

## Omega-3 fatty acids induce biochemical changes in the small bowel of rats before and after resection

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*In the present study, the effect of dietary fish oil, a highly unsaturated fat source, on the intestinal mucosa before and after proximal small bowel resection in rats was studied. Forty Wistar rats were fed defined diets containing fish oil (experimental group) or corn oil (control group). After 2 weeks, animals underwent a 50% proximal small bowel resection. Mucosal disaccharidases, alkaline phosphatase, aminopeptidase, protein, DNA, and TBARs levels were assessed in samples immediately before and 6 and 12 days after surgery. Disaccharidase activities were reduced in the ileal mucosa from rats in the fish oil-fed group compared to the control group before and 12 days after surgery. The other parameters showed no significant differences. The results indicate that fish oil (omega-3 fatty acids) alters the functional parameters of the normal intestinal mucosa and after intestinal resection compared to omega-6 polyunsaturated fatty acids, suggesting that caution should be taken to use omega-3 fatty acids as adjuvants in the adaptive process after resection.*

### Uniterms

- Omega-3 PUFA
- Intestinal resection
- Intestinal adaptation

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## INTRODUCTION

In western countries, individuals consume 100-200 g fat per day, which corresponds to 35-45% of the dietary energy. Animal fat makes up 60-80% of the daily intake. The most important lipids in the human diet are triacylglycerols (TAGs). There is a wide variety of types of TAG-fatty acids consumed in the diet. The most abundant fatty acids consist of even number straight chains ranging from 4-carbon fatty acids in milk to 30-carbon fatty acids in some fish oils (Gunstone, Harwood, Padley, 1994).

The effects of composition and amount of fatty acids ingested by organisms have been studied for a long time. However, this interest has intensified in the last decades due to the demonstration that fatty acids play a very important role in the development of many diseases including cardiovascular disease and cancer (Landmark, 1998; Hubbard, Lim, Reickson, 1998). In almost all models studied the animals treated with omega-6 polyunsaturated fatty acids showed a high incidence of tumors of increased size (Hillyard, Abraham, 1979; Connolly, Gilhooly, Rose, 1999), and of tumorigenesis (Wynder, 1987). In contrast,

omega-3 fatty acids have opposite effects, i.e., they can inhibit cell division, cause cell cycle arrest, and induce cell death in malignant cells of various tissue origins *in vitro* (Mollerup, Haugen, 1996). In human beings, it was demonstrated that esters of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) could reduce sporadic colonic adenomas (Anti *et al.*, 1994). However, little is known about the effects of long-chain polyunsaturated fatty acids on normal epithelial cells.

The normal intestinal epithelium has a high cell turnover and extensive small bowel resection promotes structural, kinetic, and functional changes in the remaining intestine to allow the animal to adapt to the shortened intestine (Williamson, 1978). Increase in mucosal mass reaches a peak 7 to 12 days after intestinal resection (Hanson, Osborne, Sharp, 1977), and this compensatory response is more marked in the ileum than in the jejunum (Williamson, 1978). For this reason, in the present investigation we chose a period of 6 and 12 days after enterectomy to conduct our studies. A 50% proximal resection was performed since it has been documented that the maximal increase in villus height is obtained after resection of 45% of the initial intestinal length and that after more extensive resection no further increment occurs (Menge, Robinson, 1978).

Thus, considering the high cellular turnover of the small bowel and its resection as a hyperproliferative physiological model, the present study was conducted to evaluate the effect of a fish oil concentrate (a source of EPA and DHA) in some intestinal mucosal parameters, such as specific enzymatic activities of disaccharidases (sucrase, lactase and maltase) and alkaline phosphatase, and DNA and TBARs (thiobarbituric acid reactive substances) levels before and after 50% small bowel resection in normal rats receiving corn oil or fish oil for 15 days, and on the adaptation of the remnant intestinal epithelium after 50% proximal small bowel resection.

## MATERIAL AND METHODS

The animals used in this study were maintained in accordance with the norms of the National Research Council (1996).

