PAPERS

Influence of dietary fat on duodenal resistance to acid

A Lugea, A Salas, F Guarner, J-R Malagelada

Abstract

Three experimental diets were prepared from a standard formula plus pure oleic, linoleic, or eicosapentaenoic acid (2% by weight). Mucosal resistance to acid was tested in anaesthetised rats fed the experimental diets for at least four weeks (60 rats per diet) by duodenal infusion of HCl (200 to 700 μ mol) 30 minutes after pretreatment with either saline or 100 μ mol HCl (used as a mild irritant). Rats were killed one hour after the test and the duodenal damage was assessed 'blindly' using a combined macroscopic and histological score. Differences were tested by analysis of covariance of the dose-response curves. Mucosal resistance was similar in the three groups when the acid challenge was given after saline pretreatment. Resistance to acid in all three groups was significantly increased by previous exposure to 100 μ mol HCl ($p < 0.01$). Interestingly, rats fed a linoleic or eicosapentaenoic supplemented diet after pretreatment with HCl developed significantly higher resistance to acid than those fed the diet with oleic acid ($p < 0.05$). Pretreatment with indomethacin suppressed the difference between diets. In conclusion, dietary polyunsaturated fatty acids enhance duodenal resistance to acid by potentiation of adaptive cytoprotection.

(Gut 1993; 34: 1303-1309)

The duodenal mucosa is normally challenged by intermittent exposure to acid, because of the periodic emptying of the gastric content into the duodenal lumen.¹ During fasting, the intraduodenal pH fluctuates widely within a range of 1 to 7.² These fluctuations probably result from interaction between phasic gastroduodenal motility³ and intermittent pancreatic secretion of neutralising bicarbonate. In previous studies in the rat,⁴ we reproduced the intermittent duodenal acid exposure that occurs physiologically, and observed that pulses of acid entering the duodenum trigger a response of adaptive cytoprotection by the duodenal mucosa and stimulate the release of cytoprotective prostaglandins into the duodenal lumen. Hence, we postulated that adaptive cytoprotection maintains a physiological equilibrium between duodenal mucosal resistance and luminal acidity.

In experimental models of gastric damage, there is evidence that the ability of the gastric mucosa to synthesise prostaglandins plays an important part in mediating adaptive cytoprotection.⁵ Other investigators claim that endogenous

prostaglandins may not be essential since gastric cytoprotection persists after pretreatment with prostaglandin synthesis inhibitors.⁶⁻⁸ In our model of duodenal adaptive cytoprotection, a normal mucosal capacity to synthesise prostaglandins seems to be a necessary requirement, since pretreatment with indomethacin abolishes the protective response and, conversely, administration of exogenous prostaglandins restores the protection.⁴ Mucosal prostaglandin biosynthesis can be influenced by the chronic intake of precursor fatty acids such as linoleic acid or eicosapentaenoic acid. Oral administration of linoleic acid, a polyunsaturated omega-6 fatty acid, leads to noticeable increases in the release of prostaglandin E₂ into the gastric lumen in rats^{9,10} and humans.¹¹ On the other hand, a fish oil supplemented diet, rich in the omega-3 polyunsaturated fatty acid eicosapentaenoic acid, was shown to reduce the mucosal generation of prostaglandin E₂ while enhancing that of prostaglandin E₃.¹² Thus, the dietary intake of precursor fatty acids can directly influence the rate and pattern of prostaglandin generation by the gastrointestinal mucosa.

This study aimed to investigate whether the resistance to acid of the duodenal mucosa can be modulated by the dietary content of fatty acids. To this end, three experimental diets were prepared from a standard rodent diet supplemented with pure oleic, linoleic, or eicosapentaenoic acid. Mucosal resistance to acid was tested in anaesthetised rats by duodenal infusion of graded doses of HCl using a tube implanted in the proximal duodenum. In the first series of experiments, acid was administered into the duodenal lumen after pretreatment with saline to test the resistance to acid applied directly to the mucosa. A second series of experiments tested the mucosal resistance to acid after pretreatment with a mild dose of acid to induce adaptive cytoprotection. Finally, we tested the effects of indomethacin on diet induced protective changes.

Methods

ANIMALS AND DIETS

Male Sprague-Dawley rats weighing 110 to 120 g were purchased from Interfauna (Barcelona, Spain). The animals were maintained in a restricted access room with controlled temperature (23°C) and a light-dark cycle (12 hours: 12 hours), and they were housed in rack-mounted cages, each containing a maximum of six rats.

