Elucidating the mechanisms of action of short-chain fatty acids

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Chapter 2

The short-chain fatty acid uptake fluxes by mice on a guar gum supplemented diet associate with amelioration of the metabolic syndrome

Gijs den Besten, Rick Havinga, Shodhan Rao, Albert Gerding, Karen van Eunen, Albert K. Groen, Dirk-Jan Reijngoud and Barbara M. Bakker

Conditionally accepted
Abstract

Studies with dietary supplementation of various types of fibers have shown beneficial effects on symptoms of the metabolic syndrome. Short-chain fatty acids (SCFAs), the main products of intestinal bacterial fermentation of dietary fiber, have been suggested to play a key role. Whether the concentration of SCFAs or their metabolism drives these beneficial effects is not yet clear. In this study we investigated the SCFA concentrations and in vivo host uptake fluxes in the absence or presence of the dietary fiber guar gum. C57Bl/6j mice were mice fed a high-fat diet supplemented with 0%, 5%, 7.5% or 10% of the fiber guar gum. To determine the effect on SCFA metabolism, 13C-labeled acetate, propionate or butyrate were infused into the cecum of mice for 6h and the isotopic enrichment of cecal SCFAs was measured. The in vivo production, uptake and bacterial interconversion of acetate, propionate and butyrate were calculated by combining the data from the three infusion experiments in a single steady-state isotope model. Guar gum treatment decreased markers of the metabolic syndrome (i.e. body weight, glucose and insulin levels) in a dose-dependent manner. Cecal SCFA concentrations were increased compared to the control group, but no differences were observed between the different guar gum doses. Thus, no significant correlation was found between cecal SCFA concentrations and metabolic markers. In contrast, in vivo SCFA uptake fluxes by the host correlated linearly with metabolic markers. We argue that in vivo SCFA fluxes, and not concentrations, govern the protection from the metabolic syndrome by dietary fibers.
Introduction

The shift in diet in Western and developing countries from a traditional high-fiber, low-fat, low-calorie diet towards a low-fiber, high-fat, high-calorie diet is accompanied with a growing prevalence of the metabolic syndrome comorbidities: obesity, hypertension, dyslipidemia and insulin resistance (167, 168). Epidemiological and large observational studies reported an inverse correlation between dietary fiber intake and body weight, insulin resistance, hypertension and dyslipidemia (169). The dietary fiber guar gum is especially promising as it has been shown to decrease hypercholesterolemia, hyperglycemia and obesity in multiple experiments in both rodents and humans (170, 171). The molecular mechanisms by which guar gum induces these effects constitute an active field of research. Short-chain fatty acids (SCFAs), the main products of intestinal bacterial fermentation of dietary fiber, have been suggested to play a key role in these beneficial effects (Chapter 1).

SCFAs are saturated aliphatic organic acids that consist of 1-6 carbons of which acetate (C2), propionate (C3) and butyrate (C4) are the most abundant (≥95%) (8). In the last decades it became apparent that SCFAs might play a key role in the prevention and treatment of the metabolic syndrome, bowel disorders and certain types of cancer (16-22). The effects of dietary fiber on the host are mostly studied by looking at the fecal or colonic SCFA concentrations and the host physiology. However, increased concentrations of SCFAs do not always correlate to beneficial host effects. For instance, genetically obese ob/ob mice and obese human subjects had increased concentrations of cecal and fecal SCFAs as compared to lean controls (159-161), while germ-free mice and rats had low SCFA concentrations and were protected from diet-induced obesity (162, 163). Recently, Teixeira et al. (172) even suggested that human fecal SCFA concentrations in women are positively correlated with metabolic syndrome risk factors such as adiposity, waist circumference and HOMA index. These results raise the question if SCFAs are involved in the beneficial effect of dietary fibers. It is important to note, however, that at steady-state the cecal or fecal SCFA concentrations not necessarily reflect the SCFA uptake fluxes by the host. It is known that SCFAs exert their effects not only directly in the gut, but also via other organs like the liver and adipose tissue (Chapter 1). For the latter effect the cecal concentration is of less importance than the amount of SCFAs that is transported into the host. It is plausible that the SCFA host uptake fluxes are involved in the beneficial effect of dietary fibers. In vivo flux measurements, however, are challenging. Therefore, SCFA production fluxes have been measured mostly in vitro by exposing an inoculum of gut microbiota to dietary fiber. The disadvantages of this method are that (i) during isolation of the anaerobic gut microbiota the diversity decreases, (ii) raw substrates are not modified as it normally occurs in vivo in the upper part of the gastrointestinal tract, (iii) products accumulate during fermentation due to the lack of host uptake mechanisms and (iv) the uptake fluxes by the host, which we ultimately need to know, cannot be determined (173, 174).

