Rat small intestinal morphology and tissue regulatory peptides: effects of high dietary fat

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Sprague–Dawley rats (3 weeks old) were fed on isoenergetic diets in which 40 % of the total energy was provided as fat either in the form of butter (high saturated fat), olive oil (high monounsaturated fat) or maize oil (high polyunsaturated fat), with one group on low-fat (10 % of total energy) standard diet as a control. Animals were killed after 8·4 (se 0·8) weeks by cardiac puncture. Similar pieces of jejunum and ileum were prepared for morphometric studies. Extracts of tissue from the proximal and distal segments of the whole small intestine from four animals per group were assayed using established techniques for enteroglucagon, motilin, neurotensin, somatostatin, substance P and vasoactive intestinal peptide (VIP). We found that maize oil and olive oil increased villus height: crypt depth ratio in both jejunum and ileum. Maize oil increased tissue concentrations of somatostatin ($P < 0.05$) and substance P ($P < 0.005$) in the proximal segment. Both maize oil and olive oil increased tissue concentrations of neurotensin and substance P ($P < 0.005$) in the distal segments. These observations may explain the improvement of intestinal absorption of fluid following supplementation with polyunsaturated fat.

Small intestinal morphology: Gut peptides: Rat

Numerous studies have been reported relating the response of the small intestinal morphology to alteration in the feeding pattern (Fabry & Kujalove, 1960), subnutrition (Hindmarsh *et al.* 1967), starvation (Hopper *et al.* 1968) and hyperphagia (Brownlee & Moss, 1959). They demonstrated that the gut mucosa is remarkably sensitive to alteration in its microenvironment, starvation markedly affecting the epithelial renewal of rat duodenum (Hooper & Blair, 1956) and food stimulating cell proliferation in the epithelial crypt of the small intestine (McManus & Isselbacher, 1970). However, there are very few reports on the specific effects of dietary fat on small intestinal morphology. It is likely that any such effects are mediated by 'regulatory' peptides.

There are several circulating peptides released in response to feeding which have been demonstrated in high concentration in gut tissue (Schultzberg *et al.* 1980) and have been shown to be essential for normal intestinal and pancreatic functions (Adrian & Bloom, 1985).

Six peptides were chosen for study on the basis of their possible release by fat and their influence on one or more of the following intestinal functions: intestinal cell proliferation, local mucosal immunity, ion transport and intestinal motility. They were enteroglucagon, motilin, neurotensin, somatostatin, substance P and vasoactive intestinal peptide (VIP).

* For reprints.

Table 1. *Composition of the basic diet (g/kg complete diet)*

	Control (low-fat diet)	High-fat diet
Fat	35	200*
Protein	173·2	200
Carbohydrate	549	500
Vitamin mix	11·3	20
Salt mix	43·2	40
Fibre (non-nutritive)	31	40

* In the high-fat diet, the fat consisted of olive oil (high in monounsaturated fatty acids), butter (high in saturated fatty acids) or maize oil (high in polyunsaturated fatty acids).

Table 2. *Fatty acid profile of diets**

Diet... Fatty acid	High-fat (200 g/kg)			Low-fat (35 g/kg)
	Olive oil	Butterfat	Maize oil	
6:0	—	0·28	—	—
8:0	—	0·86	—	—
10:0	—	2·32	—	—
12:0	—	2·89	—	—
14:0	0·12	17·02	0·26	0·57
14:1	—	1·32	—	—
15:0	—	1·21	—	—
16:0	9·22	31·25	8·46	13·87
16:1	—	1·72	—	—
17:0	—	0·86	—	—
18:0	2·94	10·38	1·76	2·15
18:1	79·36	24·58	29·78	22·51
18:2 *n*-6	6·99	2·39	57·78	54·13
18:2 *n*-3	0·89	0·58	1·97	6·28
20:1	—	—	—	—
Others	—	1·84	—	—
Saturated	12·28	67·57	10·48	16·59
Monounsaturated	80·16	27·62	29·78	23·01
Polyunsaturated	7·88	2·97	59·75	60·41

* Diets were analysed by the Department of Food Biochemistry, Faculty of Agriculture, The Queen's University of Belfast, Belfast.

