Propionate as a health-promoting microbial metabolite in the human gut

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Propionate is a major microbial fermentation metabolite in the human gut with putative health effects that extend beyond the gut epithelium. Propionate is thought to lower lipogenesis, serum cholesterol levels, and carcinogenesis in other tissues. Steering microbial propionate production through diet could therefore be a potent strategy to increase health effects from microbial carbohydrate fermentation. The present review first discusses the two main propionate-production pathways and provides an extended gene-based list of microorganisms with the potential to produce propionate. Second, it evaluates the promising potential of arabinoxylan, polydextrose, and L-rhamnose to act as substrates to increase microbial propionate. Third, given the complexity of the gut microbiota, propionate production is approached from a microbial-ecological perspective that includes interaction processes such as cross-feeding mechanisms. Finally, it introduces the development of functional gene-based analytical tools to detect and characterize propionate-producing microorganisms in a complex community. The information in this review may be helpful for designing functional food strategies that aim to promote propionate-associated health benefits.

INTRODUCTION

The microbial community in the human gastrointestinal tract plays a substantial role in health and disease.[1] This intestinal microbiota elicits a beneficial relationship with the human host by modulating immunological functions[2] and by affecting the growth and functioning of host cells.[1] On the other hand, the gut microbiota may negatively affect the host through increased obesity,[3] inflammatory bowel diseases,[1] and colorectal cancer.[4]

Short-chain fatty acids (SCFAs) are the major products of colonic bacterial fermentation of dietary carbohydrates. The main compounds are acetic, propionic, and n-butyric acid, occurring roughly in molar ratios of 60:20:20 in the colon.[5] These anions play a crucial role in both intestinal morphology and function.[6] Butyrate has received much attention as an energy source for colonocytes.[7] Furthermore, it has been described as an anticarcinogenic agent preventing growth[8,9] and stimulating differentiation[10] of the colon epithelial cells. Acetate is used as a substrate for liver cholesterol and fatty acid synthesis,[11,12] increases colonic blood flow and oxygen uptake, and enhances ileal motility by affecting ileal contractions.[6]

The present review focuses on the potential health effects of just one SCFA – propionate. Although propionate is less frequently studied compared to other microbial metabolites, such as butyrate, it has some distinct health-promoting properties. The objective of this review is therefore to focus on the potential health effects of propionate and provide more insight into the propionate production mechanism in the gut and how one can modulate propionate production with dietary substrates. Possible microbial interactions between propionate producers and other intestinal microorganisms are discussed, as are some current and new strategies for the detection and identification of propionate producers.

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POTENTIAL HEALTH EFFECTS ATTRIBUTED TO PROPIONATE

Excess propionate and an inability for it to convert to methylmalonyl CoA through propionyl-CoA causes propionic acidemia. Propionic acidemia, as the most frequent disorder of organic acid metabolism in humans, is an inborn error of metabolism caused by the genetic deficiency of propionyl-CoA carboxylase.[13] Despite this toxicity aspect, propionic acid has been shown to have antilipogenic and cholesterol-lowering effects.[14] It also elicits strong effects towards weight control and feeding behavior.[15–17] Furthermore, there is evidence that propionate exerts, just as butyrate, an antiproliferative effect towards colon cancer cells.[10,18] It must be stressed that knowledge of in vivo colonic propionate concentrations or SCFA concentrations in general is insufficient to deduce health effects. Comparison of portal blood with colon content shows that colonocyte sorption of SCFA is highly efficient, with portal blood concentrations being a minor fraction of the colonic concentrations. Yet, unlike butyrate, which is used by the colonocytes as an energy source, propionate is found in higher concentrations in the circulation.[19] For propionate, the effects of which in the colonocyte are less known, physiological concentrations are still found to have health effects in both human and animal cell cultures.[11,20] Therefore, the biological activity of propionate may not be restricted to the colon itself, but extend to other parts of the human body. The specific health effects of propionate are summarized in Table 1.

Propionate influences lipid synthesis by hepatocytes

Lipid synthesis by the liver includes the conversion of diet-derived fatty acids and glycerol into cholesterol and triglycerides with different fatty acid compositions. These hepatic lipid molecules are then incorporated into lipoproteins, to allow distribution to various tissues through the circulation. Interestingly, lipid synthesis in hepatocytes is strongly affected by the amounts and types of SCFAs produced through fiber fermentation in the gut.[12,13] Propionate, in particular, has been determined to play a substantial role in some of these processes; however, debate remains about the exact mechanism of its cholesterol-lowering and antilipogenic effects.

Early observations of dietary modulation of hepatic lipid synthesis revealed a strong correlation with dietary fiber intake. There is extensive in vivo information on the correlation between plant fiber ingestion and the synthesis of cholesterol and triglycerides in experimental animals as well as in humans.[21,22] These studies have shown that oral administration of soluble fibers, such as pectin or guar gum, significantly decreased serum cholesterol concentrations. This effect was partially explained by the following: 1) increased fecal excretion of choles-

Table 1 **Health effects attributed to propionate.**

Study	Effect	Reference
Lipogenesis in hepatocytes		
In vivo study with inulin-fed rats	Decreased serum cholesterol levels	Illman et al. (1988)[23]
In vitro study with isolated rat hepatocytes	Inhibition of fatty acid synthesis	Nishina and Freedland (1990)[12]
In vivo study with inulin-fed rats	Decreased liver lipogenesis	Delzenne and Williams (2002)[14]
In vivo rat study	Decreased hepatic and plasma cholesterol levels	Adam et al. (2001)[24]
Satiety		
In vivo rodent study	Upregulation of GLP-1 and PYY	Zhou et al. (2008)[17]
In vivo rat study	Increased levels of GLP-1 and PYY in cecal pool, proximal colon, and portal serum, decreased levels of ghrelin	Delzenne et al. (2005)[29]
In vivo study with lactating dairy cows	Decreased energy intake and meal size, increased intermeal interval	Oba and Allen (2001)[15]
In vivo human study	Greater feeling of fullness, less hungry, and reduced desire to eat	Ruijschop et al. (2008)[16]
In vivo mice study	Doubled plasma levels of leptin	Xiong et al. (2004)[20]
In vitro mice study	Stimulated leptin expression in both a mouse adipocyte cell line and mouse adipocyte tissue in primary cultures	Xiong et al. (2004)[20]
In vitro study with human visceral adipose tissue	Induced leptin production in both mRNA and protein levels	Lahham et al. (2008)[33]
Cancer		
In vitro study with colon cancer cell lines	Antiproliferative effect	Scheppach et al. (1995)[34]
In vitro study with colorectal carcinoma cells	Induction of apoptosis	Jan et al. (2002)[18]

terol and bile acids from the gut; 2) higher hepatic conversion rate of cholesterol into bile acids; and 3) optimized peripheral metabolism of lipoproteins by decreasing the chylomicron size and lowering the incorporation of cholesterol into chylomicrons.[21]

Further analysis of dietary fiber experiments pointed to a specific role of SCFAs, as end products of microbial carbohydrate fermentation, in hepatic lipid synthesis.[12,13] Yet, the effect of SCFAs on fat synthesis and cholesterol levels should be viewed in terms of the type of SCFA produced. More specifically, propionate as a product of intestinal fiber fermentation has been shown to reduce serum cholesterol levels when fed to rats.[23] In vitro research with isolated rat hepatocytes showed an inhibitory effect of propionate on fatty acid synthesis, but not on cholesterol synthesis, although propionate decreased the incorporation of [1-^{14}C]acetate into sterols by 90%.[13] In addition, propionate has been identified as a molecule that decreases liver lipogenesis in inulin-fed rats.[14] Other rat experiments demonstrated that inclusion of whole-flour diets decreased both hepatic and plasma cholesterol levels, as well as cholesterol in plasma triglycerides, whereas hepatic triglycerides were not affected. Besides several mechanisms proposed in this study, the authors also mentioned the effect of increased propionate concentration in the portal vein, associated with whole-fiber diets, on cholesterol and fatty acid synthesis.[24]