In this study we present a novel method to determine in vivo SCFA fluxes under different dietary conditions. The method was based on infusion of tracer amounts of 13C-labeled acetate, propionate or butyrate into the cecum and the results of all three infusions were combined in a single steady-state isotope model. This allowed us to quantify how the intake of 0%, 5%, 7.5% or 10% of the dietary fiber guar gum affected the SCFA fluxes and how these correlated with...
markers of the metabolic syndrome.

**Materials and Methods**

**Ethics Statement**

The national and institutional guidelines for the care and use of animals were followed, and the experimental procedures were reviewed and approved by the Ethics Committees for Animal Experiments of the University of Groningen, The Netherlands (ethics registration code 5887). All efforts were made to minimize suffering.

**Animals and Experimental Design**

Male C57Bl/6J mice (Charles River, L’Arbresle Cedex, France), 2 months of age, were housed in a light- and temperature-controlled facility (lights on 6:30 a.m. to 6:30 p.m., 21 °C). During 6 weeks mice were fed a high-fat semi-synthetic diet (based on D12451 (175), Research Diet Services, Wijk Bij Duurstede, The Netherlands) in which 0, 5, 7.5 or 10% (w/w) guar gum (Viscogum™ MP 41230, Cargill, United States) replaced an equivalent amount of corn starch. In this way we ensured that only the fiber content was varied while the total polysaccharide and calorie content of the diets remained equal. Mice had free access to food and drinking water. Experimental procedures were approved by the Ethics Committees for Animal Experiments of the University of Groningen.

**Plasma sampling**

Mice were fasted from 8-12 a.m. Blood glucose concentrations were measured with a EuroFlash meter (Lifescan Benelux, Beerse, Belgium). Blood samples were drawn by tail bleeding into heparinized tubes. Blood was centrifuged (4000 x g for 10 min at 4 °C) and plasma was stored at -20 °C. Plasma insulin levels were determined using ELISA (ALPCO Diagnostics, Salem, United States) and HOMA-IR was calculated (IR = (fasting insulin in mU/L x fasting glucose in mM) / 22.5).

**Cecal infusion experiment**

After 6 weeks on diet, mice were equipped with a permanent cecum catheter and allowed a recovery period of at least 5 days as described previously (Chapter 3). Cecal canulas were flushed daily with phosphate buffered saline. On the day of the experiment, mice were individually housed and fasted from 6:00 to 10:00 a.m. All infusion experiments were performed in conscious, unrestrained mice. For each dietary treatment group, three different groups received solutions of phosphate buffered saline containing either sodium [1-13C] acetate (3 mM, 99 atom %, Sigma-Aldrich), sodium [2-13C]propionate (1.5 mM, 99 atom %, Sigma-Aldrich) or sodium [2,4-13C2]butyrate (0.6 mM, 99 atom %, Sigma-Aldrich) via the cecum catheter at an infusion rate of 0.2 ml/h. After 6h of infusion, animals were sacrificed by cardiac puncture under isoflurane anesthesia. Cecum content was removed quickly, frozen in liquid nitrogen, and stored at -80 °C for SCFA enrichment determination.
**Determination of SCFA concentrations and enrichments**

Cecal concentrations and enrichments of SCFAs were measured as previously described (Chapter 3). In short, cecum content was centrifuged and 25 µl of supernatant was spiked with 25 µl of internal standard (17.3 mM hydroxyisocapronic acid) and 5 µl of 20% 5-sulfosalicylic acid. After a 10 min centrifugation the supernatant was acidified with 2.5 µl 37% HCl and SCFA were extracted with 2 ml diethylether. Derivatization was performed overnight with 500 µl supernatant and 50 µl of N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA).

Mass isotopologue distributions were measured in an Agilent 5975 series GC/MSD (Agilent Technologies). The gas chromatograph was equipped with a ZB-1 column (Phenomenex). Mass spectrometry analysis was performed by electron capture negative ionization with methane as the moderating gas. Ions monitored were $m/z$ 117-118 for acetate, $m/z$ 131-132 for propionate and $m/z$ 145-147 for butyrate. The normalized mass isotopologue distributions measured by GC-MS ($m_c$-$m_x$) were corrected for natural abundance of $^{13}$C by multiple linear regression according to Lee et al. (176) to obtain the excess fractional distribution of mass isotopologues ($M_0$-$M_x$).

**Gene expression levels**

RNA was extracted from cecum tissue using Tri reagent (Sigma-Aldrich, St. Louis, MO) and converted into cDNA by a reverse transcription procedure using M-MLV and random primers according to the manufacturer’s protocol (Sigma-Aldrich). For quantitative PCR (qPCR), cDNA was amplified using the appropriate primers and probes. Taqman RT-PCR primer and probe were used to determine mRNA for MCT-1 (Mm01315398_m1) and SMCT-1 (Mm00520629_m1). mRNA levels were calculated relative to 36b4 (Mm00725448_s1) expression and normalized for expression levels of mice fed the control diet.

