Short-chain fatty acids produced by intestinal bacteria

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The colon is the major site of bacterial colonisation in the human gut and the resident species are predominantly anaerobes. They include potential pathogens but the greater proportion appear to be organisms which salvage energy through the metabolism of undigested carbohydrates and gut secretions. The major products of carbohydrate metabolism are the short chain fatty acids (SCFA), acetate, propionate and butyrate. In addition to general effects (such as lowing of pH) individual acids exert specific effects. All of the major SCFA appear to promote the flow of blood through the colonic vasculature while propionate enhances muscular activity and epithelial cell proliferation. Butyrate appears to promote a normal cell phenotype as well as being a major fuel for colonocytes. Important substrates for bacterial fermentation include non-starch polysaccharides (major components of dietary fibre) but it seems that starch which has escaped digestion in the small intestine (resistant starch) is the major contributor. Oligosaccharides are utilised by probiotic organisms and in the diet, act as prebiotics in promoting their numbers in faeces. High amylose starch is a form of RS and it appears to act as a prebiotic also. Although there is evidence that probiotics such as *Bifidobacteria* metabolise oligosaccharides and other carbohydrates, there appears to be little evidence to support a change in faecal SCFA excretion. It seems that any health benefits of probiotics are exerted through means other than SCFA.

Short chain fatty acids and bowel health

There is general acceptance of the role of the intestinal microflora in gastrointestinal health, especially in the colon which is the major site of bacterial colonisation of the human gut in terms of both numbers and species[1]. Most of these bacteria are anaerobes and include potential pathogens such as coliforms. However, by far the greater proportion are those microorganisms which salvage energy through the metabolism of dietary components (especially carbohydrates) which have escaped digestion in the small intestine and also endogenous gut secretions (which seem to be proteins mainly). Among the products generated by the metabolism of dietary carbohydrates are gases (H_2, CH_4 and CO_2), an increased biomass and short chain fatty acids (SCFA) -- the latter being the principal anions in this viscus[2]. The major acids are acetate, propionate and butyrate and are present at the same concentrations and approximate molar proportions as they are in the ruminal fluid of obligate herbivores such as sheep and cattle. In general terms, acetate appears to contribute 50-60% of total SCFA and propionate and butyrate 20-25% and 15-20%, respectively (depending on variables such as diet). There are other acids present including lactate isomers, valerate and also branched chain SCFA such as *iso*-butyrate and *iso*-valerate (formed by the catabolism of amino acids). Their concentrations are considerably lower than those of the principal acids[2].

SCFA are absorbed and enter the portal vein and appear to make a signficant contribution to energy through hepatic metabolism. However, it is their impact on the health of the colon which is addressed in this review. Indeed their quantitative importance in colonic digesta appears to be matched by those health benefits and it is clear that some of the effects ascribed to food components such as dietary fibre are due to these acids as well as to the processes which generate them[3]. It is the aim of this review to identify the interrelationships between colonic substrate supply, its bacterial population (especially probiotics) and SCFA production.

Health effects of SCFA

It is accepted that SCFA have general health benefits while individual acids exert specific effects (Table 1)[3]. Among the former are a lowering of colonic pH, a change which is thought to protect against colonic carcinogenesis through reducing the bioavailability of toxic amines. One of the side effects of the proliferation of bacteria which is a prerequisite for SCFA production is a fixing of nitrogen as bacterial protein. The main source of this is ammonia (a known cytotoxic agent) derived from urea. This leads not only to a raising of digesta pH but also a lowering of blood urea concentrations. The latter is very useful in individuals with problems of nitrogen metabolism such as hepatic coma. Lowering of intracolonic pH is believed to reduce the risk of the overgrowth of pathogenic micro-organisms and this attribute has been exploited in the management of iatrogenic infections in long-stay hospital patients. However, it must be noted that in co-culture experiments with *Bifidobacteria,* some of the suppression of growth of

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Table 1. General and specific health benefits of SCFA in the colon

