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Alterations in the amounts of microbial metabolites in different regions of the mouse large intestine using variably fermentable fibres

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ABSTRACT

Understanding the fermentation profiles of dietary fibres in the different regions of the large intestine is needed to design dietary strategies for desired outcomes related to health such as targeting region-specific diseases. In order to test how variably fermentable dietary fibres affect microbial fermentation metabolites in different regions of the large intestine, mice were fed with fructooligosaccharides (FOS, fast fermentation rate), resistant starch type 2 (native potato, RS2, moderate fermentation rate), starch-entrapped microspheres (SM, very slow fermentation rate), and lignin (control) for 14 days. Analysis of intestinal contents obtained from the cecum, proximal colon, and distal colon revealed that short-chain fatty acid (SCFA) metabolites as well as branched-chain fatty acids (BCFA) levels in different regions of the large intestine were influenced by fibre type and rate of fermentation. Slower fermenting resistant starch-based fibres (RS2 and SM) produced more butyrate than FOS in the proximal and distal colon regions. SM substantially lowered the amount of total BCFAs in the distal colon compared to FOS, suggesting that SM promotes delayed saccharolytic activity throughout the colon. Moreover, the microbial analysis done with length-heterogeneity-PCR showed that all fermentable substrates shifted the microbial community with respect to the control group. Overall, our findings show that the type and amount of SCFAs and BCFAs generated in different regions of the large intestine is dietary fibre type and fermentation rate dependent, which should be considered for increasing the health promoting effects of dietary fibres.

1. Introduction

Although the body itself provides some fermentative substrate for the gut microbiota by way of mucosal polysaccharides and sloughed off cells, dietary fibre, including oligosaccharides, is by far the most significant source of energy-providing substrate (Hamaker & Tuncil, 2014). Among the factors affecting microbiota composition and health, diet can be conveniently manipulated and tailored to specific physiological conditions. For instance, diets supplemented with dietary fibres have been attempted for the management of irritable bowel syndrome, inflammatory bowel diseases, colon cancer, duodenal ulcers, diverticular disease, pathogen susceptibility, and constipation to name a few (Albenberg & Wu, 2014; Aune et al., 2011; Aziz, Dore, Emmanuel, Guarner, & Quigley, 2013; Crowe et al., 2014; Desai et al., 2016; Smith et al., 2013). Yet, much is still not known about how dietary fibre can be used to positively affect the gut microbiome and how selection of dietary fibres will be crucial to gut health through fermentation pattern, fermentation metabolites, and shifts in bacterial communities. For instance, rapidly fermentable dietary fibres, such as fructooligosaccharides, lead to beneficial short chain fatty acid (SCFA) products and promotion of beneficial bacteria, though can also lead to excessive bloating, flatulence, diarrhea, and inflammation in some cases (Gibson & Shepherd, 2010).

Generally, changes in the gut microbiota upon introduction of a new dietary fibre depend on the initial microbiota composition (Chen et al., 2017; Tuncil et al., 2017; Walker et al., 2011). Apart from the composition of microbiota, the type of substrate and its availability in the different regions of the large intestine significantly affect fermentation and its byproducts. This is due to the fact that different regions of the large intestine are colonized by different microorganisms (Gu et al.,

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2013; Van den Abbeele et al., 2010) whose dietary fibre specificities vary. Furthermore, different regions exhibit distinct anatomical and physiological characteristics of their own (Mowat & Agace, 2014). Understanding how individual dietary fibres that differ in chemical and physical structures and in fermentation rate, are utilized by gut microbiota in different regions of the large intestine will enable the design of dietary fibre compositions for desired outcomes related to health and specific disease conditions associated with the gut microbiome.

In addition, there are two distinct microbiota populations within a region of the large intestine: the luminal microbiota, a dynamic community residing in the lumen and which continuously changes in form and number; and the mucosa-associated microbiota, a static community adhering to mucosal lining of the colonic epithelium (Donaldson, Lee, & Mazmanian, 2016; Zoetendal et al., 2002). Characterization of luminal (Eckburg et al., 2005; Ley et al., 2005; Manichanh, Borruel, Casellas, & Guarner, 2012; Yatsunenko et al., 2012) and mucosa-associated microbiota (Eckburg et al., 2005; Poxton, Brown, Sawyerr, & Ferguson, 1997; Shen et al., 2010; Zhang et al., 2007) has been reported in both healthy and diseased states. In comparison, the effects of ageing, genetics, diet and intestinal disorders on the microbiota are less well known (Ridaura et al., 2013; Tachon, Zhou, Keenan, Martin, & Marco, 2013; Yatsunenko et al., 2012; Zoetendal, Vaughan, & de Vos, 2006), particularly on the mucosa-associated microbiota that reside in a relatively difficult-to-sample location.

Considering the impact of carbohydrate substrate dependence on the microbiota population, amount and type of SCFAs produced, and rate and site of fermentation, the present study employed three fermentable dietary fibres – a rapidly fermenting oligosaccharide (fructooligosaccharide, FOS), a conventional resistant starch type 2 (RS2), and physically-entrapped resistant starch microspheres (SM, also a type 2 RS) in a 14-day mouse feeding trial. Lignin, a largely non-fermentable dietary fibre, was used as a control. The three fibre types were chosen to ferment at different rates [FOS, fast fermenting; conventional RS2, moderate fermentation rate; microsphere RS2 or SM, very slow fermentation rate as previously demonstrated using in vitro models of colonic fermentation (Kaur, Rose, Rumpagaporn, Patterson, & Hamaker, 2011; Rose et al., 2009; Rose, Venema, Keshavarzian, & Hamaker, 2010)]. The hypothesis tested in this study was that the three dietary fibres having different chemical and physical structures would reveal distinct microbial byproduct formation in different regions of the large intestine.

