Clarification of the link between polyunsaturated fatty acids and *Helicobacter pylori*-associated duodenal ulcer disease: a dietary intervention study

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Epidemiological evidence has suggested that the declining prevalence of duodenal ulcer disease may be attributable to rising consumption of polyunsaturated fatty acids, a hypothesis supported by *in vitro* evidence of toxicity of such substances to *Helicobacter pylori*. The objective of the present study was to establish whether this association is causal. Forty patients with proven infection with *H. pylori* and endoscopic evidence of past or present duodenal ulcer disease were randomized to receive either polyunsaturated fatty acids (PUFA group), in the form of capsules and margarine, or a placebo (control). Both groups received concurrent H_2 antagonist therapy. Efficacy of therapy was determined endoscopically by assessment of ulcer healing while *H. pylori* status was determined by antral biopsy, urease (*EC* 3.5.1.5) culture and histological assessment of the severity of *H. pylori* infection. Antral levels of prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) were quantified. Compliance was monitored. Before treatment, both groups were comparable for severity of *H. pylori* infection, smoking status and levels of LTB_4 and PGE_2. Despite a significant difference in consumption of linoleic acid (19·9 (SE 1·6) g for PUFA group *v.* 6·7 (SE 0·8) g for controls ($P < 0·01$) and linolenic acid (2·6 (SE 0·2) g *v.* 0·6 (SE 0·03) g ($P < 0·01$) there was no significant change in either the severity of *H. pylori* infection or prostaglandin levels in either group at 6 weeks. Consumption of a considerable amount of PUFA does not inhibit the colonization of the stomach by *H. pylori* nor does this alter the inflammatory changes characteristic of *H. pylori* gastritis. We conclude that the association between duodenal ulceration and a low level of dietary PUFA is likely to be spurious, probably reflecting the effect of confounding factors such as affluence, social class or smoking.

Helicobacter pylori: Polyunsaturated fatty acids: Duodenal ulcer

It is about 10 years since it was noted that the continuing decline in peptic ulcer morbidity and mortality in, at least, the UK and the USA was associated with a rising consumption of polyunsaturated fatty acids (PUFA) (Hollander & Tarnawski, 1986). A plausible hypothesis to relate these two phenomena was supported by a number of *in vitro* and *in vivo* studies which suggested that dietary PUFA inhibited gastric acid secretion while increasing cytoprotective prostaglandins (Hollander *et al.* 1982; Tarnawski *et al.* 1987; Grant *et al.* 1988, 1990; Hunt *et al.* 1988). It is now well recognized that prostaglandins, plentiful throughout the gastrointestinal mucosa, play a major role in gastric cytoprotection, increasing mucosal blood flow, decreasing gastric acid secretion, stimulating mucus secretion, and preventing gastric mucosal barrier disruption through altering transmucosal potential difference and mucosal permeability (Robert *et al.* 1979; Miller, 1983). Inhibitors of prostaglandin synthesis are also known to exert a deleterious effect on the gastric mucosa. Dietary essential fatty acids, particularly linoleic acid, a precursor for arachidonic

acid and prostaglandins, have been demonstrated to increase gastric prostaglandin levels after acute dosing and to modify the gastric mucosal injury induced by a number of toxic substances (Miller, 1983). *Helicobacter pylori* is one such agent toxic to the gastric mucosa. It has been shown to lead to thinning of the mucus layer and to diminish the hydrophobicity of the mucosal surface (Spychal *et al.* 1990; Goggan *et al.* 1992; Mauch *et al.* 1993). Recently linoleic acid has been shown to inhibit *H. pylori* growth *in vitro* (Thompson *et al.* 1994). Together these studies suggest that essential fatty acids, particularly linoleic acid, by a variety of mechanisms, may be important in the inhibition of *H. pylori*-induced peptic ulceration. The clinical significance of these findings is suggested by epidemiological studies which have revealed a lower concentration of PUFA in the adipose tissue of duodenal ulcer patients; this is an indirect measure of the long-term average fatty acid composition of the diet (Hollander & Tarnawski, 1986; Staveren *et al.* 1986; Kearney *et al.* 1989). Despite this, and the otherwise unexplained downward trend in duodenal ulcer disease, no *in vivo* trials of PUFA therapy in *H. pylori*-positive duodenal ulcer patients have been published. Given the extremely high prevalence of *H. pylori* infection and the impracticability of pharmacological treatment for entire populations, the discovery of a dietary method of modifying consequences of *H. pylori* infection would be extremely valuable. This led us to undertake a direct study of the effect of increasing the intake of PUFA.

