

## Commentaries

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### Haute cuisine and the colon

The stimulation of the colon after eating is within most individuals' experience and an exaggerated response is frequently a problem in patients with functional diarrhoea or irritable bowel syndrome. Early studies showed that fatty meals stimulated colonic pressure waves more than carbohydrate meals, whereas amino acid meals tended, if anything, to inhibit motility. Although these initial studies recorded only from the rectum and distal sigmoid colon we now know that the whole colon responds, though the response is less pronounced and of shorter duration in the proximal compared with the distal colon. As well as stimulating phasic activity, eating also increases colonic tone both proximally and distally.<sup>1</sup>

Modern, solid state manometric systems, which use strain gauges mounted on tubes as in the study by Rao *et al* (see page 205), have a much smaller diameter and are more comfortable for the patients than the older water perfused manometry catheters and permit prolonged ambulatory studies. Twenty hour recordings have shown a noticeable circadian rhythm, with pronounced activation on waking and after eating. These recordings also make it possible to get an accurate estimate of the frequency of infrequent forceful propulsive waves, known as high amplitude propagated contractions (HAPCs). These are characterised by high amplitude (>100 mm Hg) and prolonged duration (>12 seconds), with a relatively rapid rate of propagation (0.8–1.3 cm/s).<sup>2</sup> They are more likely to occur soon after waking (33%) or after meals (50%) and some are followed by a desire to defecate.

Most colonic pressure waves are in fact non-propagated, isolated contractions which are responsible for mixing and facilitating absorption, shifting luminal contents only short distances to and fro.

Rao *et al*'s study has many advances compared with earlier studies, including the use of solid state technology which allowed prolonged studies to be performed without the infusion of water which, with multichannel recording, has often been substantial (60 ml/hour or 1.4 litres/24 hours). After a tap water enema the probes were placed colonoscopically, with the tip positioned 60 cm from the anus. The colon was then allowed 10 hours to re-establish its normal function before the first meal was consumed and 27 hours before the second meal. The position of the probe in the descending colon was confirmed fluoroscopically at the end of each meal. The two meals tested contained 4.18 MJ (1060 kcal) of which 60% was provided as either fat or carbohydrate. Unlike some previous meals they were equally palatable and appetising. The analysis was done by investigators blind to which meal had been received, an important point as a substantial amount of the analysis was performed manually and could otherwise be subject to investigator bias. The authors provide clear criteria in the text to identify the various pressure wave patterns. These were non-propagated pressure waves, by far the commonest type, propagated pressure waves to be distinguished from specialised propagated pressure waves which the authors defined objectively as pressure waves with an amplitude and a

duration above the 95% confidence interval for non-specialised pressure waves (>103 mm Hg and >13 seconds respectively). They also defined simultaneous pressure waves and much less frequent retrograde pressure waves.

The key findings were that the fatty meal increased the number of pressure waves for three hours, whereas the carbohydrate response was shorter lived, returning to baseline by two hours. The fatty meal also increased propagated pressure waves one to three hours post-prandially, whereas simultaneous pressure waves were increased in the first hour only. Lesser responses were seen with the carbohydrate meal, which increased the propagated pressure waves but did not increase the simultaneous pressure waves. Importantly, retrograde waves also increased after the fatty meal but not after the carbohydrate meal. Eleven of the 18 subjects showed the specialised propagated pressure waves in the first hour after the fatty meal, whereas only nine showed these after the carbohydrate meal.

Many authors have observed this enhanced effect of fat but so far the cause has eluded explanation. Fatty meals tend to empty more slowly, and are associated with a larger increase in visceral blood flow and greater release of cholecystokinin (CCK), neurotensin and peptide YY. However, recent studies with CCK-A receptor antagonists appear to indicate that CCK is not an important component.<sup>3</sup> The colonic response to feeding has both a cerebral and gastric component, being elicited both by sham feeding and gastric distension with a balloon.<sup>4</sup> These immediate effects, however, appear to be transient and the more prolonged effects probably originate from the stimulation of enteroendocrine cells and possibly nerves further down the gut.