The mechanism of propionate-induced inhibition of lipid synthesis has been investigated by Lin et al[11] using rat hepatocytes and [^{14}C]acetate. The researchers observed a 50% inhibition in cholesterol and triglyceride synthesis in the presence of a propionate concentration of 0.1 mmol/L. Using 10–100-fold higher levels of labeled acetate, they rejected the possibility that propionate competed with labeled acetate and decreased cholesterol and fatty acid synthesis due to the dilution of the precursor pool. It was therefore suggested that propionate may affect the activity of a common key enzyme, such as acetyl-CoA synthetase.[12] Indeed, when acetate enters the hepatocytes, it is mainly converted to acetyl-CoA by acetyl-CoA synthetase and then enters the cholesterol and fatty acid synthesis cycle. Propionate also has a competitive effect towards the protein that is allocated at the entry of acetate into liver cells. This inhibition would thereby contribute to a decrease in cholesterol and fatty acid synthesis.[14]

Despite the convincing results of these studies, it was not always possible for other studies to confirm an inhibitory effect of propionate on lipid metabolism. For example, a daily dietary supplementation of 9.9 g sodium propionate in bread did not change lipid metabolism in six healthy volunteers and even resulted in increased triglyceride concentrations in five of the subjects.[25] In another study, the effect of propionate towards lipid metabolism was compared between human and rat hepatocytes.[12] An inhibitory effect of propionate was found, at a concentration of 0.1 mmol/L, on lipid synthesis from acetate in rats. However, in human hepatocytes, a higher concentration of propionate, of about 10–20 mmol/L, was required to obtain the same inhibitory effect. This value is 100–200-fold higher than the concentration of propionate in portal blood, indicating that the rat models cannot be completely extrapolated to the human situation.[12] In some other studies, the administration of propionate in the cecum of pigs, or perfusion of propionate to the human colon, did not affect serum cholesterol at all.[26,27]

Propionate as a molecule influencing satiety

In addition to having cholesterol-lowering and antilipogenic effects, propionate may be involved in weight control by stimulating satiety. The roles of SCFAs (acetic acid, propionic acid, butyric acid) as satiety-inducing triggers have been claimed in previous studies.[17,28] There is evidence that bacterial regulation of gut peptides such as glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) is mediated by SCFAs produced from indigestible substrates, such as inulin and oligofructose.[29] In addition, physiological concentrations of acetate, propionate, and butyrate, but also a pH decrease from 7.5 to 6.0, significantly increased proglucagon and PYY in the enteroendocrine colon cell line STC-1.[17] GLP-1 and PYY are satiety-stimulating hormones that are released in response to nutrient intake by L-cells, mainly in the ileum and colon. GLP-1 promotes insulin secretion and proliferation of pancreatic β-cells in addition to controlling glycogen synthesis in muscle cells,[30] while PYY slows down gastric emptying. In contrast, ghrelin stimulates appetite and is mainly produced by P/D1 cells in the stomach.[31] Non-digestible carbohydrates, such as oligofructose,[32] lactitol,[28] and resistant starch,[17] are effective for inducing satiety by modulating production of the gut peptides GLP-1, PYY, and ghrelin through a mechanism that also involves modulation of the intestinal microbial community.[32]

Among SCFAs, propionate, in particular, has been investigated as a satiety-inducing agent with strong effects on energy intake and feeding behavior. Human and animal trials have shown that propionate administration (in a range of 130–930 mmol/L in vivo and 0.01–10 mmol/L in vitro) results in a significantly greater feeling of fullness and lower desire to eat.[15–17,24]

One of the satiety signals triggered by propionate, in particular, is leptin, a potent anorexigenic hormone that suppresses food intake through receptors expressed in the central nervous system. Xiong et al.[20] demonstrated that the administration of sodium propionate at a dose of

500 µmol/day almost doubled the plasma concentration of leptin in mice. Furthermore, SCFAs, and propionate in particular, stimulated leptin expression in both a mouse adipocyte cell line and mouse adipose tissue in primary culture.[20] In another study, propionate at a concentration of 3 mmol/L induced leptin production in human visceral adipose tissue on both the mRNA and protein levels.[33] These data suggest that the modulating effect of gut microbiota towards obesity may be partially mediated by SCFAs, particularly propionic acid, which is derived from microbial carbohydrate fermentation.

Potential role of propionate in cancer development

The effect of SCFAs on cancer, more specifically colon cancer, has been investigated extensively.[10,18,34] Butyrate is able to modulate gene expression and has an impact on the key regulators of apoptosis and cell cycle. Several mechanisms contribute to the regulatory effect of butyrate on gene expression. These include hyperacetylation of histones and non-histone proteins as well as alteration of DNA methylation, resulting in enhanced accessibility of transcription factors to nucleosomal DNA.[35] In another study by Jan et al.,[18] propionate and acetate (at levels of 26–40 and 9–16 mmol/L, respectively) induced typical signs of apoptosis in human colorectal carcinoma cell lines. This effect included a loss of mitochondrial *trans*-membrane potential, the generation of reactive oxygen species, caspase-3-processing, and nuclear chromatin condensation.

SCFAs have paradoxical effects on colonic epithelial cell proliferation. While these anions stimulate proliferation of normal crypt cells, n-butyrate, and to a lesser extent propionate, they inhibit growth in colon cancer cell lines.[34] Butyrate and propionate are also the most potent fatty acids to induce differentiation[36] and apoptosis.[37] They are therefore protective against cancer development in general[10,36] and against colorectal cancer in particular.[18,37] Although butyrate is more effective than propionate,[38] it is mainly taken up by the colonocytes as an energy source.[8] In contrast, propionate and acetate each reach the circulation in a much higher concentration than butyrate, and they are significantly taken up by the liver (about 60%).[19] Because of the high concentrations of these anions in the liver, it is not unlikely that they affect liver cancer cells as well as other typical cancer cells known to cause metastasis in the liver, such as breast and colon cancer.[39] Further uptake of SCFAs occurs in peripheral tissues, resulting in a 47% decrease of SCFAs in peripheral venous blood. Yet, a study on sudden death victims has shown that the amounts of SCFAs in peripheral blood are still quantifiable.[19] Therefore, the anticarcinogenic effect of this circulating propionate, along with acetate and butyrate, would be a matter of interest to investigate; for example, to what extent might the effect extend beyond the small or large intestine and the liver and thus affect different tissues?

SUBSTRATES AFFECTING PROPIONATE PRODUCTION

Non-digestible carbohydrates resistant to enzymatic digestion in the small intestine are further broken down by the intestinal bacteria. Prebiotics are defined as indigestible carbohydrates that beneficially affect host health through selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon.[40] Although the effect of substrates on SCFA production in the distal gut has been denied in some studies,[41,42] many studies have demonstrated their SCFA-increasing properties. Some actual and potential prebiotic compounds influencing propionate production are described in detail in the following section. Due to differences in the experimental setup, compound structure and concentration, and intestinal microbial community of the studies, the variability in the propionate modulatory effects of these compounds is high and performing comparisons among different substrates is difficult. Importantly, a direct link between propionate production and luminal propionate concentration can only be made in an in vitro context in the absence of intestinal absorption. A summary of the main substrates inducing propionate production is given in Table 2.