Digestive System
Research Unit and
Department of
Pathology, Hospital
General Vall d'Hebron,
Autonomous University
of Barcelona, Barcelona,
Spain

A Lugea
A Salas
F Guarner
J-R Malagelada

Correspondence to:
Dr F Guarner, Digestive
System Research Unit,
Hospital General Vall
d'Hebron, Barcelona 08035,
Spain.

Accepted for publication
26 January 1993

TABLE I Fatty acid composition of the experimental diets

	Oleic acid diet (%)	Linoleic acid diet (%)	Eicosapentaenoic acid diet (%)
Linoleic acid	17*	43	17
Eicosapentaenoic acid	—	—	26
Oleic acid	69	43	43
Other saturated fatty acids	13	13	13

*Values represent the percentage of total fat content, which was 77.6 g/kg in all three diets.

The diets differed substantially in their fat composition. The oleic acid and eicosapentaenoic acid diets were equal in linoleic acid content but differed from the linoleic diet. Oleic and linoleic acid diets were equal in eicosapentaenoic acid content but differed from the eicosapentaenoic acid diet. Linoleic and eicosapentaenoic acid diets were equal in the ratio polyunsaturated/saturated fatty acids but differed from the oleic diet.

The experimental diets were prepared from a standard formula that included the following semisynthetic components (wt/wt): 20% casein, 1% methionine, 32% corn starch, 15% sucrose, 5% methyl-cellulose, 12% fat emulsion (Calogen, Scientific Hospital Supplies, Liverpool, UK; consisting of 50% water, 11% linoleic acid, 28% oleic acid, and 9% saturated fatty acids), 3% balanced salt mixture, 0.1% α -tocopherol (Roig Farma, Barcelona, Spain), and a 10% supplement including vitamins, minerals, and dextrin (Seravit, Scientific Hospital Supplies). The basic diet was supplemented with 2% by weight of either oleic acid (18:1 omega 9; 95% purity, Roig Farma), linoleic acid (18:2 omega 6; 95% purity, Sigma, St Louis, Mo, USA) or eicosapentaenoic acid (20:5 omega 3; 96% purity, Nissui Pharmaceutical, Yuki, Ibaragi, Japan), to obtain the oleic, linoleic, or eicosapentaenoic acid diets respectively. Table I shows the final fatty acid composition of the experimental diets. The mixture was thoroughly blended with 150 ml/kg of a 2% solution of polyvinylpyrrolidone (Roig Farma) in ethanol:water (50:50) used as compactant, and dried out at 40°C into biscuits. Batches were prepared twice a week and stored at 4°C for a maximum of four days.

PROTOCOLS

One hundred and eighty rats were included in this study. They were randomly assigned to receive either the oleic, linoleic, or eicosapentaenoic acid supplemented diet and were continued on the same diet for at least four weeks before entering the experimental protocols. Body weight gain was checked every week.

The study included four protocols as follows:

Protocol 1 investigated the influence of the experimental diets on the resistance of the duodenal mucosa to acid as assessed by direct instillation of acid into the duodenal lumen.

Protocol 2 studied the phenomenon of adaptive cytoprotection in response to mild irritation of the duodenal mucosa in the three experimental groups.

Protocol 3 studied the effects of indomethacin pretreatment on adaptive cytoprotection in the three experimental groups.

Protocol 4 quantified the *in vivo* intraluminal release of prostaglandin E₂ (PGE₂) in response to duodenal perfusion with acid.

Protocol 1: effect of diet on duodenal resistance to acid

After an overnight fast in cages designed to avoid coprophagia, rats were anaesthetised (urethane, 1.25 g/kg given intraperitoneally, Fluka Chemie, Buchs, Switzerland) and a tracheostomy was performed. The abdomen of each rat was incised and the stomach and duodenum were exposed. A double lumen tube, prepared from polyvinyl chloride tubing (ID: 1.40, OD: 1.90 mm; Abbot, Sligo, Eire), was inserted through the forestomach into the gastric lumen and passed through the pylorus. The tube was held in place by ligatures around the entry point (forestomach) and the pylorus. One of the lumens was used to drain the gastric contents by siphonage. The second lumen opened just beyond the pylorus and was used for pulse instillations of normal saline or acid into the duodenal lumen. Finally, the abdominal wall was sutured.