### Experimental design

We used 52 male Wistar rats weighing 150-180 g obtained from the animal house of Ribeirão Preto Campus, University of São Paulo. The rats were allowed to adapt themselves to the local animal house conditions for 3 days

before the experiment. They were housed individually in stainless steel wire-bottomed cages, and received a standard rat chow diet and water *ad libitum* during the acclimatization period, in a room with a 12 h light/dark cycle, at  $25\pm 1$  °C. The rats were randomly divided into two dietary groups and fed a modified AIN-93 chemically defined diet (Reeves, Nielsen, Fahey, 1993) containing 10% (w/w) corn oil (control diet) or a fish oil concentrate (experimental diet). The latter was prepared to provide the required levels of essential fatty acids to the animals and contained 75% fish oil (equivalent to 16.8% EPA, and 6.3% DHA) plus 25% corn oil. Both diets were isonitrogen (10% casein, w/w) and isocaloric. Rats were fed with these diets for 2 weeks. The composition of oils used in our study is presented in Table I.

Six (6) rats from each group (corn oil and fish oil) were sacrificed under ether anesthesia, blood was collected by cardiac puncture and the serum was used for the determination of cholesterol and triglycerides.

The remaining 40 rats underwent a 50% resection of the proximal small bowel under ether anesthesia; 25% of the proximal bowel starting from the ligament of Treitz, and 25% of the distal bowel starting from the ileocecal valve were maintained. The intestine removed in the surgery was divided into two fragments named proximal jejunum and distal ileum. The fragments were carefully washed with cold 0.9% (w/v) NaCl, dried out with paper towels, and then opened longitudinally on a cold glass plate. The mucosa was scraped off with the help of glass slides, and frozen in liquid nitrogen. After surgery, the animals were given 5% (w/v) glucose in 0.9% (w/v) NaCl for the first 24 hours postoperatively, and then placed back on their respective study diets. Throughout the preoperative and postoperative period, food intake of both groups was measured daily and their weight gain weekly. The diets were previously prepared and stored in a freezer in tightly sealed plastic bags. The fish oil was added every day to prevent oxidation. The animals received fresh food every day.

The animals were killed 6 and 12 days after resection under superficial ether anesthesia. Blood was drawn by cardiac puncture at the time of sacrifice, and serum was obtained for the determination of triglycerides and cholesterol levels. Fragments of remnant jejunum and ileum were removed, and their mucosae were harvested as described above.

### Biochemical analysis

Mucosal scrapings were homogenized in 2 mM Tris-Hepes buffer, pH 7.0, containing 100 mM mannitol. The mucosal protein was determined by the method of

**TABLE I** - Composition in fatty acids of oils used in diets. The data are presented as percentage.

Formula	Fatty acid	Sample	
		Corn oil	Fish oil
08:0	Caprylic acid	-	-
10:0	Capric acid	-	-
12:0	Lauric acid	-	-
14:0	Myristic acid	-	7.72±0.09
15:0	Pentadecanoic acid	-	Traces
16:0	Palmitic acid	13.55±0.16	16.16±0.69
16:1	Palmitoleic acid	0.13±0.01	10.55±1.56
17:0	Heptadecanoic acid	-	1.25±0.00
18:0	Stearic acid	1.96±0.03	2.65±0.04
18:1	Oleic acid	34.05±0.19	17.03±1.08
18:2	Linoleic acid	48.83±0.08	1.40±0.01
18:3	Linolenic acid	0.78±0.01	Traces
18:4	Octadecatetraenoic acid	-	1.96±0.04
20:0	Arachidic acid	0.52±0.01	-
20:1	Eicosanoic acid	0.21±0.03	0.80±0.01
20:4	Eicosatetraenoic acid	-	1.50±0.14
20:5	Eicosapentaenoic acid	-	22.35±0.30
22:0	Behenic acid	-	-
22:1	Erucic acid	-	-
22:5	Docosapentaenoic acid	-	2.57±0.11
22:6	Docosahexaenoic acid	-	8.40±0.18
Ni	Not identified	-	5.70±0.57
	Saturated	16.03	27.78
	Unsaturated	34.39	28.38
	Polyunsaturated	49.61	38.18

Fish oil: 22.35 g eicosapentaenoic acid + 8.40 g docosahexaenoic acid = 30.75 g omega-3 fatty acids/100g