SCFA	Specific effect	Benefit
Total SCFA	Lowering of pH	Diminished bioavailability of alkaline cytotoxic compounds
		Inhibition of growth of pH sensitive organisms
Acetate	Possible increase in Ca and Mg absorption	Diminished faecal loss of Ca and Mg
	Relaxation of resistance vessels	Greater colonic and hepatic portal venous blood flow
Propionate	Enhanced colonic muscular contraction	Easier laxation, relief of constipation
	Relaxation of resistance vessels	Greater colonic and hepatic portal venous blood flow
	Stimulation of colonic electrolyte transport	Greater ion and fluid absorption, prevention of diarrhoea
	Colonic epithelial proliferation	Possible greater absorptive capacity
Butyrate	Relaxation of resistance vessels	Greater colonic and hepatic portal venous blood flow
	Metabolism by colonocytes	Maintenance of mucosal integrity, repair of diversion and ulcerative colitis, colonocyte proliferation
	Maintenance of normal colonocyte phenotype	Diminished risk of malignancy
	Stimulation of colonic electrolyte transport	Greater ion and fluid absorption, prevention of diarrhoea

species such as *E. coli* and *C. perifringens* was pH independent and due to the secretion of an inhibitory substance other than SCFA[4].

In addition to their general effects, a number of specific properties have been identified for the major SCFA. In passing, it should be noted that this does not mean that the minor SCFA are not important but rather that their specific effects await to be elucidated. Acetate (like the other major SCFA) promotes the relaxation of resistance vessels in the colonic vasculature[5], a change which assists in the maintenance of the flow of blood to the liver as well as the colon. Acetate enhances the effects of propionate and butyrate in stimulating the absorption of Mg and other cations in the colon[6]. This stimulation is believed to assist in more efficient fluid absorption and prevention of diarrhoea. Propionate has been shown to enhance colonic muscular contraction[7], an effect which contributes to the promotion of laxation and the relief of constipation. Propionate also stimulates the proliferation of the colonic epithelium[8] which may enhance the absorptive capacity of the colon. One possible effect of propionate was reduction of plasma cholesterol concentrations[9]. It was thought that propionate, formed in the large bowel, was absorbed through the portal vein and inhibited hepatic cholesterol synthesis. This does not appear to be the case and there appears to be no major role for colonically-derived propionate in the control of plasma cholesterol[10].

Butyrate has attracted a great deal of interest as it appears to be the SCFA which makes the greatest contribution to the integrity of the colon. It appears to be the preferred metabolic fuel for colonocytes and so contributes directly to energy production[11]. The supply of butyrate appears to assist in the maintenance of mucosal integrity as its infusion leads to the rapid remission of ulcerative and diversion colitis[12]. Further, it appears that butyrate plays an important role in the maintenance of a normal cell phenotype and reduction of the risk of colonic carcinoma[13]. This conclusion has been reached from studies *in vitro* where butyrate at concentrations which are relevant physiologically has been shown to inhibit the growth of transformed colonocytes and to promote DNA repair. Studies *in vivo* support this role for butyrate although it must be noted that direct evidence for a protective role this acid in large bowel tumour formation is lacking[14].

Substrates for short chain fatty acids production

Given the importance of SCFA, the processes which control their production are of some considerable significance. Clearly, substrate supply is most likely to be one of these regulatory factors. The principal fuels for colonic SCFA production are carbohydrates and include the non-starch polysaccharides (NSP, "fibre") which are intrinsically resistant to the digestive enzymes of humans and other animal species[2]. Direct evidence of their role in regulating SCFA production has been obtained in numerous animal studies with increases in their concentration and pools in large bowel digesta after feeding diets enriched in NSP. Similar studies in humans have shown increased faecal excretion after ingestion of diets contained high fibre foods.

