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Starch digestion, large-bowel fermentation and intestinal mucosal cell proliferation in rats treated with the α-glucosidase inhibitor acarbose

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Acarbose (Glucobay®; Bayer) is an α-glucosidase inhibitor used to treat diabetes and which may have a role in the prevention of type 2 diabetes. The present study investigated the effects of acarbose treatment on the site and extent of starch digestion, large-bowel fermentation and intestinal mucosal cell proliferation. Eighteen young male Wistar rats were fed 'Westernised' diets containing 0, 250 and 500 mg acarbose/kg (six rats/diet) for 21 d. For most variables measured, both acarbose doses had similar effects. Acarbose treatment suppressed starch digestion in the small bowel but there was compensatory salvage by bacterial fermentation in the large bowel. This was accompanied by a substantial hypertrophy of small- and large-bowel tissue and a consistent increase in crypt width along the intestine. Caecal total SCFA pool size was increased more than 4-fold, with even bigger increases for butyrate. These changes in butyrate were reflected in increased molar proportions of butyrate in blood from both the portal vein and heart. There was little effect of acarbose administration on crypt-cell proliferation (significant increase for mid-small intestine only). This is strong evidence against the hypothesis that increased fermentation and increased supply of butyrate enhances intestinal mucosal cell proliferation. In conclusion, apart from the increased faecal loss of starch, there was no evidence of adverse effects of acarbose on the aspects of large-bowel function investigated.

Acarbose: Starch digestion: Butyrate: Crypt width: Cell proliferation

With the inexorable rise in obesity worldwide, the incidence of non-insulin-dependent diabetes mellitus (type 2 diabetes) is expected to double over the next decade. Given the toll of morbidity and mortality associated with type 2 diabetes and the high costs of treatment, the prevention of this epidemic is a high public health priority. Acarbose is a pseudo-tetrasaccharide composed of a cyclitol unit bound to 4,6-dideoxy-4-amino-D glucopyranose, derived from the genus *Actinoplanes*, which is an effective α-glucosidase inhibitor suppressing the digestion of starch and sucrose in the small bowel (Bischoff, 1994). There is evidence that acarbose is not only an effective treatment for type 2 diabetes (Dimitriadis *et al.* 1985; Coniff *et al.* 1994; Holman *et al.* 1999) but also may help to prevent its development by improving insulin sensitivity (Laube *et al.* 1998) in those with impaired glucose tolerance. A very recent randomised, placebo-controlled trial has demonstrated the effectiveness of acarbose in preventing or delaying the development of type 2 diabetes in those with impaired glucose tolerance (Chiasson *et al.* 1998).

Whilst acarbose is a relatively safe drug and, therefore, is potentially suitable for use in chemoprevention, it may be contraindicated in Crohn's disease (Kast, 2002). The most frequent side effects of acarbose treatment are flatulence and diarrhoea (Chiasson *et al.* 2002), probably due to the flow of extra starch and sucrose to, and their fermentation in, the large bowel (LB). The diarrhoeal side effects are believed to result from the up regulation of the production of the prostaglandin E series because of an increased production of butyrate by the LB flora (Kast, 2002). It is well established that an increased fermentation of starch in the LB leads to an increased production of SCFA with a higher proportion of butyrate (Englyst *et al.* 1987; Goodlad & Mathers, 1988; Mathers *et al.* 1997). Such stimulation of LB fermentation has been accompanied by increased LB mucosal cell proliferation (Goodlad *et al.* 1987). Because increased cell division is an early event in the development of many human cancers (Preston-Martin *et al.* 1990), and there is evidence that enhanced cell proliferation in the LB mucosa is associated with greater colorectal cancer risk (Terpstra *et al.* 1987), there is an hypothetical possibility that dietary manoeuvres or drug treatments which enhance butyrate production and cell proliferation could carry an enhanced risk of colorectal cancer.

Abbreviations: CCP, crypt-cell proliferation; LB, large bowel; SI, small intestine.

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The objectives of the present study were to determine the effects of acarbose on the site and extent of starch digestion, LB fermentation and mucosal crypt-cell proliferation (CCP) in rats. The animals were fed high-starch diets rich in fat and animal protein to simulate aspects of human 'Western' diets. A brief account of part of the present study has been published (Kooshkghazi & Mathers, 1998).