METHODS
Diet

The basic diet contained 200 g fat/kg, which provided 40% of the total energy as fat, in the form of butter (high in saturated fatty acids), olive oil (high in monounsaturated fatty acids) or maize oil (high in polyunsaturated fatty acids) (Table 1). The diets were isoenergetic and nutritionally adequate, providing for all known essential requirements (Special Diet Service, Witham, Essex). The only variables were the degree of fat saturation and the *n*-6:*n*-3 fatty acid ratio (Table 2). A fourth (control) group was fed on a low-fat (10% of energy) standard diet supplied by Robert Morton & Company Ltd, Ballymena, Co. Antrim.

Animals

Fifty-nine Sprague–Dawley rats were individually housed and weaned from the age of 3 weeks on to one of three synthetic diets, fourteen on butter, nineteen on olive oil, twelve

on maize oil, and fourteen on the control diet. To reduce coprophagy, the animals were kept in cages with wire mesh bottoms, and they were subjected to 12 h alternate darkness and light. Their intake was calculated daily. After 8·4 (SE 0·8) weeks, rats were anaesthetized by sodium pentobarbitone (60 mg/kg) intraperitoneally and killed by cardiac puncture.

Small intestinal morphometric studies

Similar segments of jejunum and ileum were removed gently from the mesenteric border and processed for light microscopy. Each segment was fixed in 10 % formal saline (9 g sodium chloride/l), dehydrated, cleared and embedded in paraffin wax. The segments were cut at 5 μm and stained using haematoxylin and eosin. A total of 100 villi and 100 crypts were studied from each group (three sections per region and three animals per group).

The villus height and crypt depth were measured using computer-aided microscopy (Slavin *et al.* 1980; Challacombe *et al.* 1983).

The villus height was measured (only in villi attached to lamina propria) as the distance between the upper border of muscularis mucosa and the tip of each villus structure seen in the slide. The crypt depth was measured as the distance between the open mouth of a crypt in the section through the centre of the crypt to the basement membrane zone at the basis of the crypt (Slavin *et al.* 1980).

Tissue regulatory peptides

The whole small intestine was removed from four animals of each group, freed from the mesentery, divided into two equal proximal and distal segments and stored at −20°.

Each segment was extracted separately by an acid-alcohol method in 0·4 M-phosphate buffer, pH 7·4 (Bryant & Bloom, 1982) before measurement of individual peptides by radioimmunoassay. Each sample was assayed for each gut peptide in duplicate, the average of which was taken as representative of the tissue concentration in each rat.

VIP. Natural porcine VIP was used as standard. Antibodies to VIP were raised in New Zealand white rabbits. The assay can detect 5 ng/l with 95 % confidence and is sensitive over a range 0–300 ng/l. There is no cross-reactivity in the assay with glucagon, secretin or gastric inhibitory peptide (GIP) and the antibody is predominantly C-terminal reactive (Ardill, 1979).

Somatostatin. The standard was synthetic cyclic somatostatin (Serono). The antibody OB5 (1) was raised in rabbits to cyclic somatostatin. No cross-reactivity with any other gut or islet peptide was found. The antibody is C-terminally directed, although reaction with fragments is low, suggesting that the whole molecule is required for full cross-reactivity. The assay can detect 3 ng/l with 95 % confidence.

Motilin. The standard was synthetic porcine motilin. The antibody was raised in rabbits and cross-reacts 100 % with porcine motilin. It does not cross-react with C-peptide, VIP, gastrin, GIP, glucagon or insulin. The assay can detect 10 ng/l with 95 % confidence.