It had been thought that NSP were the dominant fermentative fuel as it was presumed that all starch was digested in the small intestine. This was inferred from the observation that little or no dietary starch is recovered in human faeces which was taken to mean complete digestion in the small intestine. This assumption is known now to be incorrect and studies with human ileostomists, animal models and *in vitro* suggest a substantial fraction of ingested starch can escape into the colon where it is fermented[2]. These studies indicate also that quantitatively it is the most important substrate for the large bowel microflora. The starch which is not digested in the small intestine is known as resistant starch (RS) on the basis that it is resistant to the action of human digestive enzymes. Studies *in vitro* had shown that some starch was not hydrolysed by amylases for a number of reasons including chemical structure and physical inaccessibility and it was presumed that the same situation obtained *in vivo*. What has emerged is that starch digestibility in the small intestine is influenced by many factors[15]. For example, starch can escape into the colon by virtue of the incomplete mastication of food which simply renders the starch inaccessible to amylases. Raw starches (such as in unripe bananas) are more indigestible than those in cooked foods where starch has been gelatinised through heating with water. This process lead to hydration of the starch and increased access for α-amylases. The chemical structure of starches also is important but largely in the context of retrogradation. Starch occurs in two forms-- amylose and amylopectin. The former is an unbranched polymer while

amylopectin has a highly branched structure. It appears that amylopectin gelatinises relatively easily when heated with water compared with amylose. While there seems to be relatively little intrinsic difference in digestibility between fully gelatinised amylose and amylopectin they respond differently to processing. During cycles of heating followed by cooling, RS can be generated in a process known as retrogradation during which the starch molecules become packed together. The straight chains of amylose allow this packing to occur more easily, giving a structure which is relatively resistant to amylolysis. This may explain the fact that in the presence of water and heat, high amylose starches gelatinise less readily than those high in amylopectin which packs less easily[15]. Finally, the RS content of a food can be increased by the number of heating and cooling cycles to which it is subjected.

In addition to NSP and RS, simpler carbohydrates can contribute to fermentation. In the case of lactose and fructose this occurs when their dietary level exceeds the digestive and/or absorptive capacity of the small intestine[2]. Generally, their contribution is low but this does not appear to be the case with oligosaccharides which are homo- and hetero-polymers with a degree of polymerisation of up to 10 monosaccharide units. Found widely in nature, oligosaccharides generally are resistant to human digestive enzymes and the evidence available indicates that they are fermented to short chain fatty acids and CO_2 and are incorporated into the colonic biomass[16].

One aspect of large bowel physiology which is not always appreciated deserves mention, that is the distribution of fermentative activity and the products along the length of the colon. Animal studies have shown that the concentrations and pools (the product of concentration x digesta mass) of total and individual SCFA are highest in the proximal colon and fall towards the distal colon[15]. A similar distribution pattern of SCFA excretion has been found in humans volunteers with colostomy[17]. The animal data suggest also that the concentrations and pools of SCFA in the distal colon cannot be predicted always from their values in the proximal colon. This has important consequences for degenerative bowel disease (which predominates in the distal large bowel) in that greater fermentation in the proximal colon bowel need not be associated with increased SCFA availability in the region at greater risk. Equally importantly, it is may not be possible always to infer SCFA in the caecum and colon from their faecal values.

Probiotics, carbohydrates and large bowel fermentation

There is an obvious connection between substrate supply and colonic bacterial metabolism. However, substrate availability regulates not only the overall rate of fermentation but also can change the relative proportion of the individual SCFA produced. This is especially important in the case of RS, the bacterial metabolism of which may favour butyrate formation. Studies *in vitro*[18] and in humans[19] have shown greater production and greater faecal excretion of butyrate, respectively, when the supply of RS to the colonic microflora was increased.

Due attention must be given also to the bacterial population of the colon as it those organisms which are responsible for effecting fermentation. Manipulation of the bacterial population to maximise health and minimise disease risk offers considerable promise both for public health as well as clinical practice. Obviously, this is the general principle of probiotics and there is good evidence that oral ingestion of live bacteria such as *Bifidobacteria* leads to changes including altered faecal bacterial enzyme activities[20], reduced side effects of antibiotics[21] and inhibition of experimentally-induced tumours in rodents[22]. That such ingestion leads to modification of the colonic population is supported by the appearance of live organisms in faeces[23]. All of these observations are consistent with colonisation of the gastrointestinal tract. We have cultured *Bifidobacteria* in samples from the proximal, median and distal colon of the pig after ingestion of live *Bifidobacterium longum* (Topping DL, Playne M, Warhurst M, Crittenden R, Davies D and Illman RJ; unpublished observations). In this experiment, bacteria were cultured also from faeces supporting the view that faecal recovery is indicative of colonisation. Of particular interest is the observation that counts were highest in the proximal colon, the region of greatest substrate availability and the highest SCFA concentrations.