Materials and methods

Animals, diets and feeding

The present study was carried out under licence in compliance with the Animals (Scientific Procedures) Act 1986 in the UK. Eighteen male Wistar rats (initial weight approximately 90 g) were housed individually in metabolism cages which allowed the complete separation of urine and faeces. Room temperature was maintained at 20–23°C with a 12 h light–12 h dark lighting regimen within the Comparative Biology Centre, University of Newcastle upon Tyne. Six rats were allocated at random to each of three experimental groups receiving a basal high-fat, semi-purified diet (Table 1) which differed only in acarbose content; 0, 250 and 500 mg acarbose/kg respectively (provided as Glucobay®; Bayer AG, 51368 Leverkusen, Germany). The diets contained chromic oxide (2 g/kg diet) as an indigestible marker to allow the quantification of starch digestion in the small bowel and LB. Each animal was offered 15 g diet/d for a 14 d adaptation period preceding a 7 d balance period during which food residues, faeces and urine were collected quantitatively.

Sample collection

At 2 h before being killed, each rat was injected intraperitoneally with vinicristine sulfate (1 mg/kg body mass) to arrest cells in metaphase for the estimation of CCP. At 10 min before tissue collection, terminal anaesthesia was induced by intraperitoneal injection (Hypnorm–Midazolam cocktail at 1 ml/300 g body mass). Blood samples from the portal vein (1 ml) and heart (2 ml) were collected into heparinised syringes and placed on ice. The gut (from stomach to rectum) and liver were removed and weighed and small intestine (SI) and colon lengths were measured. Caecal pH was recorded and digesta samples were collected from the terminal ileum (Goodlad & Mathers, 1990), caecum and colon for DM, organic matter, starch and chromic oxide determinations. Two samples of caecal digesta (each approximately 0·5 g) were mixed with deproteinising solution (Mathers *et al.* 1990) at 0·5 ml/g digesta in preparation for SCFA measurements. For CCP determinations, approximately 10 mm lengths of intestine from 10, 50 and 90 % of the lengths of the SI and colon and a 10 mm square from the body of the caecum were excised. These samples were washed with saline and fixed in Carnoy's solution (ethanol–acetic acid–chloroform, 6:1:3, by vol.) for 2–6 h and then transferred to 70 % ethanol for storage.

Table 1. Composition of the basal experimental diet

Ingredient	Content (g/kg diet)
Maize starch*	595
Maize oil	50
Lard	100
Casein†	200
Mineral premix‡	40
Vitamin premix§	10
D, L-Methionine‖	3
Chromic oxide	2

* Product no. S4126, Sigma Chemicals, Poole, UK.
† Product no. C7078, Sigma Chemicals, Poole, UK.
‡ Product no. 905455, ICN Biochemicals, Aurora, OH, USA.
§ Product no. 960098, ICN Biochemicals, Aurora, OH, USA.
‖ Product no. M95000, Sigma Chemicals, Poole, UK.

Sample processing and analysis

Faeces were freeze-dried, sieved gently to remove any spilled food particles and milled using a coffee grinder. Organic matter in food and faeces was determined by ashing (450°C overnight) the previously dried samples. Chromic oxide concentrations in food, freeze-dried digesta and faeces were measured as described by Goodlad & Mathers (1990). N in food, faeces and urine was determined by a micro-Kjeldahl method and SCFA in caecal contacts were determined by packed column GLC (Mathers *et al.* 1990). For SCFA in blood determinations, plasma samples were deproteinised by treatment with metaphosphoric acid solution (16 %, w/v) and by heating to 60°C before the separation and quantification of SCFA by capillary column GLC (F. Brighenti, individual communication). The starch contents of the diets, digesta and faeces were determined by acid hydrolysis (Englyst & Cummings, 1988) followed by determination of the released glucose using an enzymic colorimetric assay kit (Glucose GDH; Hoffmann-La Roche & Co., Basel, Switzerland) on a Cobas Mira automatic clinical analyser (Hoffmann-La Roche & Co., Basel, Switzerland). Metaphase-arrested cells in whole microdissected crypts were determined according to Goodlad & Wright (1982) and crypt lengths and widths measured using a graticule in the microscope eyepiece.