Rats received a 1 ml saline bolus through the duodenal line every 30 minutes for two hours to achieve full equilibration and to avoid any effects of laparotomy.¹³ Thereafter, an additional 1 ml pulse of hydrochloric acid at a dose of 200, 300, 400, 500, or 600 μ mol was instilled, each dose level was given to four rats from each dietary group. Rats were killed by cervical dislocation under anaesthesia one hour after the instillation of acid. The stomach and duodenum were removed and immediately fixed by intraluminal perfusion of a neutral buffered 10% formalin solution for 30 minutes via the double lumen tube used in the experiments. Thereafter, the specimens were carefully opened along the greater curvature, laid on and pinned out on a flat surface. The mucosa was examined and lesions scored macroscopically according to the presence of petechiae or necrotic mucosa (Table II). The length of the damaged area was measured. Longitudinal sections along the duodenum were stained with haematoxylin and eosin for histological examination. Macro- and microscopic evaluation and scoring of the lesions were performed by a pathologist who was unaware of the treatment administered to each rat. A global score of duodenal damage was obtained by

TABLE II Criteria for assessment of duodenal damage

	Macroscopic score	
Mucosal appearance:	Normal	0
	Petechiae	1
	Necrotic	2
Length of damaged area:	0 cm along the duodenum	0
	<3 cm	1
	3–7 cm	2
	>7 cm	3
Maximum score		6
	Histological score	
Depth of mucosal necrosis:	None	0
	<33% of the villi	1
	33%–66%	2
	>66%	3
Thrombotic phenomena:*	Absent	1
	Rare	2
	Frequent	3
Maximum score		9

*Refers to the presence of vessels occluded by fibrin thrombi. The macroscopic score was obtained by the product of mucosal appearance times length of damaged area scores. Likewise, the histological score was the product of depth of necrosis times thrombotic phenomena scores. A global score was obtained by summation of macroscopic and histologic scores (0 to 15).

summation of macroscopic (mucosal appearance, length of damaged area) and histological (depth of mucosal necrosis, vascular thrombosis) findings (Table II).

Protocol 2: effect of diet on adaptive cytoprotection

Matched sets of rats from each dietary group were prepared according to the surgical procedure described in the previous protocol. This protocol tested mucosal resistance to acid after mild irritation. The rats received a 1 ml saline bolus through the duodenal line every 30 minutes for two hours to achieve full equilibration. Thereafter, two additional 1 ml pulses were instilled: a 100 μ mol HCl bolus, as a mild irritant, and a pulse of 300, 400, 500, 600, or 700 μ mol HCl after a period of 30 minutes. Each dose level of HCl was given to four rats from each dietary group. A bolus of 100 μ mol HCl was used as mild acid irritant since this amount and concentration of acid is comparable with the average gastric acid secretion of the conscious male Sprague-Dawley rat.¹⁴ The rats were killed 60 minutes after the last pulse and the duodena were removed to determine the macro- and microscopic scoring of the mucosal lesions as discussed above.

Protocol 3: effects of indomethacin

To study the effect of cyclo-oxygenase inhibition by indomethacin on the adaptive response induced by mild acid irritation, rats were surgically prepared as above. They received saline pulses through the duodenal line for two hours, and thereafter a 100 μ mol HCl pulse followed by 200, 300, 400, or 500 μ mol HCl 30 minutes later. The protocol was performed in 16 rats from each dietary group that received indomethacin (5 mg/kg subcutaneously) 30 minutes before the 100 μ mol HCl pulse. Sixty minutes after the last pulse, the rats were killed and their duodena subjected to evaluation of the mucosal lesions as detailed above.

Protocol 4: duodenal release of prostaglandin E₂

Four rats from each dietary group were included in this study. After an overnight fast, rats were anaesthetised (urethane, 1.25 mg/kg intraperitoneally) and a tracheostomy was performed. The abdomen of each rat was incised and a perfused duodenal loop was prepared using a double lumen polyvinyl chloride tube (ID: 1.40, OD: 1.90 mm). The first lumen was used for drainage of gastric contents, and the second, which opened just beyond the pylorus, was used for duodenal perfusion. The intestinal wall was

transected 3 cm distally to the pylorus and ligated to a glass cannula for collection of the perfusate. Pancreatic and bile ducts were ligated, and the abdominal wall was sutured.

Isotonic saline heated at 37°C was continuously perfused at a rate of 8 ml/h into the duodenal loop, and the perfusate was collected in 15 minute fractions. After a 90 minute baseline period, we instilled a 100 μ mol HCl pulse (1 ml volume) through the duodenal line and the perfusion was stopped. After a two minute exposure to acid, the perfusion was resumed for a further 90 minute period. Samples collected during the first 10 minutes after the acid pulse were discarded.