L-rhamnose

L-rhamnose or 6-deoxy-L-mannose is a naturally occurring deoxy sugar. It is found in several animal, plant, and bacterial polysaccharides. Commercially available rhamnose is produced by chemical hydrolysis of arabic and karaya gums, or from rutin or citrus fruits that contain, by weight, 10–30% rhamnose. In short-term in vitro experiments, L-rhamnose has been shown to increase propionate production by four times the amount produced by lactulose.[43] Similar results were obtained in a human in vivo study in which subjects were given 25 g of L-rhamnose, lactulose, or D-glucose on three different occasions. Serum propionic acid was measured 24 h after ingestion and was significantly higher after L-rhamnose than after lactulose or D-glucose.[44] The propionate-inducing effect of L-rhamnose has also been confirmed in one longer-term study in which ingestion of 25 g of sugar significantly increased serum propionate in humans over 28 days as compared to ingestion of D-glucose as a control.[45]

D-tagatose

D-tagatose is a stereoisomer of D-fructose, which is normally used as an alternative to sucrose because of its low

Table 2 **Substrates affecting propionate production.**

Substrate	Study	Effect*	Treatment duration	Reference
L-rhamnose	In vitro study with human feces	Selective increase of propionate production	24 h	Fernandes et al. (2000)[43]
	In vivo human study	Selective increase of serum propionate compared to control	28 days	Vogt et al. (2004)[44]
D-tagatose	In vivo pig study	Proportional increase of propionate in different segments of large intestine	18 days	Laerke and Jensen (1999)[47]
Resistant starch	In vivo rat study	Selective increase of serum propionate levels associated with decreased hepatic triglyceride and cholesterol levels	4 weeks	Cheng and Lai (2000)[49]
Inulin	In vitro study with human gut simulator	Higher SCFA production, particularly propionate and butyrate	5 weeks	Van De Wiele et al. (2004)[50]
	In vivo rat study	Proportional increase of luminal concentration of propionate, decreased plasma triglyceride levels	21 days	Brouns et al. (2002)[48] Levrat at al. (1991)[52]
Polydextrose	In vitro study with 4-stages colon-simulator	Increased SCFA production, particularly propionate	48 h	Makelainen et al. (2007)[53]
Arabinoxylans (AX)	In vivo rat study	Dropped cecal pH, SCFA accumulation (particularly propionate), decreased cholesterol absorption	Variable between subjects	Lopez et al. (1999)[54]
Arabinoxylan oligosaccharides (AXOS)	In vitro study with human gut simulator	Higher SCFA production, particularly propionate in transverse compartment of the colon, concomitant decreased lactate production in the same compartment indicating probable production of propionate through acrylate pathway	3 weeks	Grootaert et al. (2009)[56]
Ispaghula	In vivo rat study	Higher SCFA production, particularly propionate in the cecum, proximal and distal colon and feces	28 days	Edwards and Eastwood (1992)[58]
Manno-oligosaccharides (MOS)	In vitro study with human feces	Selective increase of propionate production	24 h	Asano et al. (2003)[60]
	In vivo rat study	Higher cecal SCFAs, particularly propionate	28 days	Asano et al. (2004)[61]
Oligo-laminarans	In vitro study with human feces	Higher propionate production, anticarcinogenic effect on several cancer cell lines	NA	Michel et al. (1999)[62]

* The term "selective" is used when an absolute increase in propionate production occurred. "Proportional increase" is used in case of a higher increase in propionate production compared to other SCFAs.
Abbreviation: NA, no information available.

energy content.[46] The indigestibility of this carbohydrate in the small intestine and its high fermentability in the large intestine of pigs was studied by Laerke and Jensen.[47] Besides some other metabolic effects, such as lower pH levels and higher ATP concentrations in the cecum and proximal colon, significant increases of up to 34.5 mmol/L of propionate were observed in the cecum and in several segments of the large intestines of the pigs that were fed D-tagatose (100 g/kg diet) compared to a sucrose-fed control group.

Resistant starch

Resistant starch consists of a large number of glucose units linked together by α-(1,4) or α-(1,6) glycosidic bonds and is resistant to amylase degradation. Depending on the origin of the starch, it is fermented to butyrate[48] or propionate.[49] In particular, resistant starch from rice is associated with increased propionate production. Fermentation of this compound in different proportions was investigated in rats by Cheng and Lai.[49] Hepatic triglyceride and total cholesterol concentrations in rats fed rice starch (630 g/kg feed) were found to be significantly lower (1.5 fold) than in the control group without starch. This was in parallel with a significant increase in serum propionate concentration.

Inulin

This oligosaccharide belongs to the fructan family and mainly consists of β-(2,1)-linked fructosyl-fructose. It naturally occurs in flowering plants such as chicory and Jerusalem artichoke as storage carbohydrate. As a prebiotic, inulin has been demonstrated to be very effective for increasing both butyrate and propionate production. The propionate-increasing effect of inulin has been investigated in vitro using the simulator of human intestinal microbial ecosystem (SHIME). A metabolic shift for SCFA production was observed after 1 week of inulin supplementation (5 g/d). The higher concentration of SCFAs originated from increased production of propionate and butyrate.[50] When administered to the same SHIME reactor, oligofructose and inulin with different degrees of polymerization (DP) (2–20 and 3–60 for oligofructose and inulin, respectively), resulted in 2 times greater propionate production for inulin compared to the start-up period.[51] An in vivo study with rats fed with inulin (10%) also resulted in a considerable increase in propionate production of up to 58.4 mmol/L.[52]

Polydextrose

Polydextrose is a branched, randomly polymerized polysaccharide (DP, 6–32), which is synthesized mainly from dextrose and is not digested in the upper part of the gastrointestinal tract. Modulation of the colon microbial composition and metabolic activity by this substrate was investigated using a four-stage colon simulator.[53] A significant increase in SCFA production was observed, especially for propionate (22.9 mmol/L) compared to the control sugar xylitol (8.3 mmol/L).

Arabinoxylans and arabinoxylan oligosaccharides

Arabinoxylans are the main non-starch polysaccharides found in many cereals and are part of dietary fiber. Arabinoxylans consist of β-(1,4)-linked D-xylopyranosyl residues to which α-L-arabinofuranose units are linked as side chains. Some arabinoses can be substituted with ferulic acid. In the in vivo study by Lopez et al.,[54] rats fed with a control diet (containing 710 g/kg wheat), an arabinoxylan-supplemented diet (610 g/kg wheat starch plus 100 g/kg maize arabinoxylan), and a cholesterol-supplemented diet (without or with 2 g/kg cholesterol) were compared. The cecal pH level dropped from 7 to 6 due to the accumulation of SCFAs, especially propionic acid (>45% in molar percentage). The butyrate production, however, was unaffected.

Arabinoxylan oligosaccharides are derived from hydrolysis of highly polymerized arabinoxylans. They are characterized by their DP and the average degree of arabinose substitution (DS).[55] In a study by Grootaert et al.[56] the prebiotic potential of arabinoxylan oligosaccharides was compared with inulin in two SHIME reactors. Arabinoxylan oligosaccharides and inulin degradation mostly occurred in the transverse and ascending compartment of the reactor, respectively. Lactate levels (5.5 mM/L) increased in the ascending colon during supplementation with arabinoxylan oligosaccharides, while propionate levels (5.1 mM/L) increased significantly in the transverse colon. The concomitant decrease in lactate in the transverse colon suggested that propionate was partially formed over the acrylate pathway. Inulin treatment had moderate effects on lactate, propionate, and butyrate levels.

Psyllium or ispaghula is a source of soluble fiber providing polysaccharides comparable to wheat bran arabinoxylans but with a higher variability in side-chain composition and linkage.[57] In an in vivo rat study, the effect of ispaghula (5%) on cecal and colon fermentation was compared with that of wheat bran (10%). It was noticed that is paghula fermentation resulted in higher SCFA production, particularly more propionic acid in the cecum and in all the colon fragments.[58]

Mannooligosaccharides

Mannan is one of the water-insoluble hemicelluloses comprised of linear or branched polymers derived from

sugars such as D-mannose, D-galactose, and D-glucose.[59] Mannooligosaccharides (MOSs) are fractionated through thermal hydrolysis of mannan. These carbohydrates are resistant to human salivary α-amylase, artificial gastric juice, porcine pancreatic enzymes, and rat intestinal mucous enzymes.[60] Using in vitro digestion methods, MOS (10%) fermentation by human fecal bacteria was examined. A significantly higher level of propionate was found to be produced from MOSs compared to fructo-oligosaccharides and β-1,4-mannobiose.[60] Similar results for coffee MOSs were obtained in an in vivo rat study.[61] The addition of 5% MOSs to the diet for 28 days resulted in a proportional increase of up to 36.5 mmol/L of propionate in the cecum.