Calculations and statistical analyses

Flows of DM and starch through the intestine were calculated with reference to the indigestible marker chromic oxide (Goodlad & Mathers, 1990):

$$\text{DM flow rate (g/d)} = (\text{chromic oxide intake (mg/d)})/(\text{chromic oxide in digesta DM (mg/g DM)});$$

$$\text{Starch flow rate (g/d)} = (\text{DM flow rate (g/d)}) \times (\text{starch concentration in digesta DM (g/g)}).$$

Data were examined by ANOVA with the following *a priori* orthogonal contrasts: (i) basal diet *v.* acarbose-containing diets, and (ii) 250 *v.* 500 mg acarbose/kg diet, each tested against the between-animals within-diets error term with fifteen degrees of freedom. For almost all the variables investigated, there was no difference in response between 250 and 500 mg acarbose/kg diet.

Results

Food intake and growth

All animals remained healthy throughout the study. Those treated with acarbose ate significantly more food but gained about 30 g less weight than the control rats (Table 2). This reduced growth rate was associated with an almost 18-fold higher faecal DM output. Urine volume was unaffected by treatment (average 4·4 ml/d) but both urinary and faecal N outputs were higher in the acarbose-fed animals resulting in significantly ($P<0·001$) lower N retention (Table 3). It is noteworthy that the water content of the faeces from the acarbose-treated rats was approximately one-third greater than for those fed the basal diet.

Site and extent of starch digestion

Only 0·03 of the ingested starch appeared in the ileal effluent from the animals fed the basal diet whilst 0·76–1·08 flowed through the terminal ileum of the rats treated with acarbose (Table 4). During passage through the LB, 0·36 and 0·15 of the ileal-delivered starch disappeared with the 250 and 500 mg acarbose/kg diets respectively. Whole-gut starch digestibility was virtually 100 % for the basal diet but only approximately 30 % for the acarbose-treated animals. In addition to the extra starch, acarbose treatment increased the faecal output of non-starch DM approximately 3-fold (Table 4), some of which was in the form of N-containing compounds (Table 3).

Gastrointestinal compartments and liver

Acarbose treatment increased the masses of all gastrointestinal compartments with no significant ($P>0·05$) difference between the 250 and 500 mg acarbose/kg diet doses (Table 5). For the stomach, all of the increase was accounted for by the increased digesta content, which averaged 4-fold more in the drug-treated rats. For the SI, caecum and colon, the increases in organ masses included

Table 2. Effects of acarbose (ACA) on food intake, growth and output of faeces and urine*
(Mean values with pooled standard errors of the mean for six rats per treatment)

	ACA (mg/kg diet)				Probability of effect	
	0	250	500	SEM	Basal *v.* ACA	250 *v.* 500 mg ACA
Food DM intake (g/d)†	12·5	13·8	13·9	0·25	<0·001	0·8
Initial body mass (g)	91	93	89	1·4	0·13	0·3
Final body mass (g)	227	196	197	5·5	<0·001	0·9
Body-mass gain (g/d)	6·6	5·0	5·0	0·3	<0·001	0·9
Faecal DM output (g/d)†	0·4	6·3	6·1	0·24	<0·001	0·7
Faecal water (g/kg)†	299	395	391	15	<0·001	0·8
Urine (ml/d)†	4·3	4·1	4·7	0·27	0·63	0·1

* For details of diets and procedures, see Table 1 and p. 358.
† Measured over 7 d balance period.

Table 3. Whole-body nitrogen balance in rats treated with acarbose (ACA) at two doses*
(Mean values with pooled standard errors of the mean for six rats per treatment)

	ACA (mg/kg diet)				Probability of effect	
	0	250	500	SEM	Basal *v.* ACA	250 *v.* 500 mg ACA
N intake (g/7 d)	2·47	2·70	2·75	0·04	<0·001	0·46
Urinary N output (g/7 d)	1·20	1·66	1·84	0·09	<0·001	0·19
Faecal N output (g/7 d)	0·11	0·34	0·35	0·02	<0·001	0·70
N retention (g/7 d)	1·16	0·70	0·56	0·08	<0·001	0·27

* For details of diets and procedures, see Table 1 and p. 358.