However, there are some aspects to these dietary trials with live organisms which pose difficulties for therapy and management. Chief of these is the apparent refractoriness of the colonic bacterial population to the ingestion of probiotics[23]. It appears also that once subjects stop consuming live probiotics, faecal numbers of viable organisms decline very rapidly[24]. Clearly, there are a number of possible reasons for these findings of which the availability of substrate is one. Indeed, it has been shown that this may be the key factor in probiotic colonisation. Colonisation of the gut by *Bifidobacteria* in young infants is promoted by bifidus factor, a breast milk glycoprotein[25], and it is likely that the major fuels for these organisms are lactose and other milk components including oligosaccharides. Feeding trials in adult humans have confirmed the importance of the provision of substrate with selective increases in faecal numbers of bifidobacteria in humans fed inulin or a derived oligosaccharide, oligofructose[26]. It seems that bifidobacteria have the β-fructosidase which is necessary to cleave the β-1,2 glycosidic bonds for further metabolism but that competitor species do not[27].

The potential of suitable substrates to promote colonisation of the large bowel by probiotic microorganisms has been formalised by Gibson and Roberfroid[28] as the concept of 'prebiotics' which are nondigestible food ingredients which affect the host beneficially by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health. It is a very attractive idea in that it helps to explain the apparent difficulty experienced by investigators in effecting significant colonisation of the large bowel. Clearly, if probiotic bacteria were to lack the enzymes to metabolise dietary components such as NSP, then they would be at a competitive disadvantage vis-à-vis those bacteria which do possess them. The metabolism of

Table 2. Faecal *Bifidobacteria* excretion and short chain fatty acid concentrations in pigs fed *Bifidobacterium longum* and either a high amylose or low amylose maize starch

Faecal variable	Low Amylose		High Amylose	
	- Bifido	+ Bifido	- Bifido	+ Bifido
Faecal *Bifidobacteria* excretion (log_{10} cfu/day)	None detected	8.11 ± 0.21	None detected	8.89 ± 0.09
Faecal SCFA concentrations (mmol/L)				
Total	88.0	85.4	125.0	111.5
Acetate	51.6	50.9	60.2	54.5
Propionate	21.7	20.9	37.3	33.1
Butyrate	11.3	10.2	18.9	16.2

Mean ± SEM for *Bifidobacteria*, averages for SCFA, 11 animals per group of observations.

oligosaccharides by species such as *Bifidobacteria* lends support to the potential of such carbohydrates to function as prebiotics. However, a note of caution must be added in the interpretation of data on faecal bacteria. In our unpublished experiments described earlier, low faecal counts of *Bifidobacteria* were not necessarily representative of those in the proximal colon where significant numbers were noted (Topping DL, Playne M, Warhurst M, Crittenden C, Davies D and Illman RJ; unpublished observations). These data suggest that ingested probiotics might colonise the proximal colon without passage in excreted stool. These data are similar to those for with digesta and SCFA masses where values in the proximal colon cannot be extrapolated from those in the distal large bowel.