Enteroglucagon. The antibody used in the assay was YY 57 raised in rabbits. The antibody reacts with the N-terminal region of glucagon (Flanagan *et al.* 1974). This antibody can detect 5·9 ng/l. No cross-reactivity has been noted with other gut and islet hormones including secretin, VIP, GIP, insulin, motilin, cholecystokinin and gastrin.

Neurotensin. The standard was synthetic neurotensin (Bachem Torrance, CA, USA). The antibody NT3 was raised in New Zealand rabbits (Shaw & Buchanan, 1983). This antibody reacts with the whole molecule. It detects 3 pmol/l with 95 % confidence and exhibits no cross-reactivity with other regulatory peptides.

Substance P. The standard was synthetic substance P (Bachem Torrance, CA, USA). The antibody GSP 10 was raised in guinea-pigs. It reacts with the C-terminal region. The antibody can detect 0·2 pg/assay tube with 95 % confidence. It cross-reacts (1 %) with neurokinin A and neurokinin B.

Statistical analysis

One-way analysis of variance (ANOVA) was used throughout. Unpaired Student's t test was used to analyse the statistical significance of the differences between the mean values of villus heights and crypt depths in each group compared with controls.

The results on regulatory peptides were not normally distributed so for these the non-parametric one-way analysis of variance (Kruskal–Wallis) and the Mann–Whitney U test were used. The results are reported as median and range.

RESULTS

Characteristics of animals

The weight of the animals at the start and at the end of the feeding period, their daily intake and the duration of feeding did not significantly differ among the four dietary groups (Table 3).

Dietary fat and small intestinal morphology

Feeding rats on high dietary fat altered the basic morphology of the small intestine (Table 4). Maize oil increased the villus height in both jejunum and ileum ($P < 0.001$). Olive oil markedly increased the villus height in jejunum and ileum ($P < 0.001$) and decreased the crypt depth in jejunum ($P < 0.05$) and ileum ($P < 0.05$). Butterfat markedly reduced the villus height in jejunum ($P < 0.01$) and ileum ($P < 0.05$) and reduced the crypt depth in both jejunum ($P < 0.001$) and ileum ($P < 0.01$).

Dietary fat and tissue regulatory peptides

There were no statistically significant differences in tissue VIP and motilin.

Comparing the proximal with the distal segments in each group, we found that the tissue concentrations of enteroglucagon were significantly higher distally in the control group ($P < 0.05$), in the olive oil group ($P < 0.001$) and in the maize oil group ($P < 0.025$). Neurotensin was significantly higher distally in the maize oil ($P < 0.01$) and in the olive oil ($P < 0.001$) groups.

Maize oil significantly increased the proximal small intestinal tissue concentration of substance P ($P < 0.001$, v. control) and somatostatin ($P < 0.05$, v. control).

Both maize oil and olive oil increased distal small intestinal tissue concentrations of substance P and neurotensin ($P < 0.001$, v. control). Butterfat did not significantly alter the tissue concentration of any of the six peptides investigated (Table 5).

DISCUSSION

The simultaneous occurrence of endocrine, paracrine and neurocrine functions of the gut peptide cells makes it difficult to judge the local activity of these cells only by estimation of plasma levels of the different peptides. Measurement of the total tissue concentration of gut peptides may serve to evaluate the possible effects of dietary fat on the potential release of these peptides with local paracrine and neurocrine functions.

Gut peptides are contained in the granules of neurones and endocrine cells and stimulated from the lumen side to be released locally and into the circulation. Accordingly, composition, load, transport, digestion and absorption of food would be expected to influence the release of tissue peptides. The pattern of feeding ad lib. in the present study might be expected to eliminate the surges of peptide release that follow set meals, but correspondingly makes it impossible to determine specific effects of dietary variation in individual meals.