Others

Laminarans are a group of water-soluble β-(1,3)-D-glucan polysaccharides with low molecular weight isolated from seaweeds. The biological activities and fermentation characteristics of oligosaccharides obtained from laminarans were examined by Michel et al.[62] Oligolaminarans (as a potential prebiotic) induced propionate production and demonstrated an antiproliferative effect on colorectal cancer (Caco-2), monocytic (THP1), and lymphocytic T (Jurkat) cell lines.[62]

MECHANISMS OF PROPIONATE FORMATION

Propionate produced as a result of microbial fermentation of indigestible carbohydrates in the gut is the major source of propionate available in the body. Numerous studies have focused on this metabolite as a product of intestinal fermentation[7,45,49,52,54,63,64] rather than from dietary intake.[16,25] Therefore, the next section of this review includes a detailed discussion of the two major pathways of propionate production in the intestine and the main microbial species involved in these specific pathways. Interactions between bacteria in the propionate pathways and new and current strategies to detect and identify propionate-producing bacteria are also illustrated.

Succinate or randomizing pathway

The first pathway suggests that propionate is formed through decarboxylation of the symmetrical compound, succinate. First, oxaloacetate is formed by pyruvate carboxylase from pyruvate. Then, oxaloacetate is reduced to malate by malate dehydrogenase followed by dehydration of malate to fumarate by fumarate hydratase. Fumarate is then reduced to succinate by the action of succinate dehydrogenase. It is hypothesized that further metabolism of

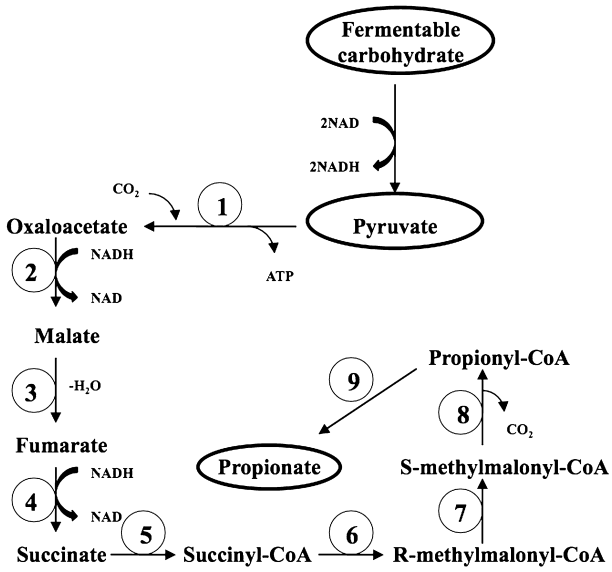

Figure 1 **Propionate formation through succinate or randomizing pathway based on Macy et al.**[66] Enzymes involved in the above reactions: 1) pyruvate carboxylase, 2) malate dehydrogenase, 3) fumarate hydratase, 4) succinate dehydrogenase, 5) succinyl-CoA synthetase, 6) methylmalonyl-CoA mutase, 7) methylmalonyl-CoA epimerase, 8) methyl malonyl-CoA decarboxylase, 9) propionate CoA-transferase.

succinate to propionate occurs through the decarboxylation of methylmalonyl-CoA, which is converted to propionyl-CoA by methylmalonyl-CoA mutase activity (Figure 1).

Pathways of succinate and propionate production were extensively examined in *Bacteroides fragilis*, an important anaerobe in the human intestine. Normally, between 10^{10} and 10^{11} cells of this species are found per gram of feces.[65] For *Bacteroides fragilis*, a CO_2-dependent mechanism for propionate production was suggested by Macy et al.[66] Enzyme assays performed with *Bacteroides fragilis* revealed that oxaloacetate formation is catalyzed by phosphoenolpyruvate carboxykinase (PEP carboxykinase) and is energy independent. Therefore, the high energy of the phosphate bond in PEP is conserved in the form of ATP during the CO_2-dependent formation of oxaloacetate. This is in contrast with other species such as *Propionibacterium shermanii* and *Veillonella* spp. in which oxaloacetate formation is catalyzed by transcarboxylase and pyruvate kinase (both ATP-dependent enzymes), respectively. In culture, succinate dehydrogenase activity (Figure 1, enzyme 4) was dependent on the presence of hemin, an iron-containing compound essential for the growth of *Bacteroides* spp.[64]

Propionibacterium spp. is another common propionate producer that uses the succinate pathway. Decarboxylation of succinate is the main method of propionate

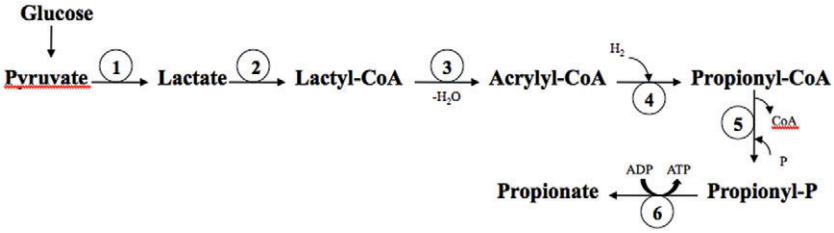

Figure 2 **Acrylate pathway in *Clostridium neopropionicum* X4 based on Tholozan et al.**[71] Enzymes involved in the above reactions: 1) L-lactate dehydrogenase, 2) propionate CoA-transferase, 3) lactoyl-CoA dehydratase, 4) acyl-CoA dehydrogenase, 5) phosphate acetyltransferase, 6) propionate kinase.

production in *Propionibacteria*, since the amount of propionic acid formed depends on the concentration of CO_2 in the medium. Higher ratios of propionate:acetate due to increased CO_2 concentration was explained by bacterial secretion of succinic acid at pH values above 6.5, but until the pH drops below that level, cellular uptake is impossible. In the case of increasing CO_2 concentrations, more succinic acid is formed, with the excess being released into the medium, and decarboxylated only when the pH decreases with glucose as substrate, resulting in a higher propionate:acetate ratio.[67]

The mechanism of propionate formation in *Propionibacterium pentosaecum* was investigated by Delwiche.[68] In this study, propionate production from succinate was higher than that from pyruvate, and equimolar amounts of CO_2 and propionate were formed. In the same study, at the presence of 0.3 mol/L malonate, propionate production was inhibited by 90%. Considering these observations, it becomes evident that *Propionibacterium pentosaecum* possesses a succinate decarboxylase system sufficiently effective to be considered as the main factor for propionate production.

Decarboxylation of succinate is found in cell-free extracts of *Micrococcus lactilyticus* in the presence of specific cofactors, such as biotin and adenylic acid. *Micrococcus lactilyticus* or *Veillonella gazogenes* are strictly anaerobic bacterium producing acetic acid, propionic acid, CO_2, and H_2 through the fermentation of lactate. They fail to ferment glucose, fructose, arabinose, and some other sugars, which might be due to a lack of the enzyme necessary to carry out the primary phosphorylation of the glucose in this species.[69] However, washed suspensions of this microorganism grown on lactate were able to convert pyruvate, oxaloacetate, L-malate, fumarate, and succinate under anaerobic conditions.