Bensadoun and Weinsten (1976) and expressed as mg protein/g wet tissue. Mucosal DNA was extracted by serial precipitation with cold perchloric acid and assayed according to Burton (1956) as modified by Croft and Lubran (1965), using *E. coli* DNA as standard. DNA was expressed as µg/mg protein. Mucosal disaccharidase activity was determined using sucrose, maltose and lactose as substrates by the method of Dahlqvist (1964), and expressed as nmol glucose liberated/mg protein/min. Mucosal alkaline phosphatase activity was determined using *p*-nitrophenylphosphate as substrate by the method of Murer *et al.* (1976), and expressed as nmol *p*-nitrophenyl liberated/mg protein/min. Leucine aminopeptidase activity was determined using L-leucyl-beta-naphthylamide as substrate; the color was developed as described by Goldbarg, Pineda, Rutemberg (1959), and enzymatic activity was expressed as µmol beta-naphthylamine liberated/mg protein/min. TBARs were determined by the method of Buege and Aust (1978), and

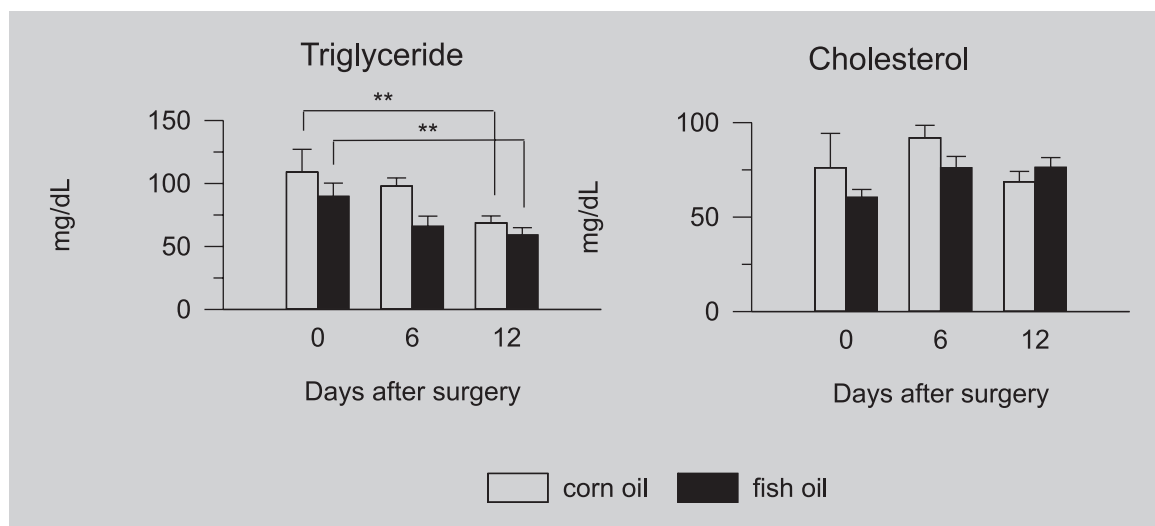
expressed as nmol TBARs/mg protein. Cholesterol and triglyceride levels were determined by an enzymatic method using a commercial kit from Labtest (São Paulo, Brazil).

### Statistical analysis

All data were reported as arithmetic mean ± SEM and analysed by analysis of variance with the level of significance set at 5%. The significant differences among means were further analysed by the Duncan test.

### RESULTS

There were no differences in diet ingestion or body weight between the two dietary groups during preoperative and postoperative periods (data not shown). Triglyceride levels were reduced but not significantly so in the fish oil-fed group and cholesterol levels presented any differences among groups (Figure 1).



**FIGURE 1** - Serum triglyceride and cholesterol levels in animals before and after 50% proximal bowel resection. \* corresponds to a significant difference between diets; \*\* correspond to a significant difference compared to day 0, in same diet. (n = 6 rats for 0 day end 8-12 rats for each period).

