LACTOSE INTOLERANCE REVISITED


Short-chain fatty acid formation at fermentation of indigestible carbohydrates

By Åsa Henningsson, Inger Björck and Margareta Nyman

ABSTRACT
Short chain fatty acids (SCFAs; acetic, propionic and butyric acid) are formed during bacterial fermentation of carbohydrates in the colon. The interest in SCFA production is related to an increasing body of knowledge of the physiological effects of these acids. SCFAs are important anions in the colonic lumen and serve locally as nutrients for the mucosa cells, stimulating mucosal proliferation and blood flow. Especially butyric acid has been emphasized. It is the main energy substrate for the colonocytes and has been suggested to play a role in the prevention and treatment of diseases of the colonic mucosa, such as distal ulcerative colitis and cancer. SCFA production decreases the luminal pH, and may thereby stimulate mineral absorption and reduce secondary bile acid formation in the colon. Colonic generation of SCFAs has also been related to systematic and metabolic effects, e.g. SCFAs may influence the motility along the gastrointestinal tract and propionic acid has been suggested to inhibit the cholesterol synthesis from acetic acid in the liver. The SCFA formed at fermentation is quantitatively and qualitatively influenced by the type and amount of carbohydrate substrate. Further, certain combinations of carbohydrates may have synergistic effects on the SCFA pattern and may also shift the site of fermentation. This opens possibilities to design foods with tailored features regarding SCFA release in the human colon with potential health implications. There is a potential that in the future it will be possible to control SCFA production in the colon regarding pattern and place for release.

Keywords: Carbohydrates, dietary fibre, fermentation, SCFA, resistant starch

Introduction
In Western societies, about 20–60 g of the daily carbohydrate intake escapes digestion and absorption in the small intestine and reaches the colon as a potential source for fermentation (1). A considerable microbial flora, which amounts to $10^{11}$ bacteria per gram content, is present in this organ. The colonic microflora contains about 400 to 500 bacterial species, but most are in small numbers (2). Most of the microorganisms are saccharolytic. The fermentation products, with carbohydrates as fermentation substrate, are mainly short-chain fatty acids (SCFAs; acetic, propionic and butyric acid) and gases (CO$_2$, CH$_4$ and H$_2$). These end products are either excreted in the stool or absorbed from the colon (Figure 1). In the 1960s, SCFAs were believed to be poorly absorbed, causing diarrhoea through osmotic fluid retention in the stool. However, it is now known that about 90% of these SCFAs are rapidly absorbed by the colon, stimulating water and sodium absorption (3). Fermentation of indigestible carbohydrate to SCFAs thus reduces the osmotic load and there is also increasing evidence that the individual SCFA may have specific roles, including beneficial health implications.

Nutritional implications of short-chain fatty acids
The SCFAs absorbed from the colon can be utilized as an energy source by the host, but they contribute only to a small part (5–10%) of total energy in healthy individuals on Western diets (4). The colonic mucosa obtains its energy by oxidizing mainly SCFAs in the order of butyric>propionic>acetic acid (5). The SCFAs that escape metabolism in the colonocytes enter the hepatic portal blood. Acetic acid is utilized by the liver where it is transferred into Acetyl-CoA, which can act as a precursor for lipogenesis (6), but also stimulates gluconeogenesis (7). Low concentrations of acetic acid can also be detected in venous blood in peripheral tissues (8).

SCFAs formed at fermentation of carbohydrates in the colon have been reported to affect carbohydrate metabolism (9). Thus, barley containing high amounts of fermentable carbohydrates improved glucose tolerance in healthy subjects compared to rice with a lower amount of indigestible carbohydrates. The effect may be ascribed to propionic acid. Propionic acid is mainly metabolized in the liver and has been shown to inhibit gluconeogenesis and increase glycolysis in rat hepatocytes (10). It has also been proposed that propionic acid may lower plasma cholesterol concentrations by inhibiting hepatic cholesterologenesis (11).

However, the results from studies examining the effects of propionic acid on cholesterol metabolism are not consistent.

Figure 1. Carbohydrate fermentation in the human colon.