The prostaglandin E₂ concentration in the perfusates was measured by specific radioimmunoassay (RIA).¹² Tritiated standard was purchased from Amersham International (Buckinghamshire, UK). Antiserum was kindly donated by Dr J A Salmon (Wellcome Research Laboratories, Beckenham, UK). Perfusate samples were assayed for PGE₂ without prior extraction and HPLC-purification. Direct PGE₂ RIA has a sensitivity of 15 pg/ml. Prostaglandin E₃ can be measured by a combined HPLC-RIA method. After extraction and HPLC-fractionation of the samples,¹² the appropriate eluates are subjected to RIA using the PGE₂ antiserum, that cross-reacts with PGE₃ at 14%. The detection limit of this method is about 100 pg/ml.

STATISTICAL METHODS

Results are presented as mean (SEM). Duodenal damage scores were plotted against the dose of acid instilled into the duodenal lumen. Dose-response curves were constructed by weighted least squares regression analysis of the data on a third order polynomial model using a computer program (Sigmaplot, Jandel Scientific, Corte Madera, Ca, USA). Statistical difference between curves was tested by analysis of covariance of damage scores per acid dose level. Differences in duodenal prostaglandin release were determined using a two way analysis of

TABLE III Daily food intake and body weight gain (mean (SEM) of 10 rats per group)

	Oleic acid diet	Linoleic acid diet	Eicosapentaenoic acid diet
Daily food intake (g/day)*	23 (1)	23 (1)	24 (1)
Body weight gain (g)			
First week	61 (2)	56 (2)	59 (2)
First month	232 (8)	234 (10)	213 (9)

*Average of the first week on diet.

Figure 1: Mucosal damage induced by intraduodenal instillation of HCl at different doses in rats fed either an oleic, linoleic or eicosapentaenoic acid supplemented diet. Mucosal resistance to acid was tested after saline pretreatment of the duodenal mucosa. Scores were obtained blindly according to the criteria stated in Table II. No difference was found between the three experimental groups.

Figure 2: Mucosal damage induced by intraduodenal instillation of HCl after pretreatment with a pulse of either saline (solid line) or 100 μmol HCl (dashed line) in rats fed the oleic acid supplemented diet. The difference between saline and mild acid pretreatment is significant ($p < 0.01$). The shift to the right after mild acid pretreatment suggests that adaptive cytoprotection rendered the duodenal mucosa more resistant to acid.

variance for multiple comparisons, and the Student's *t* test for single comparisons.

Results

EFFECT OF DIETS ON BODY WEIGHT

Ten rats from each diet group were placed in individual metabolic cages to monitor their daily food intake and body weight gain. The three diets were isocaloric and equally well accepted by the Sprague-Dawley rats. As shown by Table III, the daily food intake and body weight gain during the first week on the diet were similar in rats fed either the oleic, linoleic, or eicosapentaenoic acid supplemented diet. The rats exhibited a satisfactory weight gain over the four week period and there was no significant difference in body weight or health status between the three experimental groups. Body weight gain was satisfactory in all rats included in this study.

DUODENAL RESISTANCE TO ACID

Figure 1 shows the mean scores of duodenal damage induced by instillation of HCl into the duodenal lumen after saline pretreatment. Global scores including macroscopic and histological data were plotted against doses of HCl and the curves of duodenal resistance to acid corresponding to the three dietary groups were drawn by polynomial regression analysis. As

Figure 3: Mucosal damage induced by intraduodenal instillation of HCl after pretreatment with a pulse of either saline (solid line) or 100 μmol HCl (dashed line) in rats fed the linoleic acid supplemented diet. The difference between saline and mild acid pretreatment is significant ($p < 0.01$). The duodenal mucosa became more resistant to acid after mild acid challenge (adaptive cytoprotection).

Figure 4: Mucosal damage induced by intraduodenal instillation of HCl after pretreatment with a pulse of either saline (solid line) or 100 μmol HCl (dashed line) in rats fed the eicosapentaenoic acid supplemented diet. The difference between saline and mild acid pretreatment is significant ($p < 0.01$). The phenomenon of adaptive cytoprotection is shown by the shift of the curve to the right after mild acid pretreatment.

shown by Figure 1, the resistance of the duodenal mucosa to an acid challenge was similar among the three dietary groups at the five dose levels tested, and there was no significant difference between diets.