Enzyme specific activities in the ileal mucosa are shown in Figure 2 and protein, DNA, and TBARS levels in Figure 3. After 15 day pre-treatment with the two diets, the jejunal mucosa from animals fed corn oil or fish oil showed no differences between diets in enzyme specific activities or protein, DNA and TBARS levels (data not shown). In the ileum, mucosal disaccharidase (sucrase, lactase and maltase – the latter two activities were not shown in the figure, because they presented the same profile as sucrase) specific activities were reduced significantly in the animals fed fish oil (indicated by \* in the figure) and alkaline phosphatase and aminopeptidase specific activities did not show any differences between corn oil or fish oil based diets (Figure 2).

In the remnant ileum, the differences in disaccharidase specific activities observed during the preoperative period in animals fed fish oil were maintained after resection. The alkaline phosphatase specific activity of animals fed corn oil was significantly reduced on the 6<sup>th</sup> postoperative day and increased on the 12<sup>th</sup> postoperative day (indicated by \*\* in the figure). Animals fed fish oil showed no alterations in ileal mucosal alkaline phosphatase specific activity and on the 12<sup>th</sup> postoperative day differences among diets were significant (Figure 2, indicated by \*).

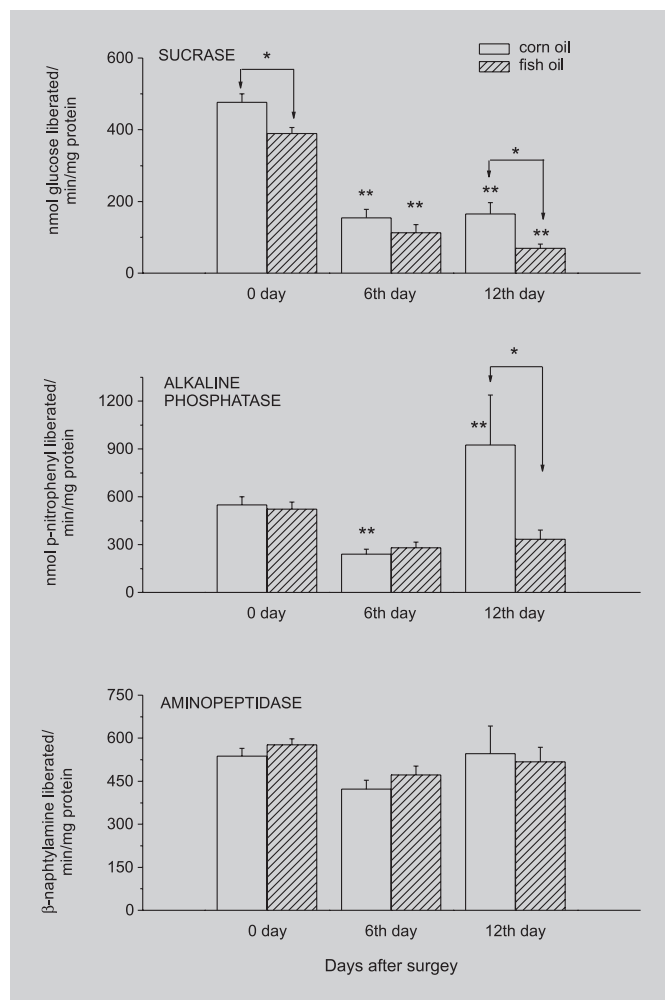
There were no differences between groups or experimental periods in aminopeptidase specific activity (Figure 2) and protein, TBARS or DNA levels (Figure 3).

## DISCUSSION

It has been described that rats treated with fish oil

tend to spill food (Halminski, Marsh, Harrison, 1991). This was not observed in the present study, and no differences were detected in average weight gain between the two dietary groups during preoperative or postoperative periods. The triglyceride levels were reduced but not so significantly in the fish oil-fed group, in agreement with the results reported by Rabbani *et al.* (2001). Cholesterol levels presented no differences among groups, in contrast to the data reported by Rabbani *et al.* (2001), who observed reduced cholesterol levels in rats treated with high doses of fish oil (5 ml of fish oil/kg/day). This difference could probably be attributed to the lower amount of fish oil ingested by the rats in our study.