**Statistics**

All data are presented as mean values ± SEM. Statistical analysis was assessed by one-way ANOVA using the Tukey test for post-hoc analysis. For analysis of correlations, Spearman’s rank test was used. Statistical significance was defined as a p value below 0.05. Data were analyzed with SPSS v.20 software.

**Results**

**Guar gum protects against diet-induced obesity and insulin resistance in a dose-dependent manner**

Supplementation of a high-fat diet with guar gum dose-dependently decreased body weight of mice after 6 weeks treatment (Figure 1A), with a maximal effect at the highest dose of the fiber (13% decrease at 10% guar gum vs. control; p<0.001). In concert, fasted plasma glucose and insulin levels decreased dose-dependently by guar gum treatment (27% decrease at 10% guar gum vs. control; p<0.001 and 49% decrease at 10% guar gum vs. control; p<0.01, respectively; Figures 1B and 1C). Accordingly, the homeostasis model assessment for insulin resistance (HOMA-IR) decreased with an increasing dose of guar gum (64% decrease at
Chapter 2

Figure 1. Effect of dietary supplementation with guar gum on mouse physiology after 6 weeks on a high-fat diet. (A) Body weight for the different guar gum groups. Plasma glucose (B) and insulin (C) levels after a 4-hour fast. Blood glucose and insulin levels were used to determine insulin sensitivity through HOMA-IR (D). Cecal content (E), cecal SCFA concentrations (F) and cecal mRNA expression (G) of SCFAs transporters. Data represent means ± SEM for n=7-8. Different letters indicate significant differences between groups (at least p<0.05).

10% guar gum vs. control; p<0.001; Figure 1D). All groups of mice treated with guar gum showed an approximately 2.5-fold increase in cecal mass content compared to the control diet (167% increase at 10% guar gum vs. control; p<0.01; Figure 1E). However, in this respect no differences were observed between the different guar gum groups. Cecal concentrations of acetate (176% increase at 10% guar gum vs. control; p<0.001), propionate (207% increase at 10% guar gum vs. control; p<0.001) and butyrate (174% increase at 10% guar gum vs. control; p<0.05) were increased in the three guar gum groups compared to the control group (Figure 1F), but again no significant differences were observed between the different guar gum groups. The cecal mRNA expression of two known colonic SCFA transporters, monocarboxylate transporter 1 (MCT-1) and sodium-coupled MCT-1 (SMCT-1), were increased in the guar gum groups compared to the control group, with the highest expressions in the 5% guar gum group (MCT-1; 62% increase at 5% guar gum vs. control; p<0.001, SMCT-1; 355% increase at 5% guar gum vs. control; p<0.001; Figure 1G)

Short-chain fatty acid concentrations correlate with cecal transporter mRNA expression but not with metabolic syndrome markers

To investigate if the concentration of cecal SCFAs associate with markers of the metabolic syndrome (i.e. body weight, glucose, insulin and HOMA-IR), we plotted the concentration of
the three SCFA against the metabolic syndrome markers of the different treatment groups. Cecal acetate, propionate and butyrate concentrations did not correlate with body weight, glucose, insulin and HOMA-IR (p>0.05; Figures 2A and S1). In contrast, all three SCFAs correlated significantly with SMCT-1 and MCT-1 expression (p<0.05; Figures 2B and S1). We hypothesized that the physiological effect of guar gum was not exerted via the colonic SCFA concentration, but rather via the amount of SCFAs that is produced by the microbiota and taken up by the host. Therefore, we set out to determine these fluxes in vivo.

A model to determine short-chain fatty acid production and uptake fluxes

We designed an animal experiment to determine in vivo fluxes of SCFA production, interconversion and uptake by the host, based on isotope dilution and incorporation. We infused separately tracer amounts of [1-13C]acetate, [2-13C]propionate or [2,4-13C2]butyrate during 6h into the cecum of conscious, unrestrained mice and measured the label content in the cecal SCFAs at the end of the infusion period as described in Chapter 3. Infusion of any of the labeled SCFAs resulted in label incorporation in all three cecal SCFAs (Figure 3A). This indicates that there is interconversion of labeled SCFAs by the gut bacteria. To calculate the in vivo fluxes of bacterial SCFA production and consumption by the host we constructed a mathematical model that accounts for this bacterial label conversion (Figure 3B). Starting from the assumption that the isotopic measurements were performed during mass and isotopic steady state, flux balance equations were derived for each of the labeled and unlabeled SCFAs during the tracer infusions of [1-13C]acetate, [2-13C]propionate or [2,4-13C2]butyrate. We further assumed that the labeled tracers did not affect the total mass fluxes and hence these should be identical in the three infusion experiments. Based on the observation that double-labeled butyrate was detected after infusion of acetate and propionate, we assumed that 2
In vivo bacterial short-chain fatty acid production and host fluxes