Table 4. Starch intake, flows through the gut and digestibility in rats treated with acarbose (ACA) at two doses*
(Mean values with pooled standard errors of the mean for six rats per treatment)

	ACA (mg/kg diet)				Probability of effect	
	0	250	500	SEM	Basal *v.* ACA	250 *v.* 500 mg ACA
Intake (g/d)	6·22	7·02	7·36	0·3	<0·001	0·42
Ileal starch flow (g/d)	0·20	7·60	5·60	0·6	<0·001	<0·001
Faecal starch output (g/d)	0·02	4·83	4·77	0·2	<0·001	0·8
Starch digestibility (g/g)	1·00	0·29	0·32	0·03	<0·001	0·7
Non-starch DM output in faeces (g/d)	0·33	1·47	1·36	0·1	<0·001	0·7

* For details of diets and procedures, see Table 1 and p. 358.

Table 5. Tissue and digesta masses in each compartment of the intestine and liver mass in rats treated with acarbose (ACA) at two doses*

(Mean values with pooled standard errors of the mean for six rats per treatment)

	ACA (mg/kg diet)				Probability of effect	
	0	250	500	SEM	Basal *v.* ACA	250 *v.* 500 mg ACA
Stomach						
Total mass (g)	4·23	10·64	13·2	1·2	<0·001	0·2
Tissue (g)	1·59	1·28	1·49	0·1	0·15	0·2
Wet digesta (g)	2·64	9·36	11·7	1·2	<0·001	0·2
Digesta DM (g)	0·95	4·42	6·16	0·8	<0·001	0·1
Small intestine						
Length (mm)	735	908	878	25	<0·001	0·4
Total mass (g)	5·37	9·75	10·0	0·5	<0·001	0·6
Tissue (g)	5·17	8·54	8·77	0·4	<0·001	0·7
Wet digesta (g)	0·20	1·21	1·23	0·1	<0·001	0·9
Digesta DM (g)	0·04	0·68	0·62	0·3	0·05	0·2
Caecum						
Total mass (g)	1·90	13·2	13·3	0·3	<0·001	0·7
Tissue (g)	0·50	2·80	2·70	0·2	<0·001	0·98
Wet digesta (g)	1·40	10·4	10·6	0·3	<0·001	0·8
Digesta DM (g)	0·09	3·40	3·34	0·2	<0·001	0·9
Colon						
Length (mm)	138	193	205	6·0	<0·001	0·2
Total mass (g)	1·36	5·82	7·07	0·6	<0·001	0·1
Tissue (g)	1·02	2·70	3·03	0·2	<0·001	0·2
Wet digesta (g)	0·34	3·12	4·04	0·5	<0·001	0·2
Digesta DM (g)	0·11	1·43	1·95	0·2	<0·001	0·1
Liver						
Mass (g)	10·1	7·03	7·30	0·4	<0·001	0·6
Relative mass (g/kg body mass)	44·5	35	37	1·0	<0·001	0·4

* For details of diets and procedures, see Table 1 and p. 358.

significant increases in both tissue and digesta. The smallest increases were for the SI where length was increased by 21 %, tissue mass by 67 % and digesta mass by 510 %. Colon length, tissue mass and digesta mass increased by 44, 281 and 1053 % respectively in the acarbose-treated animals. A 5-fold increase in caecal tissue mass accompanied a 7·5-fold increase in digesta mass in the rats given acarbose in the diet. The increase in digesta wet masses were not due simply to extra water retention within the gut since the DM content of digesta in the acarbose-treated rats was consistently higher than for the control animals (Table 5).

In contrast to the gut, liver mass was about 30 % lower in the animals exposed to acarbose (Table 5), which was a comparatively greater decrease than that observed for whole-body mass (13·5 % lower in the acarbose-treated animals; Table 2) so that relative liver mass (g/kg body mass) was significantly ($P<0·001$) reduced by the drug treatment.

Caecal fermentation

Caecal pH was reduced significantly ($P<0·001$) in the acarbose-treated animals and was lower ($P=0·03$) in those treated with 250 compared with 500 mg acarbose/kg diet (Table 6). The only significant change in SCFA concentration was for acetate where values were about two-thirds of those seen in the control animals. However, the molar proportion of butyrate was almost doubled by the acarbose treatment ($P<0·01$). Given the large increase in caecal digesta mass in the acarbose-fed rats (Table 5), there were large increases in caecal SCFA pool sizes ranging from 5·1-fold for acetate and propionate to 10·4-fold for butyrate (Table 6).