---

*Correspondence: E-mail: Margareta.Nyman@inl.lth.se

The article is based on a lecture presented at the meeting Lactose intolerance revisited, February 1-2, 2001, Stockholm, Sweden.
Both lack of cholesterol lowering (12) and increased plasma cholesterol levels (13) have been observed in pigs caecally infused with propionate. In humans, however, the synthesis of cholesterol from acetic acid decreased when propionate was ingested, and reduced histological scores, in ulcerative colitis patients. The effect appeared to be concentration-dependent, and lower concentrations of butyric acid had no effect (23). A recent intervention study in ulcerative colitis patients has demonstrated reduced symptoms regarding abdominal pain and faecal bulk rather than contribute to SCFA production (37). RS of barley, oat and wheat bran are fermented to various extents in rats (39,40), and in rats retrograded starch has been found to be less fermented than native starch (RS2) (40).

Most mono- and disaccharides are rapidly absorbed in the gut, whereas indigestible carbohydrates such as cellulose, resistant starch and fructooligosaccharides have been shown to be protective against carcinogenesis in the colon of chemically induced cancer models (27,28). However, Zoran and co-workers found that wheat bran reduced tumour incidence in a rat model independently of butyric acid concentration (29).

In addition to serving as a nutrient to the mucosa cells, SCFAs have other specific colonic effects such as increasing mucosal blood flow (30). Further, the decreased luminal pH induced by SCFA production may stimulate mineral absorption through increased mineral solubility (31,32). A lower luminal pH may also reduce secondary bile acid formation (33). Further, SCFAs have been shown to influence the gastrointestinal motility by mechanisms that are not yet fully understood (34).

### Impact of substrate

The content and distribution of SCFAs is dependent on the colonic microflora and/or the type and amount of indigestible carbohydrate available. The bacterial species present in the colon use different fermentation pathways (35,36), leading to differences in the SCFA pattern generated. Indigestible carbohydrates that reach the colon are mainly non-starch polysaccharides (NSP), resistant starches (RS) and certain oligosaccharides. The fermentability of NSP is highly dependent on its physico-chemical properties. Soluble fibres, e.g. pectin and guar gum, are fermented more readily than insoluble fibres, e.g. cellulose and wheat bran. These types of NSP instead, create faecal bulk rather than contribute to SCFA production (37).

### Table 1. The pattern of SCFA formed from the fermentation of various carbohydrates by human faecal bacteria in vitro or in rat caecum.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Composition</th>
<th>Molar distribution of SCFA (%)</th>
<th>Model</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acetic</td>
<td>Propionic</td>
<td>Butyric</td>
</tr>
<tr>
<td>Cellulose</td>
<td>Glucose</td>
<td>61</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>β-Glucan</td>
<td>Glucose</td>
<td>61</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>(barley)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(out)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guar gum</td>
<td>Mannose and galactose</td>
<td>69</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Inulin</td>
<td>Fructose</td>
<td>43</td>
<td>31</td>
<td>26</td>
</tr>
<tr>
<td>Lactose</td>
<td>Galactose and glucose</td>
<td>62</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>Pectin</td>
<td>Galacturonic acid, rhamnose, galactose, arabinose</td>
<td>57</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>Raffinose</td>
<td>Fructose and glucose</td>
<td>57</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>Starch</td>
<td>Glucose</td>
<td>51</td>
<td>14</td>
<td>43</td>
</tr>
<tr>
<td>Sugar beet</td>
<td>Fructose and glucose</td>
<td>69</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Wheat bran</td>
<td></td>
<td>63</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>53-73</td>
<td>13-25</td>
<td>8-28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38-66</td>
<td>12-26</td>
<td>22-36</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fructose and glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fructose and glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In addition to serving as a nutrient to the mucosa cells, SCFAs have other specific colonic effects such as increasing mucosal blood flow (30). Further, the decreased luminal pH induced by SCFA production may stimulate mineral absorption through increased mineral solubility (31,32). A lower luminal pH may also reduce secondary bile acid formation (33). Further, SCFAs have been shown to influence the gastrointestinal motility by mechanisms that are not yet fully understood (34).
SCFA from carbohydrate fermentation is poorly absorbed by most of the adult population in the world. Lactose is malabsorbed in the small intestine it reaches the colon amino acid fermentation yields branched SCFAs. In vitro studies, where the substrates are incubated with human faecal inocula, are easy to perform, but lack of standardization makes comparisons difficult. Further, the food needs to be subjected to in vitro digestion to mimic the action of the small intestine prior to the fermentation tests.