The above described model-based method to measure in vivo fluxes and SCFA interconversion was applied to the different groups of mice treated with guar gum and to the control group for comparison (Figure 3C). The production and uptake fluxes of acetate were highest, followed by the propionate and butyrate fluxes. This corresponded with the order of SCFA concentrations (Figure 1F). The production flux of acetate was higher than its uptake flux, since 7.1% of the acetate was converted into butyrate (Figure 3C and Table 1), revealing microbial cross-feeding. The production and uptake fluxes of propionate were almost identical, since there...
Figure 4. *In vivo* SCFA uptake fluxes correlate inversely with metabolic syndrome markers. Correlation of acetate, propionate and butyrate host uptake fluxes with body weight (A), glucose concentrations (B), insulin concentrations (C) and HOMA-IR (D). The Spearman’s correlation coefficient was calculated and the significance level was set at p<0.05.

was little conversion of propionate into the other SCFAs and back (Figure 3C and Table 1). Interestingly, approximately 50% of butyrate was not produced directly from fibers but rather via dimerization of acetate (Figure 3C and Table 1).

The guar gum treatment increased all the fluxes of SCFA production and uptake by the host in a dose-dependent manner (Figure 3C), with the highest fluxes at the highest fiber dose. The relative increase was highest for the propionate production and uptake fluxes (787% increase at 10% guar gum vs. control; p<0.001 and 761% increase at 10% guar gum vs. control; p<0.001, respectively) followed by the acetate production and uptake fluxes (514% increase at 10% guar gum vs. control; p<0.001 and 518% increase at 10% guar gum vs. control; p<0.001, respectively) and the butyrate production and uptake fluxes (273% increase at 10% guar gum vs. control; p<0.001 and 461% increase at 10% guar gum vs. control; p<0.001, respectively).
Table 1. SCFA interconversion fluxes (mmol/kg/h) for the different Guar Gum groups. Data represent means ± SEM for n=7-8.

<table>
<thead>
<tr>
<th>Guar Gum content (%)</th>
<th>0</th>
<th>5</th>
<th>7.5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate → Butyrate</td>
<td>0.11 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>0.32 ± 0.02</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td>Butyrate → Acetate</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.01</td>
<td>0.00 ± 0.02</td>
</tr>
<tr>
<td>Propionate → Acetate</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.01</td>
<td>0.00 ± 0.06</td>
<td>0.00 ± 0.01</td>
</tr>
<tr>
<td>Acetate → Propionate</td>
<td>0.00 ± 0.01</td>
<td>0.00 ± 0.01</td>
<td>0.00 ± 0.04</td>
<td>0.00 ± 0.05</td>
</tr>
<tr>
<td>Propionate → Butyrate</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Butyrate → Propionate</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.01</td>
</tr>
</tbody>
</table>

Changes in metabolic syndrome markers were inversely correlated with in vivo short-chain fatty acid fluxes

None of the *in vivo* SCFA fluxes correlated with the cecal SCFA concentrations or the cecal mRNA expression of SMCT-1 and MCT-1 (p>0.05; Figures S2 and S3). However, *in vivo* SCFA host uptake fluxes did significantly correlate with body weight, fasted glucose and insulin levels, and HOMA-IR (p<0.05; Figure 4). Similar results were found for the *in vivo* SCFA production fluxes (Figure S3). The correlation was better for the uptake fluxes by the host than for the microbial production fluxes. The difference between uptake and production fluxes was due to microbial interconversion. The correlation was generally increasing from acetate to butyrate to propionate. Altogether, these data suggest that *in vivo* SCFA fluxes, and not concentrations, are key to understand the beneficial effects of fibers on metabolic syndrome markers.

Discussion

In this chapter we demonstrate that the rate of uptake of SCFAs directly correlates with amelioration of symptoms clustered in the metabolic syndrome. Beneficial effects of SCFAs have been frequently suggested in the literature, but evidence for this contention has been lacking since physiological effects did not correlate with luminal SCFA concentrations. By determining the *in vivo* SCFA fluxes we now show for the first time that *in vivo* SCFA fluxes rather than concentrations correlate in an inverse manner with the biomarkers of the metabolic syndrome. Here we will discuss how this adds to the evidence for a causal relation between fiber intake, SCFA fluxes and attenuation of metabolic syndrome markers.