Short-chain fatty acids in portal and cardiac blood

Acetate was the predominant SCFA in both portal and cardiac blood but propionate and butyrate were also present in measurable concentrations (Table 7). There was no effect of treatment on portal acetate concentration but acarbose feeding increased portal propionate and butyrate by 2·9- and 6·1-fold respectively, so that the molar proportion of acetate decreased whilst those for propionate and butyrate increased significantly.

Total SCFA concentration in cardiac blood was about one half of that seen in portal blood, with the proportional decrease being much greater for propionate and butyrate (Table 7). Despite the relatively small concentrations, significantly ($P<0·05$) greater concentrations of propionate and butyrate were evident in cardiac blood in the acarbose-treated animals.

Intestinal mucosal crypt dimensions

Samples were collected from three sites in the SI (10, 50 and 90 % of the length), from the caecum and from three sites in the colon (10, 50 and 90 % of the length) and the lengths and widths of whole microdissected crypts were measured. At all sites, there was a significant increase in crypt width in the acarbose-treated rats which ranged from 13 % in the proximal SI to 33 % in the mid section of the SI (responses

Table 6. Caecal digesta pH and concentrations, molar preparations and pool sizes of short-chain fatty acids in the caecum of rats treated with acarbose (ACA) at two doses*

(Mean values with pooled standard errors of the mean for six rats per treatment)

	ACA (mg/kg diet)				Probability of effect	
	0	250	500	SEM	Basal *v.* ACA	250 *v.* 500 mg ACA
pH	7·3	6·2	6·6	0·10	<0·001	0·03
SCFA concentrations (μmol/g caecal contents)						
Acetate	63	44·4	40·1	1·6	<0·01	0·6
Propionate	25	18·8	19·4	2·7	0·09	0·9
Isobutyrate	2·0	4·1	3·3	1·7	0·4	0·9
Butyrate	6·9	12·7	8·7	5·8	0·07	0·1
Isovalerate	1·9	2·0	4·0	1·3	0·5	0·2
Valerate	1·9	1·7	4·0	1·4	0·6	0·3
Total	95·2	79·1	79·6	12·8	0·04	0·5
SCFA molar proportions (mmol/mol)†						
Acetate	655	611	578	33	0·2	0·5
Propionate	269	231	287	23	0·7	0·1
Butyrate	75	159	134	21	<0·01	0·4
SCFA pool sizes (μmol/rat)†						
Acetate	83	397	360	45	<0·01	0·6
Propionate	33	168	170	27	<0·01	1·0
Butyrate	9	112	76	16	<0·01	0·1
Total	125	677	606	87	<0·01	0·5

* For details of diets and procedures, see Table 1 and p. 358.
† For major SCFA (acetate, propionate and butyrate) only.

at other sites were intermediate) (Table 8). The effect of acarbose on crypt length was much less consistent with significant changes (33 % increases) detected at the 50 % SI and 90 % colon sites only.

Intestinal crypt-cell proliferation

For all treatment groups, the highest rates of CCP were observed in the proximal SI with decreases to the caecum and in the colon (Table 9). Acarbose-treatment increased CCP by 50 % in the mid SI but was without detectable effects at other intestinal sites.

Discussion

The therapeutic effects of acarbose in the treatment of both insulin-dependent (type 1) and non-insulin-dependent (type 2) diabetes have been attributed to its action in slowing the SI digestion of carbohydrates by the competitive inhibition of brush-border α-glucosidases (Bischoff, 1994),

Table 7. Concentrations and molar proportions of short-chain fatty acids in portal and cardiac blood from rats treated with acarbose (ACA) at two doses*

(Mean values with pooled standard errors of the mean for six rats per treatment)