ADAPTIVE CYTOPROTECTION

Exposure of the duodenal mucosa to a mild dose of acid (100 μmol HCl) significantly enhanced mucosal resistance to a subsequent acid challenge. Figure 2 shows the comparison between acid induced damage scores after saline pretreatment and after mild acid irritation in rats fed the oleic acid diet. Figure 2 clearly shows that a phenomenon of adaptive cytoprotection occurred in animals fed the oleic diet at the various levels of acid injury tested, since pretreatment with a 100 μmol pulse of HCl induced a significant shift of the curve to the right ($p < 0.01$). Thus, infusion of similar doses of acid caused significantly lower damage scores after mild acid than after saline pretreatment suggesting that the rat duodenum is able to withstand higher doses of acid after a mild challenge.

Figures 3 and 4 show the curves of duodenal resistance to acid in rats fed the linoleic and the eicosapentaenoic acid diets, respectively. Again, there is a significant difference between the curves of mucosal resistance after saline and after mild acid exposure ($p < 0.01$). That is, the duodenal mucosa became more resistant to acid after a mild acid challenge, indicating that adaptive cytoprotection occurred in rats fed the linoleic or the eicosapentaenoic acid diet as observed in the oleic diet group. However, comparing Figure 2 (oleic acid diet group) with Figures 3 and 4 (linoleic and eicosapentaenoic acid groups, respectively) it is evident that the gap between the saline and mild acid pretreatment curves is wider for the two latter fatty acid groups. This would suggest that the shift to the right after mild acid pretreatment is more important in rats fed diets supplemented with a polyunsaturated fatty acid than oleic acid.

Figure 5: Mucosal damage induced by intraduodenal instillation of HCl after pretreatment with mild acid in rats fed either oleic, linoleic or eicosapentaenoic acid diet. Duodenal damage was more severe in oleic diet fed rats than in linoleic or eicosapentaenoic acid diet fed rats ($p < 0.05$). The increase in mucosal resistance by adaptive cytoprotection is enhanced among linoleic or eicosapentaenoic acid rats when compared with oleic acid supplemented diet rats.

As previously shown in Figure 1, there was no difference between the three experimental groups in mucosal resistance to acid after saline. In contrast, when comparing the adaptive cytoprotection curves (Fig 5), the difference between diet groups is evident and significant ($p < 0.05$). Thus, duodenal damage scores were significantly higher in rats fed oleic acid diet than in those fed either the linoleic or eicosapentaenoic acid supplemented diet. This finding suggests that the increase in mucosal resistance produced by adaptive cytoprotection becomes much stronger in linoleic or eicosapentaenoic acid fed rats than in those fed the oleic acid diet. There was no significant difference between the linoleic acid and the eicosapentaenoic acid supplemented groups.

Correlation between macroscopic and histological scores was very close ($r = 0.828$, $n = 168$). Thus, when considering macro- and microscopic data independently, the same results were obtained: mild acid pretreatment enhances mucosal resistance in the three experimental groups but changes are significantly more noticeable in linoleic or eicosapentaenoic acid supplemented groups than in oleic acid fed rats.

EFFECTS OF INDOMETHACIN

Figure 6 shows the curves of duodenal resistance to acid after mild acid irritation (100 μmol HCl) in rats pretreated with indomethacin. All groups exhibited higher damage scores than those

Figure 6: Effect of indomethacin treatment on adaptive cytoprotection in rats fed either oleic, linoleic or eicosapentaenoic acid diet. After indomethacin treatment, duodenal damage induced by intraduodenal instillation of HCl given 30 minutes after mild acid was similar in the three dietary groups.

TABLE IV *In vivo* duodenal release of prostaglandin (PGE_2) in response to acid (values mean (SEM) of four rats per group)

	PGE_2 release (pg/min)	
	Basal	HCl stimulated
Oleic acid diet	6.7 (0.4)	17.7 (3.3)*
Linoleic acid diet	11.5 (1.4)†	30.2 (2.7)*†
Eicosapentaenoic acid diet	6.3 (1.1)	18.4 (4.5)*

* $p < 0.05$ v basal.

† $p < 0.05$ v oleic acid and eicosapentaenoic acid diets.

observed in the adaptive cytoprotection experiments shown in Figure 5 ($p < 0.05$). On the other hand, after indomethacin treatment there was no statistical difference between the three dietary groups. Thus, indomethacin treatment suppressed both adaptive cytoprotection and the difference between dietary groups.