Acrylate pathway

Although the succinate pathway is well described for some of the most prevalent bacteria in the gut, it was suggested that the differences observed in labeled carbon distribution patterns are the result of another fermentation mechanism that is different from the succinate pathway.[70] This pathway is defined as the acrylate pathway and is presented in Figure 2. Unlike the pathway of propionate formation in *Propionibacterium shermanii* and *Veillonella* spp., where fumarate and succinate are symmetrical intermediates, none of the intermediates of the acrylate pathway are symmetrical.[71] In the acrylate pathway, pyruvate is reduced to lactate by lactate dehydrogenase. Lactate is converted to lactyl-CoA, which is then dehydrated to acrylyl-CoA by l-CoA dehydratase. Acrylyl-CoA is reduced to propionyl-CoA by acyl-CoA dehydrogenase. Phosphorylation of propionyl-CoA by phosphate acetyltransferase results in propionyl-phosphate, which is eventually converted to propionate by propionate kinase.

Live *Clostridium neopropionicum* X4 is able to ferment [1-^{13}C]ethanol and CO_2 to [2-^{13}C]propionate, [1-^{13}C]acetate and [2-^{13}C]propanol. Because the labeled positions did not change and the molecular skeleton was preserved through the synthesis of propionate, it was suggested that propionate was not formed by the succinate or randomizing pathway, but by the acrylate or non-randomizing pathway.[71]

The same pathway was also used by *Megasphaera elsdenii* (cluster IX of *Clostridia*). The latter is an important obligatory anaerobic bacterium of the rumen of cattle and sheep,[72] accounting for 21% of lactate consumption by rumen bacteria; it is also found in the human intestine.[73] *Megasphaera elsdenii* utilized lactate in preference to glucose when these two substrates were present for propionate production.[74]

MICROBIAL INTERACTIONS

In a complex mixed microbial community, cooperation between different microorganisms to produce a certain end product is not uncommon. For instance, Falony et al.[75] demonstrated that substantial cooperation exists between *Bifidobacteria* and *Roseburia* species for the fer-

mentation of inulin to butyrate. This cooperation is accomplished by means of cross-feeding, in which one species metabolizes the products of another species. The following examples suggest similar cooperation between rumen bacteria accounts for propionate production. In the rumen, several bacterial species are found to be capable of performing all of the steps in the propionate pathways, using carbohydrates as a substrate for propionate formation. Examples include *Megasphaera elsdenii* and *Selenomonas ruminantium*, which produce propionate from carbohydrates and lactate through the acrylate and the succinate pathway, respectively.[74,76] On the other hand, several rumen bacterial species, such as *Ruminococcus flavefaciens* and *Bacteroides succinogenes*, only produce succinate as a major fermentation end product when cultivated in pure culture. With such species, it was seen that succinate did not accumulate in the rumen, but was decarboxylated to propionate. Therefore, it was suggested that succinate produced by a species like *Bacteroides succinogenes* was converted to propionate by succinate-consuming species, such as *Selenomonas ruminantium*.[64]

Another example of microbial interaction between two species was provided by Hino et al.[74] When grown separately in a glucose-containing medium, a monoculture of *Streptococcus bovis* grew faster than *Megasphaera elsdenii*, but the final cell yield was lower for *Streptococcus bovis* than for *Megasphaera elsdenii*. However, when these two species were cultured together, the growth rate and cell concentration of *Streptococcus bovis* were higher than those of *Megasphaera elsdenii*. Significant propionate production in this co-culture indicated that *Megasphaera elsdenii* consumed the lactate produced by *Streptococcus bovis*. The stronger growth of *Streptococcus bovis* suggested that glucose was mostly fermented by this species and that *Megasphaera* utilized little glucose when lactate was available. This implies that the growth and propionate production in *Megasphaera elsdenii* is highly affected by glucose fermentation and lactate production of *Streptococcus bovis*.

Competition for an essential nutrient is one of the negative interactions that occur between microorganisms in a mixed community. Hydrogen (H_2) is a major intermediate in intestinal fermentation. It is utilized by hydrogenotrophic microorganisms belonging mainly to the methanogens, acetogens, and sulphate-reducing bacteria. These microorganisms therefore compete for H_2 and influence each other's growth and activity. Methanogens utilize hydrogen to reduce CO_2 and produce methane (CH_4). The latter is considered as energy loss for the host and a contributor to global warming.[77] Using bacterial interactions has already been a strategy for reducing methanogenesis in in vitro as well as in vivo studies. One way to decrease methanogenesis is to induce the pathways of fermentation in which H_2 is utilized by other microorganisms. Acetogenesis seems to be a possible alternative to reduce CH_4 production by methanogens. In a study by Morvan et al.,[78] in vitro interactions between a rumen H_2-producing microorganism, *Ruminococcus flavefaciens*, and a H_2-utilizing bacteria belonging to the cluster XIV of *Clostridia*, resulted in higher acetate production by *Clostridium* in the coculture media. This indicated an interspecies H_2 transfer resulting in less H_2 availability for methanogens. The second possibility for CH_4 reduction is through propionate formation. Some propionate producers form propionate through the succinate pathway, in which intermediates such as malate and fumarate are involved. In a study of Lopez et al.,[79] it was demonstrated that an addition of sodium fumarate (6.25 mmol/d) to the fermentation media with rumen microbiota significantly decreased, by 6%, the amount of CH_4 produced. The decrease corresponded well to the fraction of fumarate that was converted to propionate. More evidence for an inverse relationship between propionate production and methanogenesis, as well as some strategies for methane mitigation in rumen, are reviewed by Boadi et al.[77] However, the main information about the effect of propionate on methanogenesis appears to be provided by animal studies and mostly in rumen. Therefore, it would be interesting to investigate this effect in the human intestine as well.

Sulfate-reducing bacteria utilize H_2 to reduce sulfur from unabsorbed amino acids and dietary sulfate. Sulfide is mainly detoxified in the colon and red blood cells through methylation by thiol methyl transferase.[80] Yet, an important role for colonic sulfide in the pathogenesis of ulcerative colitis (UC) has been found. Higher production of hydrogen sulfide and growth characteristics of sulfate-reducing bacteria is noticed in patients with UC compared to control subjects.[80] This is due to selectively impaired oxidation of butyrate, which has an essential role in maintaining the health of the colonic epithelial cell barrier. So far, strategies for reducing hydrogen sulfide include antibiotics against sulfate-reducing bacteria, methyl donors, dietary reduction of sulfide intake, and promotion of colonic methanogenesis.[80] Besides these strategies, the potential effect of propionate on hydrogen sulfide mitigation through dietary management would be a matter of interest to investigate.

MOLECULAR MONITORING OF PROPIONATE-PRODUCING BACTERIA

More than 90% of bacteria annotated in the human intestine are unculturable.[81] Molecular techniques using 16S ribosomal RNA-targeted probes[82] or polymerase chain reaction primers[83] have been used as powerful culture-independent techniques to detect predominant bacterial

Table 3 **PCR primers for detection of propionate-producing bacteria.**

Candidate	Primer sequence	Product size (bp)	Annealing temperature (°C)	Reference
Bacteroides fragilis subgroup	ATACGGAGGATCCGAGCGTTA CTGTTTGATACCCACACT	293	65	Vanhoutte et al. (2006)[86]
Bacteroides fragilis group	ATAGCCTTTCGAAAGRAAGAT CCAGTATCAACTGCAATTTTA	501	50	Matsuki et al. (2002)[83]
Prevotella ruminicola	GGTTATCTTGAGTT CTGATGGCAACTAAAGAA	485	53	Tajima et al. (2001)[87]
Prevotella ruminicola 23	GAAAGTCGGATTAATGCTCTATGTTG CATCCTATAGCGGTAAACCTTTGG	74	53	Stevens and Weimer (2007)[88]
Selenomonas ruminantium D	CAATAAGCATTCCGCCTGGG TTCACTCAATGTCAAGCCCTGG	138	56	Stevens and Weimer (2007)[88]
Megasphaera elsdenii T81	AGATGGGGACAACAGCTGGA CGAAAGCTCCGAAGAGCCT	95	54	Stevens and Weimer (2007)[88]
Bacteroides vulgatus	GCATCATGAGTCCGCATGTTC TCCATACCCGACTTTATTCCTT	287	50	Wang et al. (1996)[89]
Escherichia coli	GACCTCGGTTTAGTTCACAGA CACACGCTGACGCTGACCA	585	50	Wang et al. (1996)[89]
Propionibacterium freudenreichii	CTTTCATCCATGACGAAGCGCAAG TGGGGTCGAGTTGCAGACCCCAAT	867	69	Rossi et al. (1999)[90]
Propionibacterium acidipropionici	GACGAAGGCATTCTTTTAGGGTGT TGGGGTCGAGTTGCAGACCCCAAT	868	68	Rossi et al. (1999)[90]