	ACA (mg/kg diet)				Probability of effect	
	0	250	500	SEM	Basal *v.* ACA	250 *v.* 500 mg ACA
Portal blood						
SCFA concentrations (μM)						
Acetate	1443	1449	1305	126	0·7	0·5
Propionate	119	276	422	44	0·03	0·1
Butyrate	16	112	83	14	<0·01	0·2
Total	1577	1837	1810	113	0·2	0·2
SCFA molar proportions (mmol/mol)						
Acetate	915	788	720	50	0·03	0·3
Propionate	75	150	233	38	0·04	0·1
Butyrate	10	60	46	17	0·05	0·6
Cardiac blood						
SCFA concentrations (μM)						
Acetate	689	949	735	111	0·3	0·2
Propionate	14	30	20	4·4	0·05	0·1
Butyrate	4	8	6	1	0·02	0·1
Total	707	987	761	145	0·2	0·8
SCFA molar proportions (mmol/mol)						
Acetate	975	961	965	7	0·1	0·7
Propionate	20	30	26	6	0·2	0·8
Butyrate	6	8	8	1	0·04	0·5

* For details of diets and procedures, see Table 1 and p. 358.

Table 8. Lengths and widths (μm) of intestinal mucosal crypts in rats treated with acarbose (ACA) at two doses*

(Mean values with pooled standard errors of the mean for six rats per treatment)

	ACA (mg/kg diet)				Probability of effect	
	0	250	500	SEM	Basal *v.* ACA	250 *v.* 500 mg ACA
Small intestine						
10 % site†						
Length	129	137	138	5·3	0·2	0·9
Width	34·2	37·2	39·8	1·4	0·02	0·2
50 % site						
Length	101	142	125	4·3	<0·001	0·02
Width	29·3	39·8	38·0	1·2	<0·001	0·3
90 % site						
Length	119	126	120	2·8	0·3	0·2
Width	29·1	35·4	38·0	1·3	<0·001	0·2
Caecum						
Length	122	124	121	3·2	0·9	0·5
Width	39·3	46·7	48·5	1·2	<0·001	0·3
Colon						
10 % site†						
Length	107	112	121	13	0·6	0·6
Width	29·1	35·1	37	1·3	<0·001	0·4
50 % site						
Length	204	212	225	13	0·4	0·5
Width	31·1	35·9	36·2	1·1	<0·001	0·8
90 % site						
Length	179	235	241	15	<0·001	0·8
Width	32·6	43·1	42·1	1·5	<0·001	0·7

* For details of diets and procedures, see Table 1 and p. 358.
† Samples taken from 10, 50 and 90 % of lengths of small intestine and colon.

leading to lower postprandial glucose and insulin responses (Couet *et al.* 1989). The present study's main aims were to investigate the effect of moderate–high doses of acarbose on LB fermentation and on intestinal mucosal cell proliferation, which are often altered when extra carbohydrate is supplied to the caecum (Calvert *et al.* 1989; Mathers *et al.* 1990; Key *et al.* 1996; Mathers *et al.* 1997). The doses of acarbose used in the present study provided 3·75 and 7·5 mg/15 g food which, on a body-weight basis, are equivalent to approximately 1000–1350 mg/d for human adults. For most of the variables measured, both doses of acarbose produced similar effects.

As anticipated, acarbose treatment suppressed starch digestion in the SI and, although there was some salvage of this energy through fermentation in the LB, about 70 % of the starch eaten was lost in the faeces. The increased faecal DM output (also seen in human subjects fed starches resistant to pancreatic α-amylase, i.e. resistant starch; Cummings *et al.* 1996) could be helpful in the prevention or treatment of constipation. Unsurprisingly, although eating 10 % more DM/d, weight gains by the rats treated with acarbose were 25 % less than those on the control treatment. The modest weight reduction seen in type 2 diabetics treated with acarbose (Dimitriadis *et al.* 1985; Hauner, 1999) is probably due to the suppression of SI carbohydrate digestion and, thus, the reduced absorption of energy-yielding nutrients.