Human randomized controlled clinical trials showed that guar gum supplementation decreased body weight and fasting plasma glucose and insulin concentrations in both healthy and metabolic syndrome patients (171, 178, 179). Here, we show that increasing the content of guar gum in a high-fat diet resulted in a dose-dependent decrease in body weight, fasting plasma glucose and insulin concentrations and HOMA-IR, together indicating an improvement of the metabolic syndrome. To date, no molecular mechanism for these beneficial effects of guar gum has been described. Two lines of evidence suggest that SCFAs exert a key role in this beneficial effect. First, SCFA are the main end products of microbial fermentation of
dietary fibers in the intestine (Chapter 1). Second, SCFA supplementation in the diet protects against dietary-induced obesity and insulin resistance (17, 122), suggesting that microbial SCFAs might do the same. The question then arises how SCFAs might mediate the observed dose-dependent effect of dietary fibers whilst their concentrations do not correlate with the fiber dose and metabolic syndrome markers (32, 161, 163, 172). It is well known that SCFAs regulate the physiology of the host not only via direct effects in the colon, but also via other organs in the host, such as liver and adipose tissue (Chapter 1). SCFAs have been shown to affect host energy metabolism activation of the G-coupled receptor (GPR) 41 and 43, which promotes catabolism of lipids and glucose leading to a decreased body weight, plasma glucose and insulin concentrations (119, 135). GPR activation beyond the intestine depends on the plasma SCFA concentrations which are, unfortunately, very hard to determine due to the very low concentrations and scarce sample options. Because plasma SCFA concentrations are for a large part determined by the host uptake from the colonic tract, in vivo SCFA uptake fluxes by the host can possibly be used to explain the dietary fiber-SCFA effect on the host.

While our data suggest that the physiological effect of guar gum is mediated via the uptake fluxes of SCFA into the host, we do not explain what causes the dose-dependent SCFA uptake fluxes in the first place. In general, the uptake rate of a metabolite depends on its own concentration as well as on the capacity and kinetics of its transporter. At steady state, neither the concentration of the SCFAs nor the mRNA expression of the SCFA transporters MCT-1 and SMCT-1 correlated with the SCFA flux. Cecum content and thereby uptake surface did not correlate with the fluxes either. We have not measured transporter kinetics, which leaves the possibility that SCFA transport is regulated at the protein level. An alternative possibility which we cannot exclude is that the concentration of another metabolite co-varies with the uptake fluxes and takes part in the actual causal mechanism. Next to SCFAs, the gut microbiota produces many metabolites which are involved in the regulation of multiple host metabolic pathways (5). However, obvious candidates such as lactate were hardly detectable (data not shown).

In conclusion, our data clearly showed that in vivo SCFA fluxes and not SCFA concentrations are inversely correlated to metabolic syndrome markers. Together with the known causal effect of increased SCFA in the diet, this provides strong evidence for a causal relation between SCFA uptake flux and metabolic syndrome. Further research should elucidate the role of additional molecular factors that mediate this effect, as well as the mechanism explaining the dose-dependency of the uptake fluxes.
Supplemental figures

Figure S1. Correlation of cecal concentrations of acetate, propionate and butyrate with plasma glucose (A) and plasma insulin levels (B), HOMA-IR (C) and MCT-1 cecal mRNA expression (D). The Spearman's correlation coefficient was calculated and the significance level was set at $p<0.05$. 
SCFA fluxes associate with metabolic markers

Figure S2. Correlation of acetate, propionate and butyrate host uptake fluxes with cecal concentrations of acetate, propionate and butyrate, respectively (A). Correlation of acetate, propionate and butyrate host uptake fluxes with cecum mRNA expression of MCT-1 (B) and SMTC-1 (C). The Spearman’s correlation coefficient was calculated and the significance level was set at p<0.05.
Figure S3. Correlation of bacterial production fluxes of acetate, propionate and butyrate with cecal SCFA concentrations (A), body weight (B), plasma glucose (C) and plasma insulin levels (D), HOMA-IR (E), MCT-1 (F) and SMTC-1 (G) cecal mRNA expression. The Spearman's correlation coefficient was calculated and the significance level was set at p<0.05.
**Text S1: Model description**

**Model construction**

We constructed a steady-state model to derive the fluxes of *in vivo* short-chain fatty acid (SCFA) production, interconversion and their uptake by the host from the measured isotope distribution patterns. Based on the reaction scheme in Figure 3B, a set of steady-state equations was constructed for all three SCFA tracer infusions, *i.e.* [1-*13*C]acetate, [2-*13*C]propionate and [2,4-*13*C] butyrate. The steady-state model was solved in Excel, which uses the Generalized Reduced Gradient (GRG) Nonlinear Solver algorithm (177). The model is based on the following assumptions: (i) All total-mass fluxes (\(v\)'s below) are independent of the infusion. This is warranted by the use of tracer amounts of labeled SCFAs and moreover, the label distribution was measured at steady state. This then allowed combining the data from the three infusion experiments into one model to calculate the fluxes. (ii) Two acetate molecules are used for the production of one butyrate molecule and two acetate molecules are produced from one butyrate molecule as shown previously by others (61, 65). The former is consistent with the detection of double-labeled butyrate in our dataset. Analogously, two propionate molecules are used for the production of one butyrate molecule and two propionate molecules are produced from one butyrate molecule. This assumption is supported by mass isotopologue distribution analysis (MIDA) algorithms as shown in Chapter 3 and again by the detection of double-labeled butyrate upon infusion of single-labeled propionate in this study. One acetate molecule is produced from one propionate molecule and *vice versa* one propionate molecule is produced from one acetate molecule, as indicated previously by others (180, 181). (iii) All fluxes are considered to be equal or higher than zero and (iv) each of the isotopologue concentrations of acetate, propionate and butyrate is at steady state.