METHODS

Forty volunteer patients referred to a routine endoscopy clinic with infection with *H. pylori* and either past or present duodenal ulcer disease, who had not received antibiotics, bismuth or omeprazole within the previous 2 months, were eligible for enrolment in the study. At endoscopy, evidence of duodenal ulcer disease (erosive duodenitis, duodenal scarring, active duodenal ulcer disease) was documented by the endoscopist. An active ulcer was defined as a mucosal breach > 5 mm in one dimension with depth and an erosion as a break in the mucosal surface not fulfilling the stated criteria. Scarring was defined as distortion and pitting of the duodenum with or without an ulcer scar. Histological assessment was performed using a modified Sydney system of classification of density of *H. pylori* infection where both mild and severe categories of infection were each subdivided into two further categories. The categories were: (1) occasional organisms identified; (2) groups of organisms, up to one-third of the mucosal surface; (3) organisms covering between one-third and two-thirds of the surface; (4) organisms covering more than two-thirds of the surface and upper pits; (5) organisms covering more than two-thirds of the surface and upper pits, and dense groups of organisms. Density of infection was defined as the cumulative total of the grades of one antral and one body biopsy. *H. pylori* infection was demonstrated by a urease (*EC* 3.5.1.5) test (CLO test, Delta West Pty Ltd, Bentley, Australia), histology of two biopsies from the body and antrum, and culture of antral biopsies. Patients were considered *H. pylori* infected if two or more tests were positive. All patients were *H. pylori* positive according to these criteria. Prostaglandin E_2 (PGE$_2$) and leukotriene B$_4$ (LTB$_4$) content of the biopsies was estimated by radio-immunoassay (Amersham TRK 431, Amersham, Bucks.) which we have previously validated (Hawkey, 1986).

Patients were then randomized in a double-blind manner to a 35 d treatment of either placebo capsules (coconut oil and vitamin E, Scotia Pharmaceuticals, Guildford, Surrey) and a low-PUFA margarine (Summer County, J.S. Sainsbury plc, Stamford St, London) or a high-PUFA regimen in the form of Efamol® capsules (Scotia Pharmaceuticals; four

Table 1. *Characteristics of subjects with* Helicobacter pylori *infection who were assigned to receive a high-polyunsaturated fatty acid diet (PUFA group) or a control diet*

	PUFA group	Control group
Sex (M:F)	14:6	16:4
Age (years)	49 (SE 13)	56 (SE 11)
Smoking status		
No. of smokers (%)	9 (45)	9 (45)
No. of non-smokers (%)	6 (30)	6 (30)
No. of ex-smokers (%)	2 (10)	4 (20)
No. unknown (%)	3 (15)	1 (5)
Duodenal ulcer:Scar:Duodenitis	12:6:3*	7:10:6*

* Dual pathology (one patient, scar + duodenal ulcer; three patients, scar + duodenitis).

Table 2. *Sources of essential fatty acids supplied to subjects with* Helicobacter pylori *infection assigned to receive a high-polyunsaturated fatty acid diet (PUFA group) or a control diet*

	Linoleic acid		Linolenic acid	
	PUFA group	Controls	PUFA group	Controls
Capsules				
(g/dose)	1·3	nil	0·2	nil
(g/d)	5·2	nil	0·8	nil
Margarine (g/kg margarine)	397	68	56	3

500 mg capsules four times daily) and a high-PUFA margarine (Soya, J.S. Sainsbury plc). Capsules were taken with or after meals and the margarine was used in place of the subjects' normal spread or oil in cooking. Characteristics of the two groups are outlined in Table 1. Both groups received a concurrent H_2 antagonist, ranitidine (150 mg twice daily), as initial treatment for their dyspepsia. The PUFA content of the capsules and spreads provided to the two treatment groups is outlined in Table 2.