Although treatment with acarbose had no effect on stomach tissue mass, it resulted in 1·7-, 5·5- and 2·8-fold increases in the masses of SI, caecal and colonic tissues

Table 9. Crypt-cell proliferation (metaphase-arrested cells/crypt per 2 h) in the intestinal mucosa of rats treated with acarbose (ACA) at two doses*

(Mean values with pooled standard errors of the mean for six rats per treatment)

	ACA (mg/kg diet)				Probability of effect	
	0	250	500	SEM	Basal *v.* ACA	250 *v.* 500 mg ACA
Small intestine						
10 % site	20·5	26·4	21·1	3·0	0·4	0·2
50 % site	15·6	26·5	20·3	2·9	0·04	0·2
90 % site	16·7	19·4	16·1	2·3	0·7	0·3
Caecum	7·3	11·1	9·2	1·3	0·1	0·3
Colon						
10 % site	3·7	1·8	1·3	0·8	0·4	0·3
50 % site	5·6	7·5	5·3	0·9	0·5	0·1
90 % site	4·8	8·2	7·5	1·5	0·1	0·8

* For details of diets and procedures, see Table 1 and p. 358.

respectively (Table 5). In support of this, we have reported (Mathers *et al.* 1997) that the inclusion of raw potato starch (largely undigested in the small bowel and so rich in resistant starch) in diets for rats also increased caecal and colonic tissue masses but was without effect on stomach tissue mass. This intestinal hypertrophy is probably an adaptive response to maximise the capacity of the gut to capture the energy in the food carbohydrates. There were significant increases in mucosal crypt width throughout the intestine, but increases in crypt length in the mid SI and distal colon only (Table 8). The latter is of interest since Weaver *et al.* (2000) found a greater number of cells per rectal crypt section in human volunteers treated with acarbose. It is probable that the growth of the SI will be accompanied by increased activities of brush-border hydrolases in the ileal mucosa as has been seen when breakfast cereals were supplemented with guar gum (Mathers *et al.* 1992) and when cooked beans (*Phaseolus vulgaris*; rich in slowly digested starch) were added to a white-bread-based diet (Key *et al.* 1996). However, when averaged across both acarbose-containing diets, the major site for salvage of carbohydrate energy in the acarbose-treated rats was the LB and hypertrophy of both the caecum and the colon facilitates the accumulation of undigested food materials for a sufficiently long time to allow extensive fermentation by the LB bacteria. No attempt was made in the present study to assess transit time through the gut but in an earlier experiment, where increased starch flow to the caecum was achieved by feeding raw potato starch, LB transit time was more than doubled with all of this effect occurring in the caecum (Mathers *et al.* 1997). It is clear that although acarbose treatment suppressed SI starch digestion, bacterial amylases were able to overcome partially the inhibitory effects of this drug so that about 30 % of the ingested starch was fermented in the LB (Table 4). Weaver *et al.* (1997) observed that, although the proportion of starch-fermenting bacteria in human faeces increased significantly ($P=0\cdot025$) in human subjects taking 600 mg acarbose/d, the faecal output of starch quadrupled, reinforcing the conclusion that LB salvage does not compensate completely for the suppression of SI starch digestion.

Following the pioneering studies of Scheppach *et al.* (1988), it is now well established that acarbose treatment leads to greater faecal outputs of SCFA, particularly of butyrate (Holt *et al.* 1996; Weaver *et al.* 1997, 2000). This probably results from greater flows of starch into the LB (Cummings *et al.* 1996) since there is substantial evidence from both *in vitro* (Englyst *et al.* 1987; Goodlad & Mathers, 1988; Weaver *et al.* 1992; Mathers & Goodlad, 1999) and *in vivo* studies (Mallett *et al.* 1988; Mathers & Dawson, 1991; Mathers *et al.* 1997) that starch fermentation yields much higher molar proportions of butyrate than do most other food carbohydrates. However, it remains unclear why starch fermentation results in such high butyrate yields. In conditions of carbohydrate excess when the availability of reduced dinucleotides could limit glycolysis, LB bacteria may synthesise more butyrate as a means of disposing of H to allow the regeneration of reduced dinucleotides (Macfarlane, 1991; Mathers & Dawson, 1991). *In vitro* fermentation of [^{13}C]glucose with faecal suspensions from subjects treated with acarbose resulted in a greater production of butyrate (Wolin *et al.* 1999). NMR analysis of fermentation endproducts from the same incubations indicated that exposure to the drug reduced the production of acetate via the Wood–Ljungdahl pathway of coupled oxidation of carbohydrates to acetate and CO_2 and the reduction of CO_2 to acetate (Wolin *et al.* 1999). It is probable that changes in H disposal are key to understanding alterations in fermentation patterns following acarbose treatment but, to our knowledge, no attempt has been made, as yet, to undertake the required H balance studies.