In the present study lactase, maltase and sucrase specific activities measured in ileal mucosa homogenates were reduced in the group that received fish oil for 15 days before enterectomy. Our results were similar to those reported by Kaur *et al.* (1996), who demonstrated a reduction of leucine aminopeptidase and sucrase activity in the ileum from rats fed fish oil for 5 weeks. However, Vanderhoof *et al.* (1994), after feeding normal rats with a chemically defined diet containing 15% menhaden oil for 4 weeks, did not observe these effects. Also, the authors did not report the composition of the menhaden oil used in their study. Although we have used a lower calorie content in fat than Vanderhoof *et al.* (1994), 23% vs. 30% energy as fat, respectively, the fish oil used in our study was enriched with EPA and DHA. Thus, our results could be probably attributed to high different relative proportions of EPA and DHA in fish oil used in our experiments. Additionally, we used the same proportion of fish oil as used

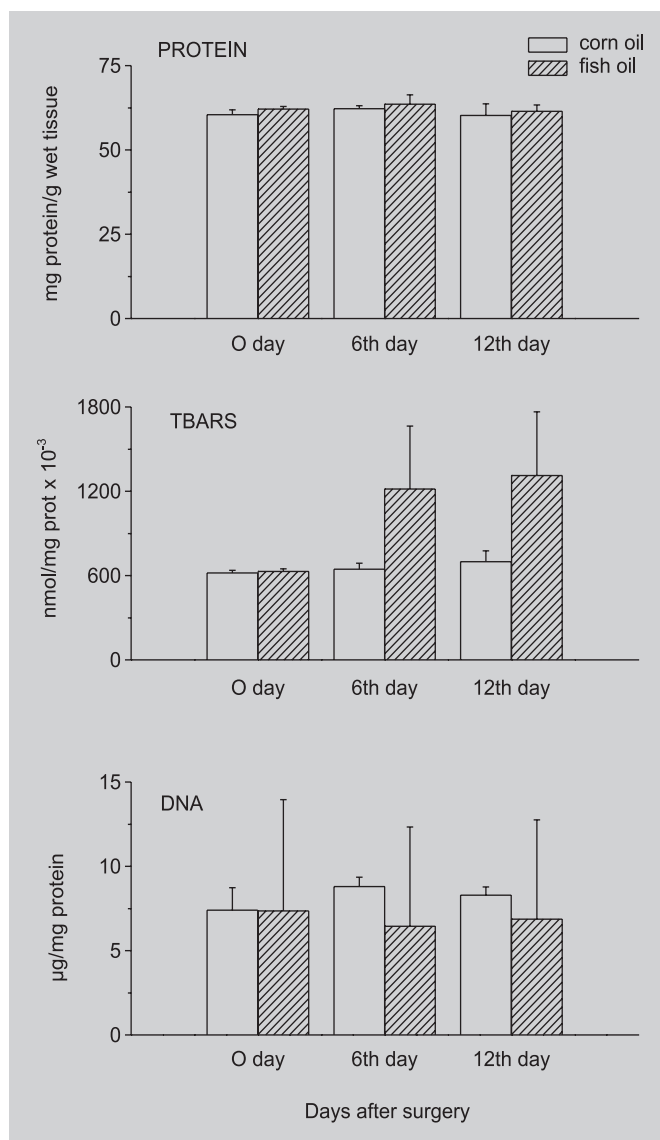


**FIGURE 2** - Effect of dietary fish oil on mucosal disaccharidase, alkaline phosphatase, and aminopeptidase specific activities before and after 50% proximal bowel resection. \* corresponds to a significant difference between diets; \*\* correspond to a significant difference compared to day 0, in same diet. (n = 20 rats for 0 day; 8-12 rats for 6<sup>th</sup> and 12<sup>th</sup> days).

by Deschner *et al.* (1990), who observed prevention of the development of an adenoma-type proliferative pattern in chemically induced cancer in rats fed fish oil.

The reduction of disaccharidase specific activity in normal ileal mucosa and no effect in normal jejunal mucosa in the group fed fish oil could be due to a higher absorption of fatty acids in the ileum than in the jejunum (Chen *et al.*, 1995).