A [^{14}C]urea breath test was performed before commencing therapy. Patients fasted overnight and were then given a fat-containing meal (Calogen, Scientific Hospital Supplies Ltd, Liverpool, Merseyside) to delay gastric emptying, just before drinking 50 ml water containing the ^{14}C-labelled urea. The [^{14}C]urea breath test involves administration of 46 kBq [^{14}C]urea which is split by *H. pylori* in the stomach to ammonium and bicarbonate ions, which, in the presence of acid, yield $^{14}CO_2$. Breath is collected and the amount of $^{14}CO_2$ expired is measured. The [^{14}C]urea is delivered orally in 50 ml water. The results are reported as the percentage of the dose excreted/mmol CO_2 at 30 min post-dosing. All other ulcer healing medications, antibiotics, aspirin, and non-steroidal anti-inflammatory drugs were proscribed. Compliance was assessed by tablet count, review of concurrent medications on follow-up visits, diary card, weighing of the returned margarine containers and review by a dietitian blinded to the randomization at the time of assessment. Each patient on entry to the study was given a daily diary form and asked to tick or cross the four treatment times each day according to whether they had or had not taken their PUFA or ranitidine capsules. Patients were asked to return these forms and all capsule bottles at their follow-up visit on day 30. On this occasion they were given a food diary and asked to record their total diet including quantities of each food consumed for a 3 d period then bring the diary with them to the dietitian for calculation of daily PUFA consumption.

At the end of the treatment period patients underwent repeat endoscopy and biopsy assessments as undertaken at the commencement of the trial. A repeat [^{14}C]urea breath test was performed and consumption of PUFA was assessed. Ethical approval was obtained from the hospital ethics committee.

Statistical methods

PUFA and control group results were all nonparametric and were compared using the Wilcoxon Signal Rank test for paired data and the Mann–Whitney U test for unpaired data.

RESULTS

Twenty patients received the active treatment and twenty the placebo. One patient from the placebo group was lost to follow-up. All treatments were well tolerated, with no significant adverse events in either group. Compliance, defined as 80 % or greater consumption of ranitidine tablets was 95 % in cases and 90 % in controls, with consumption unknown in two cases and four controls. Table 3 shows the mean consumption of PUFA for the two groups according to dietary assessment of 3 d food charts and calculation of consumption of PUFA from returned capsules and margarine. As can be seen, mean total daily intake of PUFA from all sources (dietary + margarine + capsules) in the PUFA group was 19·9 (SE 1·6) g linoleic acid and 2·6 (SE 0·2) g linolenic acid compared with 6·7 (SE 0·8) g linoleic acid and 0·6 (SE 0·03) g linolenic acid in the controls.

For six subjects (one case and five controls) compliance was unknown, as capsules were not returned. For a further case, dietary assessment was not available. [^{14}C]urea breath test results are shown in Fig. 1. There was no statistically significant difference in the breath test results at 30 min between cases and controls before treatment ($P = 0·14$). Treatment was not shown to be of benefit to either cases or controls ($P = 0·48$). In cases there was a trend to higher [^{14}C]urea breath test results after treatment ($P = 0·006$).

PGE$_2$ levels showed no statistically significant change with treatment in either cases or controls (cases: before treatment, median 75·3, interquartile range 63·4–120·0; after treatment, median 89·8, interquartile range 62·2–136·6 ($P = 0·79$); controls: before treatment, median 73·3, interquartile range 59·2–106·0; after treatment, median 64·5, interquartile range 33·4–103·9 ($P = 0·62$). LTB$_4$ levels were also not significantly affected

Table 3. *Daily consumption of polyunsaturated fatty acids (PUFA) by subjects with* Helicobacter pylori *infection assigned to receive a high-PUFA diet (PUFA group) or a control diet**

(Mean values with their standard errors for twenty subjects per group)

Daily PUFA consumption (g)	PUFA group				Control group			
	Linoleic acid		Linolenic acid		Linoleic acid		Linolenic acid	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Dietary estimation	4·7	0·7	0·6	0·1	4·3	0·8	0·5	0·1
Calculation from returned capsules	4·7	0·1	0·6	0·02	nil		nil	
Calculation from returned margarine	10·6	1·3	1·4	0·2	2·3	0·3	0·1	0·01
Total	19·9	1·6	2·6	0·2	6·7	0·8	0·6	0·03