Since most SCFA produced in the LB are absorbed (McNeil *et al.* 1978; Ruppin *et al.* 1980), enhanced pool sizes of SCFA in the caecum were reflected in greater concentrations of propionate and butyrate but, perhaps surprisingly, not acetate in portal blood (Table 6). Butyrate is readily oxidised by colonocytes (Roediger, 1982; Ardawi & Newsholme, 1985) and is reported to be a preferred energy substrate for the colonic mucosa (Roediger, 1980). However, it is clear that raising the molar proportion of butyrate in caecal contents by dietary (Goodlad & Mathers, 1990; Key & Mathers, 1993) or drug treatment (Table 6) is effective in enhancing the butyrate supply to the liver. Demigné *et al.* (1986) suggested that the rat liver removes virtually all the afferent supply of propionate and butyrate but the present results (Table 7), and those from earlier studies with rat (Goodlad & Mathers, 1990) and pig (Goodlad & Mathers, 1991) models, indicate that butyrate can bypass the liver and appear in raised concentrations in the peripheral blood. These observations are in accord with those of Wolever & Chiasson (2000) who reported higher concentrations of butyrate in the peripheral blood from human subjects with impaired glucose tolerance who were treated with acarbose for 4 months. The metabolic and health consequences, if any, of the increased circulating concentrations of butyrate remain to be established.

In the present study (Table 8), there was little evidence that the greater production of butyrate (and other SCFA) from starch fermentation following acarbose administration increased CCP rate. This is in contrast with Weaver *et al.* (2000) who observed higher rates of cell proliferation and an upward shift in the distribution of dividing cells in rectal mucosal biopsies from human volunteers given acarbose for 4 months. This treatment also raised faecal butyrate output (Weaver *et al.* 2000). Previous studies have shown that both the ileal injection of SCFA (especially butyrate; Sakata, 1987) and the provision of additional fermentable carbohydrate to the LB by intra-caecal infusion of glucose (Miazza *et al.* 1985) or feeding NSP-rich diets (Goodlad *et al.* 1987; Johnson *et al.* 1988) increased mucosal cell proliferation rate. This led to the hypothesis that butyrate stimulates colonic cell proliferation, which may be counterproductive. This is because higher rates of CCP and/or an upward shift within the crypt in the distribution of mitotic cells have been associated with the increased risk of colonic neoplasia in some (Deschner *et al.* 1963; Lipkin, 1974; Terpstra *et al.* 1987; Mills *et al.* 1995) but not all studies (Green *et al.* 1998; Mills *et al.* 2001). Such proliferative changes are of potential interest as surrogate endpoints in colorectal cancer chemopreventive studies (Kelloff *et al.* 1994). We have argued

(Key *et al.* 1996) that increased LB mucosal proliferation in response to additional SCFA is seen when the starting point is a hypoproliferative mucosa (Sakata, 1987), i.e. the increase is a return to the normal state. Alternatively, an increased proliferation may be a transient phenomenon accompanying tissue hypertrophy and would be expected to revert to normal when the animal has adapted to the new dietary situation (Key *et al.* 1996). From investigations using $Apc^{\Delta 1309}$ gene knockout mice, which develop multiple intestinal adenomas spontaneously, there is some evidence that acarbose treatment may suppress tumour multiplicity in the stomach and reduce the size of tumours developing in the more distal intestine (Quesada *et al.* 1998). Whether this is related to changes in butyrate production or to CCP remains to be established.

In conclusion, the present study has shown that acarbose treatment is a very effective means of shifting digestion of starch from the SI to the LB. The increased fermentation of starch resulted in a greater production of SCFA and, in particular, butyrate which was reflected in higher concentrations of butyrate in the portal and peripheral blood. Despite substantial intestinal hypertrophy and consistent increases in crypt width throughout the intestine, there were few significant changes in CCP at any site. Given the potential anti-neoplastic benefits of enhanced butyrate production (Williams *et al.* 2003), these results suggest that there are unlikely to be adverse effects and, indeed, possibly some amelioration of the risk of colorectal cancer when acarbose is administered in the chemoprevention of type 2 diabetes (Chiasson *et al.* 2002).

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