There were no significant effects of dietary fat on small intestinal tissue concentration of enteroglucagon, motilin and VIP. Single mixed meals are known to release more

Table 3. *Food intake (g/d) and weights (g) of rats fed on a control low-fat (35 g/kg) and three high-fat (200 g/kg) diets from the age of 3 weeks*

Diet	No. of rats	Wt at 3 weeks	Weeks on experiment	Final wt	Food intake
Control	14	42·1	8·4	277	*Ad lib.*
High-fat					
Butterfat	14	40·5	8·2	270	16·5
Olive oil	19	38·3	8·4	289	16·7
Maize oil	12	40·4	8·6	309	18·7
SD (55 df)		6·06	2·41	75·8	2·73* (42 df)

Table 4. *Mean villus heights and crypt depths (μm) of rats fed on a control low-fat (35 g/kg) and three high-fat (200 g/kg) diets from the age of 3 weeks*

	Jejunum		Ileum	
Diet	Villus height	Crypt depth	Villus height	Crypt depth
Control	408	136	237	118
High-fat				
Butterfat	364**	114***	216*	92**
Olive oil	552***	124*	358***	111*
Maize oil	547***	141	518***	113
SD (8 df)	154·5	37·3	102·3	32·3

Mean values were significantly different from those for control group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

enteroglucagon than cream or glucose alone (Ghatei & Bloom, 1981). Both test mixed meals and oral fat stimulate the release of motilin (Christofides *et al.* 1979) and intraduodenal acid (not mixed meal, nor oral or intravenous glucose) increases plasma VIP (Long & Bryant, 1982). It may be that, despite low tissue levels, the lumen or circulating levels of these peptides were elevated as they were quickly released by the diet.

However, dietary fat as maize oil markedly increased the tissue concentration of somatostatin and substance P in the proximal segment of the small intestine. Both peptides have dual paracrine and neurotransmitter functions. Somatostatin is released from D cells in the duodenum, whose morphology and direct contact with putative effector cells suggest that they deliver somatostatin directly onto the membranes of these cells. It has been found that substance P and somatostatin present in gut are able to modulate immune functions, and receptors for these peptides were found on lymphocytes (Stanisz *et al.* 1986). Since their concentration is higher in tissue than in plasma, it is possible that they may be important immunoregulatory factors (Stanisz & Bienenstock, 1986).

Neurotensin is predominantly found in the distal segment of the small intestine (Solcia *et al.* 1978; Carraway *et al.* 1976) and fat seems to be the most important nutrient in a meal in terms of effect on its release (Shaw *et al.* 1986; Rosell & Rokaeus, 1979). Lumen fat is required for neurotensin release since intravenous administration of an oily emulsion in the same oral dose did not influence the concentration of immunoreactive neurotensin in blood. Presumably neurotensin is released when neurotensin-containing cells (N cells) in the small intestine are exposed to the ingested fat (Rosell, 1982).

Intraduodenal administration of small amounts of oleic acid results in an increased

Table 5. *Concentrations (pg/10 ml per g wet tissue) of small intestinal gut peptides in rats fed on a control low-fat (35 g/kg) diet and three high-fat (200 g/kg) diets from the age of 3 weeks*

| | Proximal | | | | | | | | | | | |
| | Substance P | | | Neurotensin | | | Enteroglucagon | | | Somatostatin | | |
Diet	Med.*	Min.	Max.	Med.	Min.	Max.	Med.	Min.	Max.	Med.	Min.	Max.
Control	0·41	0·32	0·73	1·8	1·5	3·7	37·0	33·3	111	12·9	7·3	18·7
Butterfat	0·47	0·10	0·70	3·3	0·7	12·2	91·0	28·8	182	22·6	2·9	37·2
Olive oil	0·50	0·40	0·70	3·8	1·3	7·2	55·6	40·7	104	19·0	7·3	47·0
Maize oil	0·72	0·69	1·80	9·3	3·3	20·5	51·3	30·6	125	25·1	16·7	55·5
Differences†												
Maize oil	< 0·001			> 0·05			> 0·05			< 0·05		