DUODENAL RELEASE OF PROSTAGLANDIN E_2

The release of PGE_2 into a perfused duodenal loop is shown in Table IV. During the baseline period, PGE_2 was released at a significantly higher rate in linoleic acid fed rats than in the oleic acid or eicosapentaenoic acid supplemented groups. After an intraduodenal pulse of 100 μmol HCl, the release of PGE_2 appreciably increased in all three groups of rats. Changes were significant up to 45 minutes after HCl stimulation. It is interesting to note that the prostaglandin response was more pronounced in linoleic fed rats than in the other groups.

The release of PGE_3 in eicosapentaenoic acid fed rats was below the detection limit of our combined HPLC-RIA method.

Discussion

This study aimed to investigate whether changes in the type of dietary fat would influence the susceptibility of the duodenal mucosa to acid induced lesions. For this purpose, three special diets were prepared from a standard diet that was supplemented with oleic or linoleic acid or eicosapentaenoic acid. Our results suggest that diet supplementation with linoleic acid or eicosapentaenoic acid compared with oleic acid enhances the resistance of the duodenal mucosa to acid by potentiation of adaptive cytoprotection. The diets were isocaloric and hence, the body weight gain and the daily intake of food were similar in the three experimental groups. Therefore, it is unlikely that the beneficial effects of the polyunsaturated diets were the result of a difference in caloric consumption. The findings observed in this study are most probably related to differences in the fat composition of the diets.

The three experimental diets differed in their content of polyunsaturated fatty acids, since the linoleic and eicosapentaenoic acid diets were rich in polyunsaturated fatty acids compared with the oleic acid diet, although essential requirements of polyunsaturated fatty acids were amply satisfied by all three dietary regimens, as also evidenced by the normal growth of the rats. Thus, differences in the dietary intake of polyunsaturated fatty acids between the three experimental groups were probably responsible for the

variances noted in the resistance of the duodenal mucosa to acid. Either polyunsaturated fatty acids enhanced mucosal resistance or the oleic acid supplement weakened the defensive mechanisms.

It should be emphasised that when acid was applied directly to the duodenal mucosa without prior irritation, there was no difference in mucosal resistance in the three dietary groups tested in our study. Thus, changes in the physico-chemical properties of mucosal cell membranes as a consequence of the putative variations in their fatty acid composition are unlikely to be responsible for the beneficial effect of diet, since those structural changes would also intervene when the acid load is delivered to the mucosa without prior irritation. The beneficial effect of the diets supplemented with polyunsaturated fatty acids was only observed when the acid challenge took place after mild acid irritation, suggesting that the dietary manipulation increased the ability of the mucosa to promote a defensive mechanism against the acid challenge.

Diet supplementation with fatty acid precursors of eicosanoid synthesis may influence the adaptive response of the duodenal mucosa to acid. In the gastrointestinal tract, local eicosanoid synthesis and metabolism seem to be governed by intraluminal, neural, and hormonal factors. Diet may be particularly relevant as a regulator of eicosanoid biosynthesis, since the dietary intake of fatty acids can directly influence the pool of precursors in the membrane phospholipids of epithelial cells in the gastrointestinal mucosa.¹⁵ In fact, dietary linoleic acid increases the release of prostaglandin E₂ into the gastric lumen in rats¹⁰ and humans.¹¹ Previous studies by our own group showed that changes in the dietary intake of polyunsaturated fatty acids may also modify the pattern of eicosanoids synthesised by the rat gastrointestinal mucosa.¹² Thus, after four weeks on an eicosapentaenoic acid rich diet, the generation of PGE₂ and 6-keto-PGF_{1α} by gastric mucosal specimens was significantly reduced, whereas at the same time we observed substantial generation of previously undetectable PGE₃. In the present study, we have shown that instillation of 100 μmol HCl into a perfused duodenal loop triggers the release of PGE₂, and the response is quantitatively more important in rats fed the linoleic acid supplemented diet than in the other two experimental groups. The release of PGE₃, however, was below the detection limit of our combined HPLC method, and we were not able to detect if such an enhanced response is also present in eicosapentaenoic acid fed rats. An enhanced generation of either omega-3 or omega-6 derived prostaglandins possessing cytoprotective properties¹⁶⁻¹⁸ could account for the protection against acid-induced duodenal lesions in rats fed the polyunsaturated fatty acid diets, though this cannot be confirmed with the present data.