groups present in the human intestine. For propionate producers as well as other bacterial groups, the 16S ribosomal RNA gene has mainly been used to design gene-targeted species-specific primers. An overview of primers that are suitable to detect several known propionate producers are listed in Table 3. More recently, genes involved in the metabolic pathways, such as the production of SCFAs by bacteria, have become new targets in molecular analysis. To exemplify this point, a study of Louis et al.[84] reported the design of polymerase chain reaction primers using genes encoding the final enzymes of butyrate synthesis in the human large intestine. Genes encoding butyrate kinase, phosphotransbutyrylase, and butyryl-CoA:acetate CoA-transferase were used to assess the potency of bacteria to produce enzymes involved in butyrate production. In addition, the predominant route for butyrate formation in the gut could be examined.

Using a similar approach, Asanuma and Hino[85] elucidated the regulatory mechanism for propionate production in *Selenomonas ruminantium* by targeting phosphoenolpyruvate carboxykinase and pyruvate kinase genes, which are both involved in the early steps of propionate synthesis. Such a research strategy may also apply to the detection of other propionate-producing bacteria. In addition, the final enzymes in the pathways of propionate formation (Figures 1 and 2) may be used to 1) screen for a wider range of phylogenetically diverse propionate-producing bacteria from the human large intestine and 2) determine the extent to which the pathways of propionate production are employed in this ecosystem.

Further research is needed to expand the primer set for detecting genes encoding for the enzymes that are involved in the propionate production pathways, and for determining and quantifying propionate-producing microorganisms. To aid in the development of such a molecular analytical strategy, an overview of the enzymes involved in either the succinate or acrylate pathways of propionate production is provided here. One can query these enzymes in the Gene Bank DNA sequence database from the National Center for Biotechnology Information (NCBI) to look for coding genes. To illustrate, a search was performed based on the enzyme commission number (EC number) and with exclusion of archaea. Except for one enzyme that is involved in the acrylate pathway, lactoyl-CoA dehydratase, the query for the different enzymes resulted in a large number of species possessing genes, including putative genes, expressing these enzymes (Table 4). Interestingly, the number of bacterial species possessing specific enzymes was highly variable. For the enzymes involved in the succinate pathway, an increasing number of species that possess the enzymes involved in the electron transport chain was observed (Table 4; Figure 1, enzymes 1, 2, 3, 4, and 5). Further downstream, a decreasing number of species would have the potency to produce enzymes involved in the last steps of propionate formation (Table 4; Figure 1, enzymes 6, 7, 8, and 9). One possible explanation for this trend is an information gap concerning the bacterial species involved in the initial or final steps of propionate production. Alternatively, it is probable that only a limited number of bacterial species are capable of performing the

Table 4 **Results of NCBI search for enzymes involved in pathways of propionate production.**

Enzyme	EC number	No. of bacterial species
Succinate pathway		
1. Pyruvate carboxylase	6.4.1.1	243
2. Malate dehydrogenase	1.1.1.37	656
3. Fumarate hydratase	4.2.1.2	834
4. Succinate dehydrogenase	1.3.99.1	1,443
5. Succinyl-CoA synthetase	6.2.1.5	1,133
6. Methylmalonyl-CoA mutase	5.4.99.2	466
7. Methylmalonyl-CoA epimerase	5.1.99.1	99
8. Methyl malonyl-CoA decarboxylase	4.1.1.41	47
9. Propionate CoA-transferase	2.8.3.1	25
Acrylate pathway		
1. L-lactate dehydrogenase	1.1.1.27	245
2. Propionate CoA-transferase	2.8.3.1	25
3. Lactoyl-CoA dehydratase	–	–
4. Acyl-CoA dehydrogenase	1.3.99.3	424
5. Phosphate acetyltransferase	2.3.1.8	625
6. Propionate kinase	2.7.2.15	34

final metabolic steps in the propionate production pathway. In contrast, no such trend was observed for the enzymes involved in the acrylate pathway. As for the succinate pathway, information on the number of species that possess the relevant enzymes may be incomplete.

In addition to performing the search, the bacterial genera were ranked based on the total number of enzymes that can be linked to either the succinate or the acrylate pathway. This provides a better view of the particular bacterial genera that have a higher probability of producing propionate. All of the bacterial genera that possess enzymes for the succinate and the acrylate pathways are presented as Tables S1 and S2, which are available online as Supporting Information. The entire categorized list of bacterial genera with the total number of enzymes can be consulted in those tables. In addition, the supporting tables present the number of species for each specific enzyme. Repetitions of the same bacterial genus and species do occur, due to the presence of several strains of the same species, or due to the presence of a different nomenclature for the same gene within one species. Some of these genera are well known to be present in the human intestine, such as, *Veilonella*, *Lactobacillus*, *Bacteroides*, and *Propionibacterium*. However, many other genera exist for which the functional genes are determined but further investigation is needed to elucidate whether the species can be detected in the human intestine.

CONCLUSION

Propionate of gut microbial origin is known to possess biological activity at the level of the intestine and intestinal epithelium. Yet, due to its efficient transport across the gut epithelium, it may affect other organs and tissues in addition to the intestinal epithelium. This opens up the potential for the health effects of this metabolite to be modulated through the gut. The selective changes in serum propionate concentration are obtained by feeding specific fermentable substrates. These changes reflect differences in colonic production and microbial composition. Therefore, selective alteration of the colonic fermentation pattern using functional foods could yield a new strategy for modulating propionate-derived health effects. Although many prebiotic compounds have been reported to promote propionate production by gut microorganisms, the mechanisms behind propionate production need to be elucidated further. Due to the large inter-individual differences in the composition and associated metabolism of the gut microbial community, knowledge of carbohydrate structure is insufficient to predict whether propionate will be a major fermentation metabolite following prebiotic consumption. It is therefore proposed that research be directed towards the study of the microbial metabolic pathways that result in propionate production. Cross-feeding reactions between gut microorganisms must be fully uncovered and the importance of the succinate pathway and/or acrylate pathway for propionate production in a microbial consortium must be assessed. These goals can be obtained by applying novel molecular tools that are based on species-specific primers. In addition, a functional gene approach to the detection and quantification of propionate-producing microbiota in the complexity of the gut environment is a challenging but promising step when validating the potential of novel functional foods (probiotics and prebiotics) to increase gut propionate production.