| | Distal | | | | | | | | |
| | Substance P | | | Neurotensin | | | Enteroglucagon | | |
Diet	Med.	Min.	Max.	Med.	Min.	Max.	Med.	Min.	Max.
Control	0·46	0·26	0·51	3·4	0·08	6·9	114	44·3	171
Butterfat	0·31	0·10	0·40	4·6	2·2	11·1	121	64·5	206
Olive oil	0·60	0·35	0·70	8·8	7·7	14·5	206	111·8	253
Maize oil	0·90	0·80	1·30	21·4	15·2	50·0	132	68·3	236
Differences†									
Olive oil	< 0·001			< 0·001			> 0·05		
Maize oil	< 0·001			< 0·001			< 0·05		
Differences‡									
Control	> 0·05			> 0·05			< 0·05		
Olive oil	> 0·05			< 0·001			< 0·001		
Maize oil	> 0·05			< 0·01			< 0·05		

* Medians and ranges are given because the data are not normally distributed. Non-parametric tests (Kruskal–Wallis and Mann–Whitney U test) were used to determine the significance of differences between diet groups and between the proximal and distal regions of the small intestine.

† Significance of difference from control group, *P*: values not quoted in the table are all > 0·05.

‡ Significance of differences between proximal and distal regions of the small intestine, *P*: probabilities for the butterfat diet are all > 0·05.

plasma concentration of neurotensin, which is dose-responsive (Kihl *et al.* 1981). The plasma levels of neurotensin after oral administration of an amino acid mixture or oleic acid were higher than after glucose or saturated fat (Ito *et al.* 1982). The present study agrees with the previous observations and demonstrates that linoleic acid in maize oil is more potent than oleic acid in olive oil in terms of increasing distal small bowel tissue concentrations of both neurotensin and substance P.

The tissue concentration of substance P in the distal segments followed the same pattern as neurotensin. Whether their total endocrine cell mass is increased or whether neurotensin stimulates the formation of more substance P via neuronal mechanisms is not clear.

Descriptive observations based on visual assessment of mucosal sections are not sufficient and objective measurements are required to detect any changes in small intestinal morphology produced by dietary fat. Villi are taller proximally than distally (Kapadia & Baker, 1976) even after feeding high dietary fat, with the exception of the group fed on high polyunsaturated dietary fat in which the villus height in the ileum was not far different from that in the jejunum.

The crypt depth was increased proximally to a greater extent than distally as shown by some authors (Clarke, 1975; Thomson *et al.* 1986) but not others (Kapadia & Baker, 1976).

Small quantities of long-chain triacylglycerols (20% of total energy intake) promote mucosal growth equal to a complete macromolecular diet (Robinson & Dowling, 1982), and free fatty acids are even more effective than long-chain triacylglycerols (Grey *et al.* 1984). Short-chain fatty acids (acetic, propionic and butyric acids) are not only major products of fermentive digestion by intestinal micro-organisms but are also a physiological lumen trophic factor for the intestinal mucosa (Miazza *et al.* 1985).

Whether the unsaturated dietary fat produces any biochemical changes that allow the epithelial cells of the jejunum and ileum to survive longer than those exposed to the saturated-fat or low-fat diet remains unclear. Whether the enterocyte life span is determined by their being pushed off the tip of the villus (Clarke, 1977) or whether the final shape of the villi is a result of variation in cell production (Creamer, 1964) requires further studies. However, these observations may explain some of our previous results on the effects of high dietary fat on water and ion transport during in vivo jejunal perfusion studies, because rats fed on a diet high in polyunsaturated fat had both the tallest villi and the best absorption (Sagher, 1988; Sagher *et al.* 1990). However, enteroglucagon is usually regarded as the most important of the gut peptides known to have trophic effects, but the lack of effect on enteroglucagon levels in the present study suggests that it was not the agent responsible for increased villus growth in response to unsaturated fat.

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