Up to the present, it has been accepted that oligosaccharides and related carbohydrates offered the greatest prebiotic potential for microorgansims such as *Bifidobacteria* and *Lactobacilli* and that starches were of limited value[28]. This perception is understandable, given the view (which prevailed until relatively recently) that starches were digested completely in the small intestine. However, with the emerging understanding of RS, it seemed appropriate to re-examine the prebiotic potential of starches. We have done so by feeding pigs a high amylose starch which shows a significant degree of resistance to amylolysis[29]. In these experiments, significantly higher faecal counts of *Bifidobacteria* were found in animals fed *Bifidobacterium longum* with a high amylose starch than with an amylopectin-rich starch (Table 2). There are a number of possible reasons for this increase. It may be simply a consequence of the greater availability of fermentative substrate when the animals consumed the high amylose starch. This is reflected in the greater faecal bulk when the animals were fed the amylomaize starch compared with when they were fed the starch high in amylopectin. Alternatively, the high amylose starch may have offered a degree of protection to the *Bifidobacteria* on passage through the upper gastrointestinal tract. Whatever the precise reason, it appears that high amylose starches offer the potential to act as prebiotics, an attribute which may be of value in the production of novel foods.

SCFA and the health benefits of probiotics

Given the interest in the actions of probiotic organisms and SCFA, the obvious question is: do SCFA mediate the health benefits ascribed to organisms such as *Bifidobacteria* and *Lactobacilli*? Up to the present, the answer would seem to be a qualified "no". Bartram and co-workers have reported that the ingestion of live *Bifidobacterium longum* by humans does not lead to any change in faecal SCFA or in other parameters (such as the relative concentrations of secondary bile acids) which would indicate altered fermentation[23]. These authors made the point that the effects of the probiotic could be exerted in the proximal colon and any changes might not be apparent in faeces. If this were to be the case then it argues against the concept that beneficial effects in the distal colon (the site of greatest degenerative bowel disease) are mediated through SCFA. Of course, it could be that the lack of any effects was due to the absence of appropriate substrate. This is a reasonable suggestion as it is known from studies *in vitro* that probiotics can metabolise prebiotics such as oligosaccharides and that this fermentation leads to the generation of SCFA as well as a greater biomass. Studies in rats have produced similar results with a significant increase in large bowel SCFA[16]. Other animal feeding trials with oligosaccharides have shown effects such as increased Ca and Mg absorption[30] and a modest degree of proliferation of colonocytes[31]. These changes are consistent with increased SCFA generation. Further, feeding trials with oligosaccharides have shown that they raise faecal *Bifidobacteria* numbers in humans[26]. However, in this study again there was no change in SCFA excretion suggesting that substrate availability was not a limiting factor. This view is supported by our experiments with pigs fed the two starches where the high amylose starch raised faecal total SCFA and propionate and butyrate. There was no additional apparent effect of *B. longum* ingestion (Table 2). However, as has been mentioned, none of these experiments preclude the possibility of changes in SCFA in the proximal colon. Thus, it remains possible that probiotics increase SCFA generation (through the metabolism of carbohydrates) but that this increase need not lead to greater faecal excretion of SCFA.

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由腸道細菌製造的短鏈脂肪酸

摘要

在人體腸道，結腸是細菌集居的主要部位，寄生的種類以厭氧菌為主，它們包括潛在的病原菌，但大多數是通過攝取未消化的碳水化合物和腸道分泌物取得能量。碳水化合物代謝的主要產物是短鏈脂肪酸、醋酸鹽、丙酸、丁酸鹽，除一般的作用之外（例如降低pH），各種酸有其特異的作用。所有的短鏈脂肪酸似乎通過結腸脈管系統促進血流，而丙酸增強肌肉的活動和上皮細胞增生。丁酸鹽似乎促進正常細胞表型，而且是結腸細胞的主要燃料。細菌發酵的重要基質包括非澱粉多糖（食物纖維的主要成分），但未在小腸被消化的澱粉（Resistant starch）似乎是主要基質。寡糖被原生菌（Probiotic）利用，在飲食中作為前生物，增加原生菌（Probiotic）在糞質中的數量。高直鏈澱粉是抗消化澱粉（Resistant starch）的一種形態，它似乎也具前生物效應。雖然有証據表明原生菌（Probiotic），比如勞根式不規則小杆菌（Bifidobacteria）代謝寡糖和其他碳水化合物，未有証據支持糞便短鏈脂肪酸分泌的改變，看來原生菌（Probiotic）對人體健康的效益不是通過短鏈脂肪酸的方式。

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