The fluxes \(v_{A,0}\), \(v_{P,0}\) and \(v_{B,0}\) as depicted in Figure 3B in the main text are defined as the bacterial production fluxes from complex carbohydrates for acetate, propionate and butyrate, respectively. Similarly, the fluxes \(v_{0,A}\), \(v_{0,P}\) and \(v_{0,B}\) are defined as the host uptake fluxes for acetate, propionate and butyrate, respectively. The interconversion fluxes are defined as:

\[
\begin{align*}
    v_{A,A} & : 1 \text{ Acetate} \rightarrow \frac{1}{2} \text{ Butyrate} \\
    v_{B,B} & : 1 \text{ Butyrate} \rightarrow 2 \text{ Acetate} \\
    v_{B,P} & : 1 \text{ Propionate} \rightarrow \frac{1}{2} \text{ Butyrate} \\
    v_{P,P} & : 1 \text{ Butyrate} \rightarrow 2 \text{ Propionate} \\
    v_{P,A} & : 1 \text{ Acetate} \rightarrow 1 \text{ Propionate} \\
    v_{A,P} & : 1 \text{ Propionate} \rightarrow 1 \text{ Acetate}
\end{align*}
\]

Detailed reaction mechanisms are not considered and, therefore, chemical balances are not complete. There is steady state with respect to mass and isotope enrichment in Acetate (A), Propionate (P) and Butyrate (B). Therefore the sum of fluxes into and out of each pool is zero. The measured normalized mass isotopologue distribution (MID) for acetate is based on \(m_0/\nu\) \(m_1/\nu\) and are represented as \(a_0\) and \(a_1\) such that \(a_0 + a_1 = 1\). Analogously for propionate the measured MID is based on \(m_0/\xi\) \(m_1/\xi\) and are represented by \(p_0\) and \(p_1\) with \(p_0 + p_1 = 1\) and for...
butyrate the measured MID is based on \( m_0/z, m_1/z \) and \( m_2/z \) and are represented by \( b_0, b_1, \) and \( b_2 \) with \( b_0 + b_1 + b_2 = 1 \). The normalized measured MID was corrected for natural abundance of \(^{13}\text{C}\) by multiple linear regression according to Lee et al. (176) to obtain the excess normalized MID, with \( A_0 \) and \( A_1 \) for acetate and \( P_0 + A_1 = 1, P_0 \) and \( P_1 \) for propionate and \( P_0 + P_1 = 1, B_0, B_1 \) and \( B_2 \) for butyrate and \( B_0 + B_1 + B_2 = 1 \). According to the model the following mass balances can be written for acetate, propionate and butyrate during steady state in which \( v_x \) are the fluxes corresponding to Figure 3B in the main text in mmol/kg/h.

\[
\begin{align*}
\text{Acetate:} & \quad v_{\text{inf}, A} + v_{A,0} + 2 \cdot v_{A,B} + v_{A,P} - (v_{0,A} + v_{B,A} + v_{P,A}) = 0 \quad \text{(7)} \\
\text{Propionate:} & \quad v_{\text{inf}, P} + v_{P,0} + 2 \cdot v_{P,B} + v_{P,A} - (v_{0,P} + v_{B,P} + v_{A,P}) = 0 \quad \text{(8)} \\
\text{Butyrate:} & \quad v_{\text{inf}, B} + v_{B,0} + \frac{1}{2} \cdot v_{B,A} + \frac{1}{2} \cdot v_{B,P} - (v_{0,B} + v_{P,B} + v_{A,B}) = 0 \quad \text{(9)}
\end{align*}
\]

in which \( v_{\text{inf}, A}, v_{\text{inf}, P} \) and \( v_{\text{inf}, B} \) are the mass inflow rates due to infusion of acetate, propionate and butyrate, respectively.

The mass balances and the normalized excess MID are used to formulate the mass balances for each of the mass isotopomers \( A_0, A_1, P_0, P_1, B_0, B_1 \) and \( B_2 \) during the infusions of \([1-^{13}\text{C}]\) acetate \((A_0, A_1, P_0, P_1, B_0, B_1, B_2)\), \([2-^{13}\text{C}]\)propionate \((A_0, A_1, P_0, P_1, P_0, P_1, B_0, B_1, B_2)\) and \([2,4-^{13}\text{C}_2]\)butyrate \((A_0, A_1, P_0, P_1, P_0, P_1, B_0, B_1, B_2)\).