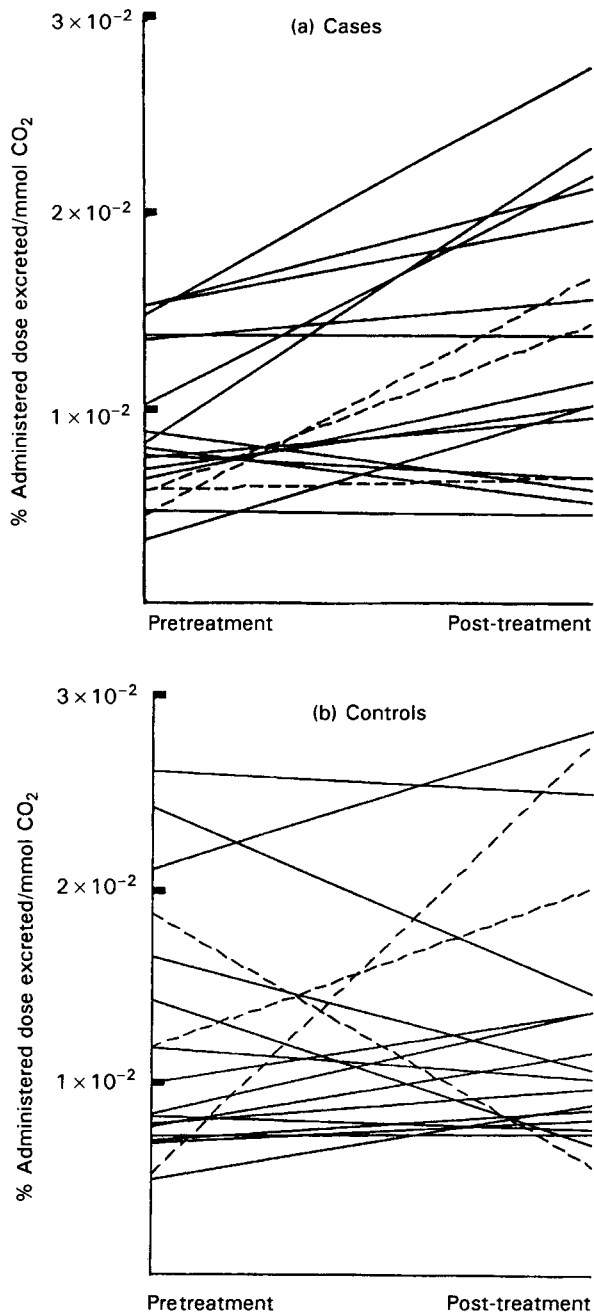* For details of diets and procedures, see pp. 516–518.

Fig. 1. Excretion of $^{14}CO_2$ during a $[^{14}C]$urea breath test by subjects with *Helicobacter pylori* infection, before and after 35 d treatment with a high-polyunsaturated fatty acid diet (cases) or a control diet (controls). For details of procedures, see p. 517.

by treatment in either cases or controls (cases: before treatment, median 0·50, interquartile range 0·38–1·3; after treatment, median 0·55, interquartile range 0·39–0·84 ($P = 0.77$); controls: before treatment, median 0·53, interquartile range 0·40–1·86; after treatment, median 0·53, interquartile range 0·40–0·77 ($P = 0.95$)). Treatment reduced *H. pylori* density in the cases, but this was not significant (before treatment, median 4, interquartile range 3–4; after treatment, median 3, interquartile range 2–5; ($P = 0.07$). There was also a decrease in *H. pylori* density in the placebo group which did not reach significance (before treatment, median 6, interquartile range 4–7; after treatment, median 4, interquartile range 3–6 ($P = 0.06$).

DISCUSSION

Several authors have noted a declining prevalence of peptic ulcer in the UK and the USA in the last almost 30 years (Coggan *et al.* 1981; Fineberg & Pearlman, 1981; Sonnenberg *et al.* 1985). A fall in the rate of surgery for peptic ulcer disease, at least in the USA, occurred before the introduction of cimetidine, the first of the H_2 antagonists. This suggests that other factors have altered the prevalence, if not the severity, of the disease in this period (Fineberg & Pearlman, 1981). The association of poor living standards with increased *H. pylori* prevalence and in turn *H. pylori* infection with peptic ulcer disease is becoming increasingly clear. Improving hygiene is also associated with improvements in nutrition and, historically at least, there is a clear relationship between increased dietary PUFA intake and declining duodenal ulceration (Hollander & Tarnawski, 1986).