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REFERENCES

1. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet. 2003;361:512–519.
2. Noverr MC, Huffnagel GB. Does the microbiota regulate immune responses outside the gut? Trends Microbiol. 2004;12:562–568.
3. Backhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004;101:15718–15723.
4. Lim CC, Ferguson LR, Tannock GW. Dietary fibers as "prebiotics": implications for colorectal cancer. Mol Nutr Food Res. 2005;49:609–619.
5. Cummings JH. Short chain fatty-acids in the human-colon. Gut. 1981;22:763–779.
6. Scheppach W. Effects of short-chain fatty-acids on gut morphology and function. Gut. 1994;35:35–38.
7. Roediger WEW. Role of anaerobic-bacteria in the metabolic welfare of the colonic mucosa in man. Gut. 1980;21:793–798.
8. Milovic V, Teller IC, Turchanowa L, Caspary WF, Stein J. Effect of structural analogues of propionate and butyrate on colon cancer cell growth. Int J Colorectal Dis. 2000;15:264–270.
9. Vecchia MG, Carnelos M, Fellipe CRC, Curi R, Newsholme EA. Acetate and propionate potentiate the antiproliferative effect of butyrate on RBL-2H3 growth. Gen Pharmacol. 1997;29: 725–728.
10. Li CJ, Elsasser TH. Butyrate-induced apoptosis and cell cycle arrest in bovine kidney epithelial cells: involvement of caspase and proteasome pathways. J Anim Sci. 2005;83:89–97.
11. Lin YG, Vonk RJ, Slooff MJH, Kuipers F, Smit MJ. Differences in propionate-induced inhibition of cholesterol and triacylglycerol synthesis between human and rat hepatocytes in primary culture. Br J Nutr. 1995;74:197–207.
12. Nishina PM, Freedland RA. Effects of propionate on lipid biosynthesis in isolated rat hepatocytes. J Nutr. 1990;120:668–673.
13. Miyazaki T, Ohura T, Kobayashi M, et al. Fatal propionic acidemia in mice lacking propionyl-CoA carboxylase and its rescue by postnatal, liver-specific supplementation via a transgene. J Biol Chem. 2001;276:35995–35999.
14. Delzenne NM, Williams CM. Prebiotics and lipid metabolism. Curr Opin Lipidol. 2002;13:61–67.
15. Oba M, Allen MS. Intraruminal infusion of propionate alters feeding behavior and decreases energy intake of lactating dairy cows. Paper presented at: Annual Meeting of the American-Dairy-Science-Association; Indianapolis, Indiana; Jul, 2001.
16. Ruijschop R, Boelrijk AEM, Giffel MCT. Satiety effects of a dairy beverage fermented with propionic acid bacteria. Int Dairy J. 2008;18:945–950.
17. Zhou J, Martin RJ, Tulley RT, et al. Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through fermentation in rodents. Am J Physiol Endocrinol Metab. 2008;295:1160–1166.
18. Jan G, Belzacq AS, Haouzi D, et al. Propionibacteria induce apoptosis of colorectal carcinoma cells via short-chain fatty acids acting on mitochondria. Cell Death Differ. 2002;9:179–188.
19. Cummings JH, Pomare EW, Branch WJ, Naylor CPE, Macfarlane GT. Short chain fatty-acids in human large-intestine, portal, hepatic and venous-blood. Gut. 1987; 28:1221–1227.
20. Xiong YM, Miyamoto N, Shibata K, et al. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. Proc Natl Acad Sci U S A. 2004;101:1045–1050.
21. Anderson JW, Chen WJL. Plant fiber-carbohydrate and lipid-metabolism. Am J Clin Nutr. 1979;32:346–363.
22. Story JA. The role of dietary fiber in lipid metabolism. Adv Lipid Res. 1981;18:229–246.
23. Illman RJ, Topping DL, McIntosh GH, et al. Hypocholesterolemic effects of dietary propionate-studies in whole animals and perfused rat-liver. Ann Nutr Metab. 1988;32: 97–107.
24. Adam A, Levrat-Verny MA, Lopez HW, Leuillet M, Demigne C, Remesy C. Whole wheat and triticale flours with differing viscosities stimulate cecal fermentations and lower plasma and hepatic lipids in rats. J Nutr. 2001;131:1770–1776.
25. Todesco T, Rao AV, Bosello O, Jenkins DJA. Propionate lowers blood-glucose and alters lipid-metabolism in healthy-subjects. Am J Clin Nutr. 1991;54:860–865.
26. Beaulieu KE, McBurney MI. Changes in pig serum-lipids, nutrient digestibility and sterol excretion during cecal infusion of propionate. J Nutr. 1992;122:241–245.
27. Wolever TMS, Spadafora P, Eshuis H. Interaction between colonic acetate and propionate in humans. Am J Clin Nutr. 1991;53:681–687.
28. Gee JM, Johnson IT. Dietary lactitol fermentation increases circulating peptide YY and glucagon-like peptide-1 in rats and humans. Nutrition. 2005;21:1036–1043.
29. Delzenne NM, Cani PD, Daubioul C, Neyrinck AM. Impact of inulin and oligofructose on gastrointestinal peptides. Br J Nutr. 2005;93:157–161.
30. Delzenne NM, Daubioul C, Neyrinck A, Lasa M, Taper HS. Inulin and oligofructose modulate lipid metabolism in animals: review of biochemical events and future prospects. Br J Nutr. 2002;87:255–259.
31. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem. 2004;50:1511–1525.
32. Cani PD, Delzenne NM. Gut microflora as a target for energy and metabolic homeostasis. Curr Opin Clin Nutr. 2007; 10:729–734.
33. Lahham SH, Weening D, Rezaee F, Roelofsen H, Venema K, Vonk RJ. Propionic acid stimulates leptin production in human adipose tissue through G-protein coupled receptor(s). Paper presented at: 10th Annual Gutday, 2008; University Medical Centre Utrecht and Gut flora Fundation, The Netherlands. 2008.
34. Scheppach W, Bartram HP, Richter F. Role of short-chain fatty-acids in the prevention of colorectal-cancer. Eur J Cancer. 1995;31A:1077–1080.

35. Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ. Review article: the role of butyrate on colonic function. Aliment Pharmacol Ther. 2008;27:104–119.
36. Kosaka M, Takeda M, Matsumoto K, Nishimune Y. F9-cells can be differentiated toward 2 distinct, mutually exclusive pathways by retinoic acid and sodium-butyrate. Dev Growth Differ. 1994;36:223–230.
37. Hague A, Elder DJ, Hicks DJ, Paraskeva C. Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. Int J Cancer. 1995;60:400–406.
38. Matthews GM. *Short-Chain Fatty Acid Modulation of Apoptosis in Gastric and Colon Cancer Cells*. Adelaide, SA: Department of Philosophy, The University of Adelaide; 2007.
39. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer. 2002;2:563–572.
40. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota-introducing the concept of prebiotics. J Nutr. 1995;125:1401–1412.
41. Kien CL, Blauwiekel R. Cecal infusion of butyrate does not alter cecal concentration of butyrate in piglets fed inulin. JPEN J Parenter Enteral Nutr. 2008;32:439–442.
42. Janczyk P, Pieper R, Smidt H, Souffrant WB. Effect of alginate and inulin on intestinal microbial ecology of weanling pigs reared under different husbandry conditions. FEMS Microbiol Ecol. 2009;72:132–142.
43. Fernandes J, Rao AV, Wolever TMS. Different substrates and methane producing status affect short-chain fatty acid profiles produced by in vitro fermentation of human feces. J Nutr. 2000;130:1932–1936.
44. Vogt JA, Pencharz PB, Wolever TMS. L-rhamnose increases serum propionate in humans. Am J Clin Nutr. 2004;80:89–94.
45. Vogt JA, Ishii-Schrade KB, Pencharz PB, Wolever TMS. L-rhamnose increases serum propionate after long-term supplementation, but lactulose does not raise serum acetate. Am J Clin Nutr. 2004;80:1254–1261.
46. Levin GV, Zehner LR, Saunders JP, Beadle JR. Sugar substitutes – their energy values, bulk characteristics, and potential health benefits. Am J Clin Nutr. 1995;62:1161–1168.
47. Laerke HN, Jensen BB. D-tagatose has low small intestinal digestibility but high large intestinal fermentability in pigs. J Nutr. 1999;129:1002–1009.
48. Brouns F, Kettlitz B, Arrigoni E. Resistant starch and "the butyrate revolution." Trends Food Sci Technol. 2002;13:251–261.
49. Cheng HH, Lai MH. Fermentation of resistant rice starch produces propionate reducing serum and hepatic cholesterol in rats. J Nutr. 2000;130:1991–1995.
50. Van De Wiele T, Boon N, Possemiers S, Jacobs H, Verstraete W. Prebiotic effects of chicory inulin in the simulator of the human intestinal microbial ecosystem. FEMS Microbiol Ecol. 2004;51:143–153.
51. Van De Wiele T, Boon N, Possemiers S, Jacobs H, Verstraete W. Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects. J Appl Microbiol. 2007;102:452–460.
52. Levrat MA, Remesy C, Demigne C. High propionic-acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. J Nutr. 1991;121:1730–1737.
53. Makelainen HS, Makivuokko HA, Salminen SJ, Rautonen NE, Ouwehand AC. The effects of polydextrose and xylitol on microbial community and activity in a 4-stage colon simulator. J Food Sci. 2007;72:153–159.
54. Lopez HW, Levrat MA, Guy C, Messager A, Demigne C, Remesy C. Effects of soluble corn bran arabinoxylans on cecal digestion, lipid metabolism, and mineral balance (Ca, Mg) in rats. J Nutr Biochem. 1999;10:500–509.
55. Swennen K, Courtin CM, Lindemans G, Delcour JA. Large-scale production and characterisation of wheat bran arabinoxylooligosaccharides. J Sci Food Agric. 2006;86:1722–1731.
56. Grootaert C, Van den Abbeele P, Marzorati M, et al. Comparison of prebiotic effects of arabinoxylan oligosaccharides and inulin in a simulator of the human intestinal microbial ecosystem. FEMS Microbiol Ecol. 2009;69:231–242.
57. Edwards S, Chaplin MF, Blackwood AD, Dettmar PW. Primary structure of arabinoxylans of ispaghula husk and wheat bran. Proc Nutr Soc. 2003;62:217–222.
58. Edwards CA, Eastwood MA. Comparison of the effects of ispaghula and wheat bran on rat cecal and colonic fermentation. Gut. 1992;33:1229–1233.
59. Moreira LRS, Filho EXF. An overview of mannan structure and mannan-degrading enzyme systems. Appl Microbiol Biotechnol. 2008;79:165–178.
60. Asano I, Hamaguchi K, Fujii S, Iino H. In vitro digestibility and fermentation of mannooligosaccharides from coffee mannan. Food Sci Technol Res. 2003;9:62–66.
61. Asano I, Ikeda Y, Fujii S, Iino H. Effects of mannooligosaccharides from coffee on microbiota and short chain fatty acids in rat cecum. Food Sci Technol Res. 2004;10:273–277.
62. Michel C, Benard C, Lahaye M, et al. Algal oligosaccharides as functional foods: in vitro study of their cellular and fermentative effects. Sci Aliments. 1999;19:311–332.
63. Campbell JM, Fahey GC, Wolf BW. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. J Nutr. 1997;127:130–136.
64. Scheifin CC, Wolin MJ. Propionate formation from cellulose and soluble sugars by combined cultures of *Bacteroides succinogenes* and *Selenomonas ruminantium*. Appl Microbiol. 1973;26:789–795.
65. Macy J, Probst I, Gottschalk G. Evidence for cytochrome involvement in fumarate reduction and adenosine 5`-triphosphate synthesis by *Bacteroides fragilis* grown in presence of hemin. J Bacteriol. 1975;123:436–442.
66. Macy JM, Ljungdahl LG, Gottschalk G. Pathway of succinate and propionate formation in *Bacteroides fragilis*. J Bacteriol. 1978;134:84–91.
67. Johns AT. The mechanism of propionic acid formation by *Propionibacteria*. J Gen Microbiol. 1951;5:337–345.
68. Delwiche EA. Mechanism of propionic acid formation by *Propionibacterium pentosaceum*. J Bacteriol. 1948;56:811–820.
69. Whiteley HR. The mechanism of propionic acid formation by succinate decarboxylation. I. The activation of succinate. Biochemistry. 1953;39:772–779.
70. Wood HG, Stjernholm R, Leaver FW. The Role of succinate as a precursor of propionate in the propionic acid fermentation. J Bacteriol. 1956;72:142–152.
71. Tholozan JL, Touzel JP, Samain E, Grivet JP, Prensier G, Albagnac G. *Clostridium neopropionicum* sp nov, a strict anaerobic bacterium fermenting ethanol to propionate through acrylate pathway. Arch Microbiol. 1992;157:249–257.

72. Marounek M, Fliegrova K, Bartos S. Metabolism and some characteristics of ruminal strains of *Megasphaera elsdenii*. Appl Environ Microbiol. 1989;55:1570–1573.
73. Brancaccio M, Legendre GG. *Megasphaera elsdenii* endocarditis. J Clin Microbiol. 1979;10:72–74.
74. Hino T, Shimada K, Maruyama T. Substrate preference in a strain of *Megasphaera elsdenii*, a ruminal bacterium, and its implications in propionate production and growth competition. Appl Environ Microbiol. 1994;60:1827–1831.
75. Falony G, Vlachou A, Verbrugghe K, De Vuyst L. Cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose. Appl Environ Microbiol. 2006;72: 7835–7841.
76. Paynter MJB, Elsden SR. Mechanism of propionate formation by *Selenomonas ruminantium*, a rumen microorganism. J Gen Microbiol. 1970;61:1–7.
77. Boadi D, Benchaar C, Chiquette J, Masse D. Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. Can J Anim Sci. 2004;84:319–335.
78. Morvan B, RieuLesme F, Fonty G, Gouet P. In vitro interactions between rumen H_2-producing cellulolytic microorganisms and H_2-utilizing acetogenic and sulfate-reducing bacteria. Anaerobe. 1996;2:175–180.
79. Lopez S, Valdes C, Newbold CJ, Wallace RJ. Influence of sodium fumarate addition on rumen fermentation in vitro. Br J Nutr. 1999;81:59–64.
80. Roediger WEW, Moore J, Babidge W. Colonic sulfide in pathogenesis and treatment of ulcerative colitis. Dig Dis Sci. 1997;42:1571–1579.
81. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005;307:1915–1920.
82. Sghir A, Gramet G, Suau A, Rochet V, Pochart P, Dore J. Quantification of bacterial groups within human fecal flora by oligonucleotide probe hybridization. Appl Environ Microbiol. 2000;66:2263–2266.
83. Matsuki T, Watanabe K, Fujimoto J, et al. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. Appl Environ Microbiol. 2002;68:5445–5451.
84. Louis P, Duncan SH, McCrae SI, Millar J, Jackson MS, Flint HJ. Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. J Bacteriol. 2004;186:2099–2106.
85. Asanuma N, Hino T. Molecular characterization, enzyme properties and transcriptional regulation of phosphoenolpyruvate carboxykinase and pyruvate kinase in a ruminal bacterium, *Selenomonas ruminantium*. Microbiology. 2001; 147:681–690.
86. Vanhoutte T, De Preter V, De Brandt E, Verbeke K, Swings J, Huys G. Molecular monitoring of the fecal microbiota of healthy human subjects during administration of lactulose and *Saccharomyces boulardii*. Appl Environ Microbiol. 2006;72:5990–5997.
87. Tajima K, Aminov RI, Nagamine T, Matsui H, Nakamura M, Benno Y. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Appl Environ Microbiol. 2001;67:2766–2774.
88. Stevenson DM, Weimer PJ. Dominance of *Prevotella* and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Appl Microbiol Biotechnol. 2007;75:165–174.
89. Wang RF, Cao WW, Cerniglia CE. PCR detection and quantification of predominant anaerobic bacteria in human and animal fecal samples. Appl Environ Microbiol. 1996;62:1242–1247.
90. Rossi F, Torriani S, Dellaglio F. Genus- and species-specific PCR-based detection of dairy *Propionibacteria* in environmental samples by using primers targeted to the genes encoding 16S rRNA. Appl Environ Microbiol. 1999;65:4241–4244.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 **Bacterial ranking based on the total number of encoding genes for the enzymes involved in the succinate pathway. Numbers of species possessing the enzymes for each genus are presented.**
Table S2 **Bacterial ranking based on the total number of enzymes involved in acrylate pathway. Numbers of species possessing the enzymes for each genus are presented.**

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