The overall effect of this stimulation in normal subjects is usually mixing with very little net movement, though this does vary with region.<sup>5</sup> More detailed examination reveals to and fro mixing movements within the ascending<sup>6</sup> and transverse colon, especially after a fatty meal. Much less movement is seen in the descending colon where the contents are normally more solid. These patterns change in disease states, thus in diarrhoeal states with more fluid colonic contents, isotope injected into the splenic flexure shows exaggerated backward and forward movements<sup>7</sup> whereas in constipation movement is much reduced.<sup>8</sup> Recent studies have suggested that although there is a decrease in mixing activity and tone, the number of propagated contractions increases in both functional diarrhoea<sup>9</sup> and ulcerative colitis.<sup>10</sup> The functional outcome evidently depends on the balance between propulsive propagated contractions, mixing non-propagated contractions, and retrograde pressure waves.

We are only just beginning to understand the pharmacology of HAPCs but they cannot be induced simply by cholinergic stimulation. HAPCs seem to involve a local reflex which starts with stimulation of mucosal afferents. Luminally active contact laxatives such as bisacodyl and senna induce HAPCs, as do irritant substances such as long chain fatty acids and glycerol, an action which can be blocked by lignocaine and atropine. Distension of the colon alone does not induce this motor pattern.<sup>2</sup> 5-Hydroxytryptamine (5-HT) may play a key role. 5-HT<sub>4</sub>

receptor agonists such as prucalopride can induce HAPCs<sup>11</sup> whereas 5-HT<sub>4</sub> antagonists impair the gastrocolonic response. Understanding how to control the colonic response to food may well be valuable therapeutically in treating both diarrhoea and constipation.

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## DMT1 expression: avoiding too much of a good thing

Haemochromatosis is a common, inherited disorder of iron metabolism. The gene (*HFE*), which is mutated in the majority of patients, has been cloned. The HFE protein, however, is not an iron transporter, but rather is thought to function as a regulator of iron absorption. The cloning of the *HFE* gene was followed rapidly by the identification of further proteins involved in the iron absorption pathway in the duodenum and transfer to the main iron storage site in the liver. The identification of a new metal ion transporter in the rat provided the first molecular information on the active absorption of metal ions by mammalian cells.<sup>1</sup> This divalent metal transporter 1 (DMT1) has a broad substrate specificity that includes Fe<sup>2+</sup> and a range of other divalent metal cations.<sup>1</sup> (DMT1 is also referred to as divalent cation transporter 1, DCT1, and natural resistance-associated macrophage protein 2, Nramp2.) DMT1 mRNA is widely expressed, with high levels in the proximal duodenum, the site of absorption of iron and most other divalent metal ions.<sup>1</sup> The biological importance of this transporter is shown by its involvement in two naturally occurring animal mutants of iron metabolism. Homozygous mutation of *DMT1* in the mouse is responsible for microcytic anaemia, and in the rat, the Belgrade phenotype of microcytic, hypochromic anaemia, with severe defects in intestinal and reticulocyte iron absorption.<sup>2–3</sup> The Belgrade reticulocyte defect seems to be a failure to transport iron out of the transferrin cycle endosomes.

Alternative splicing of exons in the primary DMT1 RNA transcript results in two classes of mature DMT1 mRNAs: DMT1(IRE) includes an iron responsive element in the 3' untranslated region; DMT1(non-IRE) does not.<sup>4–5</sup> Duodenal DMT1 mRNA increases in response to dietary iron deficiency.<sup>1</sup> This iron sensitive regulation may thus be mediated by the 3' iron responsive element of the DMT1(IRE) form, in a similar way to regulation of the transferrin receptor.<sup>6</sup> In *HFE* related haemochromatosis, duodenal enterocytes have increased duodenal iron responsive protein activity, and decreased ferritin mRNAs, with respect to controls. Thus despite body iron overload, the evidence suggests that haemochromatotic duodenal enterocytes are paradoxically

iron deficient.<sup>7</sup> Consistent with this, DMT1 mRNA was increased in duodenal mucosa from patients with haemochromatosis, suggesting a possible mechanism for the increased duodenal iron uptake and iron overload in haemochromatosis.<sup>8</sup> Similarly, in the *HFE* knockout mouse, northern blot analysis indicated an increase in the DMT1(IRE) mRNA relative to controls in duodenum. However, in the iron overloaded liver of these mice, there was no increase in either total or DMT1(IRE) mRNA, as measured by northern blot analysis.<sup>4</sup>