In previous work we and others have shown that incubation of *H. pylori* microaerophylically with a range of PUFA has an inhibitory effect on *H. pylori* (Thompson *et al.* 1994). In studies with ω-linolenic acid at concentrations of 10^{-3} M virtually all organisms were killed. Electron microscopy of *H. pylori* shows disruption of the cell membrane to be the mechanism of cell lysis (Maczulak *et al.* 1981; Thompson *et al.* 1994). At lower concentrations of these PUFA, inhibition of *H. pylori* motility has been observed (Thompson *et al.* 1994; Khulusi, 1995). While the mechanism remains unclear, animal studies have shown that long-chain fatty acids inhibit rumen bacterial metabolism (Maczulak *et al.* 1981). Our own studies have shown a relationship between the degree of unsaturation and the antibacterial effect against human colonic bacteria (Thompson *et al.* 1992).

PUFA also may be effective against *H. pylori* by stimulating prostaglandin production. Prostaglandins of the E, A, and I series have been shown to suppress basal and stimulated gastric acid secretion in rats and normal human subjects (Hollander *et al.* 1982). Furthermore, it has been demonstrated that prostaglandins of the E type are capable of preventing formation of gastric ulcers in rats induced by a wide variety of experimental techniques (Robert *et al.* 1979). Tarnawski *et al.* (1987) also showed a significant cytoprotective effect of oral administration of linoleic acid on ethanol-induced gastric injury, an effect significantly reduced by pretreatment with oral indomethacin, a known inhibitor of prostaglandin sythetase.

Our present study failed to show an effect of PUFA consumption on *H. pylori* gastritis which underlies most duodenal ulcer disease, despite levels of PUFA in excess of that which would be accounted for in a normal diet. While our own work *in vitro*, and that of other authors, has shown both toxicity of PUFA to *H. pylori* and a trophic effect on gastric prostanoid levels, our current findings suggest that these are not clinically significant effects even with a high-PUFA diet. One reason may be that PUFA are rarely ingested in

isolation. Their effect may, therefore, be attenuated by binding to other foods consumed simultaneously.

Unlike Grant *et al.* (1988) we failed to find any effect of substantial PUFA intake on gastric prostaglandin production. This difference is likely to be due to different methodologies. While Grant *et al.* (1988) assayed PGE levels from aspirated gastric juice collected over 75 min, in the present study levels were derived from biopsies. The reliability of this method is well established and is therefore unlikely to explain our failure to show a significant rise in PGE levels (Hawkey, 1986). More importantly, Grant *et al.* (1988) studied gastric PGE_2 production 1 h after linoleic acid intake and noted the effect to be declining over the 1 h. This implies a very acute, short-lived response which we would have missed since our subjects had taken no PUFA for at least 8 h overnight.

The association of PUFA with the decline in peptic ulcer incidence may be an epiphenomenon. In studies of adipose fatty acid profiles, which reflect dietary fat intake over the preceding 9–12 months, duodenal ulcer patients have been found to have a lower mean percentage of linoleic acid than controls (Grant *et al.* 1990). Using the virtually 1:1 relationship between percentage linoleic acid in adipose tissue and percentage in the diet (Staveren *et al.* 1986) we can predict that the differences in PUFA intake between ulcer patients and controls in the study of Grant *et al.* (1990) would be of the order of 30 %. However in our studies we increased the PUFA intake 400 % and yet noted no difference in either mucosal prostaglandin levels or *H. pylori* colonization. More detailed analysis of dietary intake has suggested that low PUFA intake may simply be a marker for other dietary and social habits including high salt intake and increased rates of smoking (Oliver, 1989). Furthermore, smoking may lower tissue levels of linoleic acid by altering fatty acid metabolism. Controlling for the effects of smoking greatly reduces the difference in incidence of low PUFA levels in duodenal ulcer patients (Grant *et al.* 1990). Such a difference may in fact be indicative of a broader variation in the diet of duodenal ulcer patients as compared with controls. This hypothesis warrants further investigation but the present study suggests that merely changing intake of PUFA will not alter the risk of developing a *H. pylori*-associated duodenal ulcer.

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