The experiments using indomethacin to inhibit cyclo-oxygenase activity would support the above concept, since indomethacin suppressed all differences between the three experimental groups suggesting that in the absence of normal prostaglandin generation, there is no

beneficial effect derived from the dietary supplement with polyunsaturated fatty acids. The effects of indomethacin, however, may also involve alternative mechanisms. Cyclo-oxygenase inhibition may divert the metabolism of the precursor fatty acid through the lipoxygenase pathway, favouring the production of leukotrienes with potent chemotactic activity. It has been recently shown that leukocyte accumulation within the mucosal blood vessels plays an important role in the pathogenesis of indomethacin induced gastropathy.¹⁹ Nevertheless, if in our experiments the effects of indomethacin were mediated by an increased production of leukotrienes, one would have expected that indomethacin reversed the beneficial effect of the linoleic diet but not that of the eicosapentaenoic acid diet, or at least not to the same extent, since the lipoxygenase products derived from eicosapentaenoic acid are weaker proinflammatory mediators than products derived from arachidonic acid metabolism.²⁰⁻²²

It has been shown that oral administration of lipid mixtures containing surface-active phospholipids provides significant protection to the gastric mucosa against a variety of ulcerogenic agents, including acid.^{23,24} These lipids form a hydrophobic lining on the mucosal luminal surface that repels the back diffusion of acid ions (H⁺) and therefore provide an effective 'mucosal barrier' against acid erosion. Hypothetically, such an effect could have occurred in our experiments using diets that differed in their fat composition. However, our experiments were performed after an overnight fast (16 hours), and influences derived from the presence of traces of food within the duodenal lumen can be excluded. In addition, palmitic acid, a saturated fatty acid, was shown to be the main component of the protective lipid mixtures and indomethacin pretreatment did not abolish their beneficial effects.²⁴ The latter point in particular suggests that our findings reflect a different phenomenon, since we found protection when using diets supplemented with polyunsaturated fatty acids and this protection was suppressed by indomethacin pretreatment. However, it is possible that both phenomena (adaptive cytoprotection and surfactant lipid barrier) are integrated in a common protective mechanism, as far as the enhanced mucosal ability to synthesise prostaglandins would favour the prompt accumulation of hydrophobic phospholipids on the mucosal surface²⁵ in response to mild irritation, rendering the mucosa more resistant to subsequent acid challenge.

In summary, our study shows that adaptive cytoprotection of the duodenal mucosa to luminal acid can be modulated by the dietary intake of fat. Diets rich in polyunsaturated fatty acids enhance the phenomenon of adaptive cytoprotection and render the duodenal mucosa more resistant to acid. These findings may be relevant for the prophylaxis of duodenal ulcer disease in humans, an appealing concept indirectly supported by epidemiological and experimental data suggesting a relationship between the incidence of peptic ulcer and the consumption of dietary polyunsaturated fatty acids.^{26,27}

This study was partly supported by grant PM92/0193 from Direction General de Investigación Científica y Técnica (Madrid, Spain).

Preliminary data of this study were presented at the Annual Meeting of the American Gastroenterological Association (May 1990, San Antonio, Texas) and published as an abstract in *Gastroenterology* 1990; 98: A420.