In this issue (see page 270), Trinder and colleagues report RNA in situ hybridisation and immunohistochemical studies of DMT1 in liver and duodenum of rats, in response to altered iron stores. Convincing evidence is given for the specificity of the anti-DMT1 peptide antibody. Both the riboprobe used to detect mRNA and the antibody used for protein staining are common to the IRE and non-IRE forms of DMT1, and so total DMT1 staining is described. In duodenal villi from iron deficient animals, RNA in situ hybridisation indicated an increase in DMT1 mRNA with respect to controls. Immunohistochemistry did not detect any DMT1 protein staining in the crypts in any iron status, under the experimental conditions used, but DMT1 staining was detected, beginning at the crypt–villus junction and reaching highest levels in the upper half of the villus. Protein staining of the villus was highest in iron deficiency and least in iron overload. The mechanism underlying the change in expression of DMT1 as the duodenal enterocyte migrates from crypt to villus is not yet clear, although these observations are consistent with regulation of the villus DMT1(IRE) via its 3' iron responsive element.

Trinder *et al* also describe some of the first observations on the pattern of expression of *DMT1* in the liver under different dietary iron conditions. Interestingly, a relative increase in the DMT1 protein staining and polarisation to the hepatocyte sinusoidal membranes was seen in iron excess. In liver, this different regulatory pattern (apparent upregulation and polarisation in iron excess) may indicate a difference in the regulatory response mechanism of DMT1 compared with that of the duodenal villi, probably involving expression of DMT1(non-IRE).

Polarisation of DMT1 to the sinusoidal plasma membranes of hepatocytes may explain the remarkable avidity of the liver for the highly reactive, non-transferrin bound (NTB) form of iron. This pool in normal individuals is

minute or undetectable in the serum, where iron is normally bound to transferrin. However, under conditions where transferrin is fully saturated with iron, such as in haemochromatosis, substantial serum NTB iron may be detected.<sup>9</sup> NTB iron stimulates both the formation of the highly reactive and damaging hydroxyl radical, and the peroxidation of membrane lipids. However, most serum NTB iron is extracted by the liver in the first pass.<sup>9, 10</sup> Trinder *et al* propose that DMT1 on the microvillus membrane of hepatocytes clears the portal blood of NTB iron, and so reduces the risk of unregulated iron uptake and oxidative damage to cells elsewhere in the body. Conversely, in iron deficiency, relative downregulation of DMT1 in the liver, and upregulation in the duodenal enterocyte may permit iron to bypass the liver, bind transferrin, and be transported to body cells according to transferrin receptor expression.

This tissue specific regulation of DMT1 expression pattern reflects both the bodily requirements for iron, and its potentially damaging effects. Iron is essential because it can be readily and reversibly oxidised and reduced between its two common valencies, ferrous (2+) and ferric (3+). This property is exploited by iron dependent proteins and enzymes, to permit oxygen transport and the redox reactions of respiration. However, in excess, iron catalyses formation of reactive free radicals, leading to tissue damage. Given that our regulation of body iron content is at the level of absorption, the differing DMT1

expression pattern in duodenum and liver is clearly important to ensure regulation of this essential but toxic metal ion.

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## Fibrosing colonopathy

Healing by fibrosis represents the end stage of a response to a variety of insults. Known antecedents to fibrosis in the colon include ischaemia, inflammation (e.g., Crohn's colitis), and noxious chemicals (e.g., irritant laxatives). Endogenous pancreatic enzymes may have a role in enhancing, even if not initiating, lesions which lead to fibrosis, as shown by animal experiments in which ligation of the pancreatic duct mitigated or delayed the response to ischaemia.<sup>1</sup>

It has been recognised fairly recently that a distinctive form of fibrosing colonopathy develops in young children with cystic fibrosis taking mega-doses of pancreatic enzymes, and it was suggested that the enzyme preparations themselves may be the cause.<sup>2</sup> The most striking feature is a thick band of submucosal fibrosis, suggesting that some component of the enzyme preparation must gain access through the mucosal barrier, but evidence of healed mucosal ulceration has only been observed in a few cases. Vascular damage and a moderate eosinophilic infiltration were observed in some cases, and in several there was quite noticeable serosal fibrosis. Clinically, in addition to the signs of intestinal obstruction, several patients had chylous ascites. These features suggest that translocation of a component of the enzyme preparation—whether the enzymes themselves or the enteric coating used to protect them from gastric acid—occurs across the full thickness of the colon. It may be that the higher absorption of sodium, glucose, amino acids, and water observed in the small intestine of patients with cystic fibrosis<sup>3</sup> occurs in the colon too, and physiological studies showing an increased potential differ-

ence across the rectal mucosa would support this speculation. It is not known how often subclinical colonic fibrosis occurs in cystic fibrosis, but it can be an incidental finding at autopsy.<sup>4</sup>