All ordinary differential equations (ODEs) for mass isotopomers below are based on

\[
\frac{d[X_i]}{dt} = \sum_j \left( p_{j,i, \text{production}} X_i \cdot v_{j,i, \text{conversion}} \right) - \sum_j \left( p_{j,i, \text{conversion}} X_i \cdot v_{j,i, \text{production}} \right) = 0 \quad \text{(10)}
\]

where \( X \) stands for either acetate, propionate or butyrate, \( i \) stands for the isotopologue (i.e. \( 0,1,2 \)), \( j \) stands for the reaction and \( p \) for the probability of \( X_i \) involved in reaction \( j \). Based on formula 10 we constructed in total 21 ODEs for the acetate, propionate and butyrate infusions.

\[1-^{13}\text{C}]\text{Acetate infusion}

\[
\begin{align*}
\frac{d[A_{0,A}]}{dt} & = v_{A,0} + v_{A,P} \cdot P_{0,A} + 2 \cdot v_{A,B} \cdot B_{0,A} + v_{A,B} \cdot B_{1,A} - (v_{0,A} + v_{B,A} + v_{P,A}) \cdot A_{0,A} = 0 \quad \text{(11)} \\
\frac{d[A_{1,A}]}{dt} & = v_{\text{inf}, A} + v_{A,P} \cdot P_{1,A} + 2 \cdot v_{A,B} \cdot B_{0,A} + v_{A,B} \cdot B_{1,A} - (v_{0,A} + v_{B,A} + v_{P,A}) \cdot A_{1,A} = 0 \quad \text{(12)} \\
\frac{d[P_{0,A}]}{dt} & = v_{P,0} + v_{P,A} \cdot A_{0,A} + 2 \cdot v_{P,B} \cdot B_{0,A} + v_{P,B} \cdot B_{1,A} - (v_{0,P} + v_{B,P} + v_{A,P}) \cdot P_{0,A} = 0 \quad \text{(13)} \\
\frac{d[P_{1,A}]}{dt} & = v_{P,B} \cdot A_{1,A} + 2 \cdot v_{P,B} \cdot B_{1,A} + v_{P,B} \cdot B_{1,A} - (v_{0,P} + v_{B,P} + v_{A,P}) \cdot P_{1,A} = 0 \quad \text{(14)} \\
\frac{d[B_{0,0}]}{dt} & = v_{B,0} + \frac{1}{2} \cdot v_{B,A} \cdot (A_{0,0} + A_{1,0})^2 + \frac{1}{2} \cdot v_{B,P} \cdot (P_{0,0} + P_{1,0})^2 - (v_{0,B} + v_{B,B} + v_{P,B}) \cdot B_{0,0} = 0 \quad \text{(15)} \\
\frac{d[B_{1,0}]}{dt} & = \frac{1}{2} \cdot (2 \cdot v_{B,B} \cdot A_{0,0} + A_{1,0}) + \frac{1}{2} \cdot (2 \cdot v_{B,B} \cdot P_{0,0} + P_{1,0}) - (v_{0,B} + v_{B,B} + v_{P,B}) \cdot B_{1,0} = 0 \quad \text{(16)}
\end{align*}
\]
SCFA fluxes associate with metabolic markers

\[
\frac{d[B_{2-A}]}{dt} = \frac{1}{2} \cdot v_{B,A} \cdot (A_{1-A})^2 + \frac{1}{2} \cdot v_{B,p} \cdot (P_{1-A})^2 - (v_{0,B} + v_{A,B} + v_{P,B}) \cdot B_{2-A} = 0 \tag{17}
\]

[2-\textsuperscript{13}C]Propionate infusion

\[
\frac{d[A_{0-p}]}{dt} = v_{A,D} + v_{A,p} \cdot P_{0-p} + 2 \cdot v_{A,b} \cdot B_{0-p} + v_{A,b} \cdot B_{1-p} - (v_{0,A} + v_{B,A} + v_{P,A}) \cdot A_{0-p} = 0 \tag{18}
\]

\[
\frac{d[A_{1-p}]}{dt} = v_{A,p} \cdot P_{1-p} + 2 \cdot v_{A,b} \cdot B_{2-p} + v_{A,b} \cdot B_{1-p} - (v_{0,A} + v_{B,A} + v_{P,A}) \cdot A_{1-p} = 0 \tag{19}
\]

\[
\frac{d[P_{0-p}]}{dt} = v_{P,0} + v_{P,a} \cdot A_{0-p} + 2 \cdot v_{P,b} \cdot B_{0-p} + v_{P,b} \cdot B_{1-p} - (v_{0,P} + v_{B,P} + v_{A,P}) \cdot P_{0-p} = 0 \tag{20}
\]

\[
\frac{d[P_{1-p}]}{dt} = v_{P,1} + v_{P,a} \cdot A_{1-p} + 2 \cdot v_{P,b} \cdot B_{2-p} + v_{P,b} \cdot B_{1-p} - (v_{0,P} + v_{B,P} + v_{A,P}) \cdot P_{1-p} = 0 \tag{21}
\]