- Malagelada JR, Azpiroz F. Determinants of gastric emptying and transit in the small intestine. In: Schultz SG, Wood JD, Rauner BB, eds. *Handbook of physiology. Section 6: The gastrointestinal system*. New York: Oxford University Press, 1989: 909–37.
- Ovesen L, Bendtsen F, Tage-Jensen U, Pedersen NT, Gram BR, Rune SJ. Intraluminal pH in the stomach, duodenum, and proximal jejunum in normal subjects and patients with exocrine pancreatic insufficiency. *Gastroenterology* 1986; 90: 958–62.
- Nishioka T, Sekiguchi T, Kogure M, Kusano M, Arai H, Katoh R, et al. A study on continuous simultaneous recordings of gastrointestinal motility and intraluminal pH in human healthy subjects. *Jpn J Gastroenterol* 1981; 78: 2065–73.
- Lugea A, Salas A, Guarner F, Azpiroz F, Malagelada JR. Duodenal mucosal resistance to intraluminal acid in the rat: role of adaptive cytoprotection. *Gastroenterology* 1992; 102: 1129–35.
- Robert A, Nezamis JE, Lancaster C, Davis JP, Field SO, Hancher AJ. Mild irritants prevent gastric necrosis through 'adaptive cytoprotection' mediated by prostaglandins. *Am J Physiol* 1983; 245: G113–21.
- Wallace JL. Increased resistance of the rat gastric mucosa to hemorrhagic damage after exposure to an irritant. Role of the 'mucoid cap' and prostaglandin synthesis. *Gastroenterology* 1988; 94: 22–32.
- Hawkey CJ, Kemp RT, Walt RP, Bhaskar NK, Davies J, Filipowicz B. Evidence that adaptive cytoprotection in rats is not mediated by prostaglandins. *Gastroenterology* 1988; 94: 948–54.
- MacNaughton WK, Wallace JL. A role for dopamine as an endogenous protective factor in the rat stomach. *Gastroenterology* 1989; 96: 972–80.
- Tarnawski A, Hollander D, Krause WJ, Stachura J, Zipser RD, Gergely H, Dadufalza V. Is linoleic acid (dietary essential fatty acid) cytoprotective for the gastric mucosa? *Gastroenterology* 1985; 88: 1610.
- Schepp W, Steffen B, Ruoff HJ, Schusdziarra V, Classen M. Modulation of rat gastric mucosal prostaglandin E₂ release by dietary linoleic acid: effects on gastric acid secretion and stress-induced mucosal damage. *Gastroenterology* 1988; 95: 18–25.
- Grant HW, Palmer KR, Kelly RW, Wilson NH, Misiewicz JJ. Dietary linoleic acid, gastric acid, and prostaglandin secretion. *Gastroenterology* 1988; 94: 955–9.
- Rodríguez R, Martínez M, Guarner F, Malagelada JR. Modification of gut mucosal eicosanoid synthesis by a fish oil diet. *J Clin Nutr Gastroenterol* 1990; 5: 11–19.
- Yonei Y, Guth PH. Laparotomy stimulates an endogenous gastric mucosal protective mechanism in the rat. *Gastroenterology* 1988; 95: 600–11.
- Barocelli E, Impicciatore M, Seaton J, Conter R, Kauffman G. Localization of central prostaglandin E₂ antisecretory effects. *Gastroenterology* 1991; 100: 320–7.
- Lichtenberger LM, Renooij W. Rapid de novo biosynthesis of phosphatidylcholine by the rat gastric mucosa. *Gastroenterology* 1989; 96: A301.
- Robert A. Prostaglandins and the gastrointestinal tract. In: Johnson LR, ed. *Physiology of the gastrointestinal tract*. Vol 2. New York: Raven Press, 1981: 1407–34.
- Boughton-Smith NK, Whittle BJR. Inhibition of gastric acid secretion in the rat by prostaglandins derived from arachidonic acid or eicosapentaenoic acid. *Gastroenterology* 1990; 98: A24.
- Guarner F, Vilaseca J, Salas A, Rodríguez R, Malagelada JR. Reduction of cysteamine-induced duodenal ulcers by dietary fish oil. *Eur J Gastroenterol Hepatol* 1991; 3: 239–43.
- Wallace JL, Arfors KE, McKnight GW. A monoclonal antibody against the CD18 leukocyte adhesion molecule prevents indomethacin-induced gastric damage in the rabbit. *Gastroenterology* 1991; 100: 878–83.
- Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese J, Spur BW, Robinson DR, et al. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N Eng J Med* 1985; 312: 1217–24.
- Terano T, Salmon JA, Higgs GA, Moncada S. Eicosapentaenoic acid as a modulator of inflammation. Effect on prostaglandin and leukotriene synthesis. *Biochem Pharmacol* 1986; 35: 779–85.
- Vilaseca J, Salas A, Guarner F, Rodríguez R, Martínez M, Malagelada JR. Dietary fish oil reduces progression of chronic inflammatory lesions in a rat model of colitis. *Gut* 1990; 31: 539–44.
- Lichtenberger LM, Graziani LA, Dial EJ, Butler BD, Hills BA. Role of surface-active phospholipids in gastric cytoprotection. *Science* 1983; 219: 1327–9.
- Lichtenberger LM, Romero JJ, Kao YJ, Dial EJ. Gastric protective activity of mixtures of saturated polar and neutral lipids in rats. *Gastroenterology* 1990; 99: 311–26.
- Sheiman JM, Kraus ER, Bonville LA, Weinhold PA, Boland CR. Synthesis and prostaglandin E₂-induced secretion of surfactant phospholipid by isolated gastric mucous cells. *Gastroenterology* 1991; 100: 1232–40.
- Hollander D, Tarnawski A. Dietary essential fatty acids and the decline in peptic ulcer disease – a hypothesis. *Gut* 1986; 27: 239–42.
- Grant HW, Palmer KR, Riermesma RR, Oliver MF. Duodenal ulcer is associated with low dietary linoleic acid intake. *Gut* 1990; 31: 997–8.