The levels of sucrase activity are considered as a marker of enterocyte maturation (Ferretti *et al.*, 1996). The difference in ileal disaccharidase activity between two fatty acid groups was observed before and on the 12<sup>th</sup> day post operation. Thus, these differences may have not been



**FIGURE 3** - Effect of dietary fish oil on mucosal protein, DNA and TBARS levels before and after 50% proximal bowel resection.

due only to resection since we observed no differences in protein or DNA levels. The reduction observed in our study could be interpreted as a specific adaptive response to the effect of dietary fish oil on growth rate, probably timing the growth curve of enterocyte differentiation (Smith, 1985). If dietary fish oil alter some aspects of enterocyte migration and/or differentiation from crypt to villus tip, the disaccharidases will be more affected than alkaline phosphatase, because these enzymes become well expressed soon after movement of the cell from crypt to villus, and alkaline phosphatase (Albert, Young, 1992) and aminopeptidase (King *et al.*, 1981) are specifically expressed by cells at the villus tip. Thus, while cells were

able to express a normal level of alkaline phosphatase or aminopeptidase in both groups (corn oil and fish oil), they were unable to express disaccharidases fully in the fish oil-fed group because of the reduction of differentiation process promoted by fish oil. It is possible that over a long period of time the full enterocyte differentiation process could be attained in the fish oil-fed group. However, cellular differentiation is a very complex process and should not be analyzed only on the basis of reduction in disaccharidase activity.

In our study, alkaline phosphatase specific activity was increased only in the control group (corn oil) 12 days after resection. It has been reported that the interactions of protein and lipids of the intestinal microvillus membrane can influence protein function. Enzymes such as lactase, maltase, sucrase, leucine-aminopeptidase and gamma-glutamyl transpeptidase do not appear to experience functionally the effects of lipid composition. Instead, alkaline phosphatase is an integral enzyme, intimately associated with the hydrophobic core of the intestinal microvillus membrane (Brasitus *et al.*, 1979, 1988) and can be affected by the lipid environment. Thus, it is reasonable to suggest that the reduced expression of this enzyme in the group fed fish oil observed in our study may have been caused in part by alterations of the membrane lipid composition. Additionally, Wahnnon, Coogan, Mokady (1992) described a reduction of alkaline phosphatase in isolated intestinal brush border vesicles from rats fed 15% fish oil, with no effect on the lipid fluidity of intestinal microvilli.

The differential response of enzymes (reduction of sucrase and no effect on alkaline phosphatase) in normal and hyperplastic and hypertrophic ileal mucosa to dietary fish oil observed in the present study suggests a reduction on the process of enterocyte differentiation process and on the intestinal adaptation to resection. This finding suggests that caution should be taken in using polyunsaturated fatty acids, especially omega-3 fatty acids, as therapeutical adjuvants in the treatment of intestinal resection.

## RESUMO

### Efeito do ácido graxo ômega-3 no intestino delgado de ratos antes e após ressecção

*No presente estudo, foi avaliado o efeito da dieta com óleo de peixe, uma fonte de ácidos graxos altamente insaturados, sobre a mucosa intestinal antes e após ressecção do intestino delgado proximal em ratos. Quarenta ratos Wistar foram alimentados com dieta definida contendo óleo de peixe (grupo experimental) ou óleo de milho (grupo controle). Após duas semanas, os*

*animais foram submetidos à ressecção de 50% do intestino delgado proximal. Os níveis das dissacaridases, fosfatase alcalina, aminopeptidase, proteína, DNA e TBARS foram avaliados, imediatamente antes e 6 e 12 dias após a cirurgia. As atividades das dissacaridases foram reduzidas na mucosa ileal no grupo de ratos alimentados com óleo de peixe, comparado com grupo controle antes e 12 dias após a cirurgia. Os outros parâmetros não mostraram diferenças significativas. Este resultado indica que o óleo de peixe (ácidos graxos ômega-3) altera parâmetros funcionais na mucosa intestinal normal e após ressecção, comparado ao ácido graxo ômega-6, sugerindo cautela no uso de ácidos graxos ômega-3 como adjuvante no processo adaptativo após ressecção.*

*UNITERMOS: Ácidos graxos poliinsaturados ômega-3. Ressecção intestinal. Adaptação intestinal.*

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