\[
\frac{d[B_{0-p}]}{dt} = v_{B,0} + \frac{1}{2} \cdot v_{B,a} \cdot (A_{0-p})^2 + \frac{1}{2} \cdot v_{B,p} \cdot (P_{0-p})^2 - (v_{0,B} + v_{A,B} + v_{P,B}) \cdot B_{0-p} = 0 \tag{22}
\]

\[
\frac{d[B_{1-p}]}{dt} = \frac{1}{2} \cdot (2 \cdot v_{B,a} \cdot A_{0-p} \cdot A_{1-p}) + \frac{1}{2} \cdot (2 \cdot v_{B,b} \cdot P_{0-p} \cdot P_{1-p}) - (v_{0,B} + v_{A,B} + v_{P,B}) \cdot B_{1-p} = 0 \tag{23}
\]

\[
\frac{d[B_{2-p}]}{dt} = \frac{1}{2} \cdot v_{B,a} \cdot (A_{1-p})^2 + \frac{1}{2} \cdot v_{B,p} \cdot (P_{1-p})^2 - (v_{0,B} + v_{A,B} + v_{P,B}) \cdot B_{2-p} = 0 \tag{24}
\]

[2,4-\textsuperscript{13}C\textsubscript{2}]Butyrate infusion

\[
\frac{d[A_{0-b}]}{dt} = v_{A,D} + v_{A,b} \cdot P_{0-b} + 2 \cdot v_{A,b} \cdot B_{0-b} + v_{A,b} \cdot B_{1-b} - (v_{0,A} + v_{B,A} + v_{P,A}) \cdot A_{0-b} = 0 \tag{25}
\]

\[
\frac{d[A_{1-b}]}{dt} = v_{A,b} \cdot P_{1-b} + 2 \cdot v_{A,b} \cdot B_{2-b} + v_{A,b} \cdot B_{1-b} - (v_{0,A} + v_{B,A} + v_{P,A}) \cdot A_{1-b} = 0 \tag{26}
\]

\[
\frac{d[P_{0-b}]}{dt} = v_{P,0} + v_{P,a} \cdot A_{0-b} + 2 \cdot v_{P,b} \cdot B_{0-b} + v_{P,b} \cdot B_{1-b} - (v_{0,P} + v_{B,P} + v_{A,P}) \cdot P_{0-b} = 0 \tag{27}
\]

\[
\frac{d[P_{1-b}]}{dt} = v_{P,a} \cdot A_{1-b} + 2 \cdot v_{P,b} \cdot B_{2-b} + v_{P,b} \cdot B_{1-b} - (v_{0,P} + v_{B,P} + v_{A,P}) \cdot P_{1-b} = 0 \tag{28}
\]

\[
\frac{d[B_{0-b}]}{dt} = v_{B,0} + \frac{1}{2} \cdot v_{B,a} \cdot (A_{0-b})^2 + \frac{1}{2} \cdot v_{B,p} \cdot (P_{0-b})^2 - (v_{0,B} + v_{A,B} + v_{P,B}) \cdot B_{0-b} = 0 \tag{29}
\]

\[
\frac{d[B_{1-b}]}{dt} = \frac{1}{2} \cdot (2 \cdot v_{B,a} \cdot A_{0-b} \cdot A_{1-b}) + \frac{1}{2} \cdot (2 \cdot v_{B,b} \cdot P_{0-b} \cdot P_{1-b}) - (v_{0,B} + v_{A,B} + v_{P,B}) \cdot B_{1-b} = 0 \tag{30}
\]

\[
\frac{d[B_{2-b}]}{dt} = v_{B,1} + \frac{1}{2} \cdot v_{B,a} \cdot (A_{1-b})^2 + \frac{1}{2} \cdot v_{B,p} \cdot (P_{1-b})^2 - (v_{0,B} + v_{A,B} + v_{P,B}) \cdot B_{2-b} = 0 \tag{31}
\]

Objective function

The objective function was defined as \( \sum \frac{d[X_{i}]}{dt} \) in which \( \frac{d[X_{i}]}{dt} \) are all the time derivatives of all isotopic fractions of the three SCFAs and they were summed over the three infusion experiments. This objective function was minimized.
Alternative model

To check the stoichiometric assumptions we made for the propionate-butyrate conversion, we also constructed an alternative model with different stoichiometry and compared the objective function and the fluxes to the standard model for the different guar gum diets to validate if the correlation with the metabolic markers is still valid.

Instead of reactions [3] and [4]

1 Propionate → 1 Butyrate
1 Butyrate → 1 Propionate

Statistics

The principle of Maximum Likelihood Estimation (MLE) is used for the estimation of errors in the parameters. The computed likelihood function L is given by

$$
\ln(L) = \sum_{i=0}^{2} \left( \frac{d[A_i]}{\sigma_i^2} \right)^2 + \left( \frac{d[P_i]}{\sigma_i^2} \right)^2 + \left( \frac{d[B_i]}{\sigma_i^2} \right)^2
$$

This likelihood function is optimized with respect to the different parameters in order to determine their errors (182).