 619–624.
15. Makkink, C. A., Negulescu, G. P., Qin, G. and Verstegen, M. W. (1994) Effect of dietary protein source on feed intake, growth, pancreatic enzyme activities and jejunal morphology in newly-weaned piglets. Br. J. Nutr. 72, 353–368.
16. Takase, S. and Goda, T. (1990) Effects of medium chain triglycerides on brush border membrane-bound enzyme activity in rat smaller intestine. J. Nutr. 120, 969–976.
17. Reddy, B. S. (1981) Dietary fat and its relationship to large bowel cancer. Cancer Res. 41, 3700–3705.
18. Goda, T. and Takase, S. (1994) Effect of dietary fat content on microvillus in rat jejunum. Nutr. Sci. Vitaminol. 40, 127–136.
19. Kolkenbrock, H. and Tschesche, H. (1987) A new inhibitor of elastase from the sea anemone *(Anemonia sulcata).* Biol. Chem. Hoppe. Seyleer. 368, 93–99.
20. Shiozaki, H., Yoshioka, M., Miura, S., Imaeda, H., Morita, A., Asakura, H., Tsuchiya, M. and Ishii, H. (1995) Conjugated bile salts regulate turnover of intestinal brush border membranes hydrolases. Dig. Dis. Sci. 40, 1193–1198.
21. Semenza, G. (1986) Anchoring and biosynthesis of stalked brush border membrane protein: Glycosidases and peptidases of enterocytes and renal tubuli. Ann. Rev. Cell. Biol. 2, 213–255.
22. Weaver, L. T., Desai, M., Austin, S., Arthur, H. M., Lucas, A. and Hales, C. N. (1998) Effects of protein restriction in early life on growth and function of the gastrointestinal tract of the rat. J. Pediatr. Gastroenterol. Nutr. 27, 553–559.
23. Kimura, T., Kato, T., Tsukasaki, K. and Yoshida, A. (1979) Effect of diet supplemented with amino acids on intestinal sucrase and leucine aminopeptidase activities in rats. J. Nutr. Sci. Vitaminol. 25, 195–204.
24. Dunstan, G. A. Baillie, H. J., Barrett, S. M. and Volkman, J. K. (1996) Effect of diet on de lipid composition of wild and cultured abalone. Aquaculture 140, 115–127.
25. Stefanov, K. L., Christie, W. W., Brechany, E. Y., Poppov, S. S. and Andreev, S. (1992) Lipid composition of the red and green forms of actinia equina from the black sea. Comp. Biochem. Physiol. 103B, 687–690.
26. McCarthy, D. M., Nicholson, J. A. and Kim, Y. S. (1980) Intestinal enzyme adaptation to normal diets of different composition. Am. J. Physiol. 239, 445–451.
27. Mozes, S., Lenhardt, L. and Martinkova, A. (1998) A quantitative histochemical study of alkaline phosphatase activity in isolate rat duodenal epithelial cells. Histochem. J. 30, 583–589.
28. Kimura, T., Seto, A. and Yoshida, A. (1978) Effect of diets on intestinal disaccharidase and leucine aminopeptidase activities in refed rats. J. Nutr. 108, 1087–1097.
29. Hayakawa, E. and Lee, V.H.L. (1992) Aminopeptidase activity in jejunal and ileal Peyer's patches of the albino rabbit. Pharma Research 9, 535–540.
30. Shinahara, H., Goda, T., Takase, S. and Sugawa-Katayama, Y. (1993) Feeding medium-chain triglycerides to rats decreases degradation of sucrase-isomaltase complex in jejunum. J. Nutr. 123, 1161–1167.
31. Tazi, A., Galteau, M. M. and Siets, G. (1980) Gamma-glutamyltransferase of rabbit liver: kinetic study of phenobarbital induction and *in vitro* solubilization by bili salts. Toxicol. Appl. Pharmacol. 55, 1–7.
32. Larichi, M., Ratanasavannh, D., Galteau, M. M. and Siets, G. (1982) Effect of chronic ethanol administration on gamma-glutamyltransferase activities in plasma and in hepatic plasma membranes of male and female rats. Enzyme 28, 251–257.
33. Abbot, W. A. and Meister, A. (1983) Modulation of gamma-glutamyltranspeptidase activity by bile acids. J. Biol. Chem. 258, 6193–6197.

Cristina Taboada

Department of Physiology
Faculty of Pharmacy
University of Santiago de Compostela
15706, Spain