Against this background, Bansi *et al*'s case report (see page 283) is of great interest because it describes typical fibrosing colonopathy in an adult who was not thought to have cystic fibrosis, but who had taken very high doses of pancreatic enzyme supplements. The lessons would seem to be that fibrosing colonopathy is not confined to cystic fibrosis, nor to children. There is no doubt that this patient did not have classic cystic fibrosis, but the authors have not ruled out one of the many atypical conditions that are also associated with malfunction of the cystic fibrosis transmembrane regulator protein (CFTR). The 12 commonest CFTR mutations found in the British population have been excluded, but without completely sequencing the gene, it is virtually impossible to rule out a CFTR related disorder, because to date more than 800 different CFTR gene mutations have been described. It should be noted that the recurrent attacks of pancreatitis which led to pancreatico-duodenectomy in this patient are often associated with cystic fibrosis mutations, even when only a single copy is present.<sup>5</sup> The episodes of jaundice which occurred when the patient was younger could have resulted from common bile duct stenosis, which has also been attributed to cystic fibrosis.<sup>6</sup> The surgical finding of the caecum embedded in a solid mass of fibrous tissue is reminiscent of some early descriptions of patients with cystic fibrosis and intestinal obstruction following years of abdominal pain. These reports long antedated enteric coated enzymes.<sup>7</sup> Adhesions following surgery are a frequent problem in cystic fibrosis, and it may be that exogenous pancreatic enzymes have a damaging effect

on anastomoses. In retrospect, the earlier diagnosis of Crohn's disease in this patient may have been incorrect.

Fibrosing colonopathy is usually associated with excessive doses of pancreatic enzymes. The doses of enzymes consumed by this patient were very high, and similar to those found in the UK<sup>8</sup> and US<sup>9</sup> case control studies, namely around 40 000 lipase units/kg/day. The American study demonstrated that the risk of developing fibrosing colonopathy increased correspondingly with increasing enzyme dosage. The British study also found an apparent association between fibrosing colonopathy and the type of enteric coating, but this was not seen in the American study and may be related to other factors such as institutional brand preference. In addition, the confounding effect of switching between different brands of enzymes was a notable feature of the UK series. High doses of the order seen in these cases were made possible by the high strength preparations, but a similar dosage can be achieved in young children with standard strength preparations, and has been associated with fibrosing colonopathy.<sup>10</sup> None the less, some patients with cystic fibrosis seem to tolerate very high concentrations of pancreatin without developing fibrosing colonopathy, suggesting that in affected patients there may be additional factors which render their colon more vulnerable to enzyme damage.

One such factor might be diet. The patient described by Bansi *et al* was taking a low fat diet prior to her hemicolectomy, but no mention is made of the fibre content. It is well known that short chain fatty acids derived from fibre provide a large part of the nutrition to the colon, but in the past nutritional advice to patients with cystic fibrosis has been to recommend a high calorie, high carbohydrate, high protein, and, in recent years, normal or high fat content, and to allow this intake the component generally reduced was, by implication, fibre. In a recent study it was shown that gastrointestinal symptoms in children with cystic fibrosis were inversely correlated with the fibre content. The mean daily fibre intake in children with cystic fibrosis was less than half that of controls, and in those with severe gastrointestinal symptoms, it was only about a quarter of that of healthy controls. There was also a non-significant trend towards higher pancreatin intake in those children with more severe gastrointestinal symptoms.<sup>11</sup> I have suggested elsewhere that depriving the colon of short chain fatty acids

by limiting fibre intake, and then administering unnecessarily high doses of pancreatic enzymes, may be tantamount to adding insult to injury.<sup>4</sup> Fibre reduces paracellular permeability in the rat colon,<sup>12</sup> whereas increasing intestinal permeability (by oleic acid or reserpine) and then giving excessive doses of pancreatic enzymes produced intestinal eosinophilia and necrosis.<sup>13</sup>

Finally, although in their discussion Bansi *et al* follow convention by focusing on the dose of lipase taken by their patient, it would seem intrinsically more likely that other enzymes present in pancreatic preparations may be responsible for the damage observed. These enzymes include proteases and elastase, which are present in approximately similar proportions to lipase in all the enzyme products available. The epidemiological association between high enzyme doses and fibrosing colonopathy has been established. There is now clearly a need for experimental studies which will shed light on the pathogenesis.<sup>14</sup>

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