2. Materials and methods

2.1. Animals and diet

Forty C57BL6/J male mice, aged 6 weeks, with an average weight of 21.80 ± 0.35 g were acquired commercially (The Jackson Laboratory, Bar Harbor, ME, USA). The mouse model C57BL/6 J was used because of its close similarity to the dominant bacterial divisions of the human colonic microbiota (Ley et al., 2005). Animals were housed in individual cages containing wood pulp bedding (TEK-Fresh, Harlan Laboratories Inc., Indianapolis, IN, USA) and the cages were kept in an isolated room at a temperature of 22–25 °C with a 12 h light/dark cycle. Animals were acclimatized for a period of one week during which they had free access to water and semisynthetic feed as per the guidelines of the American Institute of Nutrition (TestDiet® AIN-76A Semi-Purified Diet, Harlan Laboratories Inc., Indianapolis, IN, USA). Test diets were manufactured commercially into 1/2 in. pellets (Harlan Laboratories Inc., Madison, WI, USA). Changes in composition were based on the AIN-76A diet where 5% cellulose and 15% corn starch were replaced with 10% (w/w) dietary fibre of interest and 10% sucrose. Sucrose was used as a replacement because it does not reach the large intestine for fermentation and was the major single component of the acclimatizing mouse chow. The four dietary fibres tested in this study included the fermentable-resistant control, lignin (Indulin AT, Westvaco, Charlsten, SC, USA), and three fermentable fibres, raw potato starch/RS2 (Roquette, Keokuk, IA, USA), FOS (Orafti, Tienen, Belgium), and SM, the physically inaccessible RS2. Microspheres were prepared using waxy corn starch and 1% alginate as per Rose et al. (2009). Before incorporating these dietary fibres into animal diets, total carbohydrate was determined using the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Because alginate is not typically fermented by gut microbiota (Rose et al., 2009), total starch instead of total carbohydrate was determined for the starch-entrapped microspheres using a commercial kit according to the manufacturer's instructions (total starch method, Megazyme, Wicklow, Ireland). Microspheres were ground for 2 min in a ball mill (Brinkmann Instrument Co., Westbury, NY, USA) and then analyzed. Based on the amount of total carbohydrate in each case, 10% equivalent carbohydrate was incorporated into the diets. Composition of the diets is presented in Table S1. Animals were randomly assigned to the four diet groups (n = 10): 1) lignin, 2) FOS, 3) RS2 potato starch, and 4) SM, starch-entrapped microspheres. Mice were raised on these diets for two weeks.

2.2. Experimental design

Purdue Animal Care and Use Committee (PACUC, Purdue University, West Lafayette, IN, USA) approval was obtained for all experimental procedures before the initiation of the study. Fig. 1 shows the outline of the experimental design. During the 2 week period, animals were monitored daily for changes in fecal consistency and general well-being. Body weight was measured every third day as an indicator of feed intake and health. Fecal samples were collected at the start (day 0) and end (day 14) of the feeding trial. At the end of the study, animals were euthanized using a carbon dioxide overdose. A mid-ventral incision was given to dissect out intestines so as to collect intestinal contents as well as tissue parts. Firstly, the distal colon was identified as the segment touching the anus and the proximal colon was clamped just below the cecum. Average length of large intestine in mice is approximately 77 mm (Dahlhoff et al., 2008) from which the 5 mm part touching the anus was left out and a 24 mm part proximal to that was collected as the distal colon (Zizzo, Mule, & Serio, 2007). The remaining section of the intestine below the cecum was collected as the proximal part of the colon. After careful removal, the dissected intestine parts (cecum, proximal colon, and distal colon) were placed on a filter paper and the contents were removed. The contents were placed in a sterile pre-weighed container, weighed immediately, and were snap frozen using liquid nitrogen. A longitudinal incision was made throughout the entire length of the intestine and it was cut open and washed thrice with 1× phosphate buffered saline (PBS, pH 7.4, 37 °C). Tissues of empty cecum, proximal colon, and distal colon were weighed and snap frozen in separate containers.

2.3. Measurement of short-chain fatty acids

Short-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs) were analyzed using gas chromatography for the contents from cecum, proximal colon, and distal colon. In case of the proximal and distal colon contents, samples were pooled from two animals because of insufficient amounts available (n = 5). Fecal samples and contents were weighed, diluted 1:10 with 1× PBS and were homogenized by vortexing. Samples (400 μL) were combined with 100 μL of a standard mixture containing 50 mM 4-methyl-valeric acid (nr 277827–5G, Sigma-Aldrich Inc., St. Louis, MO, USA), 5% meta-phosphoric acid, and copper sulfate (1.56 mg/mL), and stored at –40 °C until analysis. A standard mixture with 50 mM internal standard solution was diluted 1.25 times (400 μL sample plus 100 μL standard mixture) so that the final concentration of the internal standard in the sample was 10 mM/L which was same as the external standard mentioned below. Later, thawed samples were centrifuged (3000 × g, 10 min) and the supernatants were injected into a gas chromatograph (model 7890 A, Agilent