Rats and pigs are the most frequently used animals for fermentation studies. Pigs are colon fermenters, like man, and they also have a similar microflora to man (44). However, in pigs there is also a considerable fermentation in the ileum (45), which may affect the results, and dietary fibre fermentation has been reported to be considerably higher in pigs than in man. In contrast to man, the rat is a caecum fermenter. However, the degree of fermentation of a variety of dietary fibres has been shown to correlate well between rat and man (46). Further, there are similarities in SCFA pattern when comparing in vitro fermentation data for different dietary fibres (i.e. pectin and soybean fibre), using rat and human faecal inocula (47).

The molar distribution of acetic, propionic and butyric acid produced at fermentation of indigestible carbohydrates varies between substrates, as demonstrated in Table 1. Although the relative proportion of the organic end products from fermentation may vary, acetate is the major anion. Pectin is a particularly good source for acetic acid (48,49), whilst arabinogalactan and guar gum are the poorest sources (48,50,51). By contrast, arabinogalactan and guar gum have been shown to give high amounts of propionic acid. Butyric acid production varies over a wide range. Starches have been shown to give high proportions of butyric acid in vitro (49,50,52). In humans it has been demonstrated that administration of an α-amylase inhibitor, acarbose, to subjects resulted in enhanced butyric acid in faeces, measured either as concentration or as percentage of total SCFAs (53). However, studies in rats (54-56) and pigs (45,57,58) have shown important variations in fermentation profiles for RS. An explanation could be that the production of butyric acid may vary for different sources of RS. In addition, the time allowed for adaptation of the microflora to the RS may have an impact on the SCFA pattern. We have found in rats fed raw potato starch, that an adaptation period of 4 w gave significantly higher proportions of butyric acid in caecum (19%) compared to an adaptation period of 2 w (6%) (Henningsson Å, Nyman M, Björck I, unpublished results). A prolongation of the adaptation (6 w) did not have any further effect on butyric acid proportion. β-glucans and raffinose are other substrates that have been shown to give high amounts of butyric acid upon fermentation (48,49,52).

In contrast, fermentation of lactose with human faecal inocula in vitro was reported to give mainly acetic acid, and when the amount of lactose increased, the proportion of acetic acid increased further (59). Lactulose is an indigestible disaccharide and is often used to mimic lactose malabsorption. It is completely indigestible (60), and the fermentation pattern is identical to that of lactose (42).

Most studies have been performed on substrates tested as single sources of indigestible carbohydrates, which is not representative of a human diet that contains a complex mixture of carbohydrates. The combination of different indigestible carbohydrates may influence the fermentation pattern, and a mixture of pectin and guar gum gave a higher proportion of butyric acid in the caecum of rats (15%) compared to the individual substrates (10 and 6%, respectively) (Henningsson Å, Björck I, Nyman M, unpublished results). Further, the site for SCFA generation in the colon is likely to be dependent on how rapidly fermented the carbohydrate is. Interestingly, it has been demonstrated that the fermentation of easily fermentable high-amylase maize starch could be shifted to the distal colon of rats, when fed in mixture with a slowly fermentable dietary fibre, e.g. psyllium (61). This is an interesting observation since it provides a dietary tool to increase butyric acid formation in the distal colon where most colonic cancers appear in humans (62).

Conclusions and future perspectives
There are indications that SCFAs may have health-promoting effects, both locally in the colon and systemically, e.g. on glucose and cholesterol metabolism. The design of carbohydrate foods that generate specific SCFAPatterns at controlled sites in the large bowel is a challenge for the future with possible health consequences, e.g. in the prevention and treatment of colonic diseases.

REFERENCES


